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Increased Susceptibility to Mouse Hepatitis Virus 3 of Peritoneal Macrophages Exposed to Dieldrin

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Increased Susceptibility to Mouse Hepatitis Virus 3 of Peritoneal Macrophages Exposed to Dieldrin. KRZYSTYNIAK, K., HUGO, P., FLIPO, D., AND FOURNIER, M. (1985). Toxicol. Appl. Pharmacol. 80, 397-408. Interaction of a single dose (36 mg/kg body wt) of the organochlorine pesticide dieldrin with mouse peritoneal macrophages was examined in C57Bl/6, (C57Bl/6 × A/J)F1, and A/J strains of different genetic resistance to mouse hepatitis virus 3 (MHV3) infection. In vivo studies showed increased susceptibility to MHV3 acute disease of C57Bl/6 and (C57Bl/6 × A/J)F1 animals challenged with the pesticide. Significant decrease of mean time of death in dieldrin-exposed, MHV3-infected susceptible C57Bl/6 mice was observed similarly upon po or ip administration of a single, sublethal dose of dieldrin. In addition, decrease of humoral response to the virus was quantified by determination of anti-MHV3 IgG antibodies in spleen cell supernatant fractions and in blood sera of dieldrin-exposed C57Bl/6 mice. A single dose of dieldrin did not alter the in vivo resistance of A/J animals to acute MHV3 disease. The resistant A/J mice, however, showed increased mortality upon two subsequent exposures to dieldrin followed by infection with high lethal doses of MHV3. Phagocytic activity, cell adherence capacity, and attachment and uptake of 3H-radiolabeled MHV3 by C57Bl/6 peritoneal macrophages were determined by in vitro studies. These effector activities of peritoneal macrophages were slightly decreased or unchanged in cells originating from animals exposed to the pesticide. However, the intrinsic activity of MHV3 restriction appeared to be affected in macrophages derived from dieldrin-treated animals: (i) peritoneal C57Bl/6 macrophages collected from the early phase of acute MHV3 disease contained increased MHV3 antigen and (ii) increased cytolysis was observed after in vitro MHV3 infection of macrophages originating from dieldrin-exposed C57Bl/6 mice. © 1985 Academic Press, Inc.

Exposure to certain environmental pollutants or drugs may perturb immune responsiveness and alter susceptibility of animals and humans to infectious agents, including viral infections (Crocker et al., 1976; Bradley and Morahan, 1982; Kern, 1982; Howett et al.; 1979; Casto et al., 1974). The interaction of xenobiotics with host antiviral defense mechanisms, which involve both humoral and cellular elements of immune response, is an extremely complicated system for toxicological studies (Bradley and Morahan, 1982). Therefore, the experimental model of viral infection for the toxicological studies was selected on the basis of the considerable amount of information available on the pathogenesis of viral infection (Kern, 1982).

In a preliminary study (Krzystyniak et al., 1984), we reported the increased susceptibility of C57Bl/6 and (C57Bl/6 × A/J)F1 mouse strains to mouse hepatitis virus 3 (MHV3), after exposure of the animals to the organo-
chlorine pesticide dieldrin. MHV3, a member of coronaviruses family, is one of the extensively investigated animal models of viral infection (Bang and Warwick, 1960; Le Prevost et al., 1975; Dupuy et al., 1980). Since Bang and Warwick (1960) reported that the macrophage is a target cell for MHV and since destruction of macrophages with silica was shown to affect host defense mechanisms against MHV infection (Tamura et al., 1979), it is of interest to see the effects of xenobiotics on MHV–macrophage interactions. This work was undertaken to determine the effects of dieldrin on the interaction of MHV3 with mouse peritoneal macrophages.

METHODS

Animals. Inbred female C57Bl/6 (susceptible to MHV3 acute disease), C57Bl/6 × A/J F1 (semisusceptible), and A/J (resistant) mice, 8 to 10 weeks old, were obtained from the Jackson Laboratory, Bar Harbor, Maine. Upon arrival, all mice were quarantined for 1 week prior to use. The lethal dose of dieldrin administered by ip injection was determined in female Swiss mice, 8 to 10 weeks old, obtained from the Charles River Company (Montreal, Quebec). The LD50 of ip injected dieldrin, dissolved in corn oil, calculated according to Litchfield and Wilcoxon, (1949) was 54 mg/kg body wt, which corresponded to data reported by others (reviewed by Hodge et al., 1967).

The animals received one ip dose of 36 mg/kg body wt dieldrin (99.9% purity; Supelco Inc., Bellefonte, Pa.) dissolved in 0.1 ml corn oil. In one experiment, dieldrin was administered by gavage, as a single dose (4.0 to 30.0 mg/kg body wt) of the pesticide dissolved in 0.1 ml corn oil.

Three different kinds of MHV3 virus were used for in vivo infection: (1) wild MHV3 which has a high capacity to induce acute disease and rapid mortality in the susceptible C57Bl/6 mouse (Krzyżyniak and Dupuy, 1981), (2) an L-cloned substrain of MHV3, which kills the animal after a prolonged acute disease (Dupuy and Rodrigue, 1981), and (3) a cloned YAC-MHV3 variant which has reduced in vivo pathogenicity and is nonlethal for the C57Bl/6 strain (Lamontagne and Dupuy, 1984). Four to five days after pesticide treatment, the animals were infected ip with wild MHV3 (Krzyżyniak and Dupuy, 1981) or with its L-cloned substrain (Dupuy and Rodrigue, 1981). Humoral response to the virus in dieldrin-exposed C57Bl/6 mice was determined after infection of animals with nonlethal YAC-MHV3 variant (Lamontagne and Dupuy, 1984). Untreated controls and animals receiving 0.1 ml corn oil were similarly infected with the virus. No marked effect of the vehicle on the mortality of the susceptible C57Bl/6 mice and on the survival of the resistant A/J animals was observed upon in vivo infection with MHV3, as compared to the untreated controls.

Cells. Peritoneal exudate macrophages were obtained and cultured as described elsewhere (Krzyżyniak and Dupuy, 1981). Briefly, control and dieldrin-treated mice were killed 4 days after treatment and peritoneal exudates were obtained by washing the peritoneal cavities of mice with a total volume of 8 ml of heparinized Eagle's minimal essential medium (MEM; Grand Island Biological Co., Grand Island, N.Y.). After collection, cells were centrifuged at 1800 rpm at 4°C for 10 min, resuspended in MEM, and counted. Nonadherent cells were removed after 2 hr of incubation at 37°C in Petri dishes by three washings with MEM. Adherent cells were shown to have morphological, functional, and biochemical criteria of macrophages (Dupuy et al., 1980).

L cells were grown as monolayers in MEM medium supplemented with 10% fetal calf serum and antibiotics, as described elsewhere (Krzyżyniak and Dupuy, 1981).

Cell cultures. Macrophages were incubated at 37°C in RPMI 1640 medium (Grand Island Biological Co.) supplemented with 10% fetal calf serum and antibiotics, as described elsewhere (Krzyżyniak and Dupuy, 1981). For in vitro studies, dieldrin, 0 to 10 μg/ml, was added in ethanol solution for 48 hr. The final concentration of ethanol in the medium never exceeded 2% and was shown not to affect cell viability, cellular adherence to plastic, phagocytosis, nor replication of MHV3 in cells.

Spleen cells from dieldrin-exposed, YAC-MHV3-infected C57Bl/6 animals were cultured as described elsewhere (Krzyżyniak and Dupuy, 1983). Briefly, suspensions of 2 × 10^7 cells/ml were incubated for 48 hr at 37°C with 5% CO2 in RPMI medium supplemented with 10% fetal calf serum and antibiotics. Culture supernatant fractions were then collected for determination of anti-MHV3 IgG antibodies. Exposure of C57Bl/6 spleen mice to 0 to 36 mg/kg body wt of dieldrin did not change cell viability in the cultures, which was determined by trypan blue exclusion test at the end of the 48-hr incubation period.

Virus. The MHV3 strain of mouse hepatitis virus was passaged in vitro on L cells (MHV3-L) as described by Krzyżyniak and Dupuy (1981). 3H-Radiolabeled MHV3 was prepared as described by Lai and Stohlman (1978), with slight modifications (Krzyżyniak and Dupuy, 1981). Briefly, MHV3-L-cloned virus with high yield capacity at 33°C (Lamontagne and Dupuy, 1982) was adsorbed on monolayers in 150-cm² tissue flasks (Corning Glass Work, Montreal, Canada) for 30 min at 37°C (10^7 to 10^8 plaque-forming units [pfu]/bottle). Cells were washed and MEM containing 10% fetal calf serum, antibiotics, and 200 μCi of a 3H-labeled amino acid mixture (L-leucine, L-lysine, L-phenylalanine, L-proline, L-tyrosine, sp act 70 to 100 Ci/mmol) and [5,6-3H]uridine (sp act
40 to 60 Ci/mmol; Amersham, Arlington Heights, Ill.) was added to each bottle immediately following absorption of the virus. Cultures were then incubated at 33°C for 18 hr. Purification of radiolabeled virus was carried out by successive centrifugations. The supernatant fractions were clarified by centrifugation at 12000g for 10 min and 15,000g for 30 min at 4°C. The cellular fraction was frozen and thawed three times, homogenized, and clarified by centrifugation. The virus obtained from the supernatant fraction and cellular fractions was concentrated by pelleting at 90,000g for 2 hr at 4°C, resuspended, and gently homogenized. Virus suspensions were then layered onto a discontinuous gradient consisting of 2.5 ml of 30% sucrose and 2.5 ml of 50% sucrose, and centrifuged at 100,000g for 3 hr at 4°C in a SW-65 Beckman rotor. The visible band at the 30 to 50% sucrose-gradient interface was removed and concentrated by centrifugation at 90,000g for 2 hr at 4°C. Pellets were then gently homogenized and virus fractions were stored at −70°C. In some experiments virus fractions were further centrifuged in a linear 25 to 50% sucrose gradient at 100,000g for 3 hr at 4°C. Virus titer, 3H radioactivity, and absorbance (220 to 320 nm) were determined in all fractions.

Virus titer and cytopathic effects. The MHV3 titer in culture supernatant fractions was determined on L cells and was expressed in plaque-forming units (Dulbecco and Vogt, 1954). Cytopathic effects were determined microscopically 24 and 48 hr postinfection as the percentage of the cell monolayer (Krzystyniak and Dupuy, 1984).

Attachment and uptake of 3H-MHV3. Cell monolayers were infected with 3H-MHV3 for 60 min at 0°C, washed several times with ice-cold MEM, and dissolved with 2% SDS, and the remaining radioactivity was counted and calculated as the surface-bound virus (Marsh et al., 1983). In other experiments, cells were infected for 20 min at 37°C (uptake of MHV3 was over 95%, Krzystyniak and Dupuy, 1981), washed several times with ice-cold MEM, and incubated at 0°C for 90 min with 5 mg/ml of proteinase K (Sigma) to distinguish surface-bound from internalized virus (Marsh et al., 1983). Cells were then dissolved in 2% SDS and the radioactivity was counted. Negative controls were performed by pretreatment of 3H-radiolabeled MHV3 for 3 hr at 4°C with anti-MHV3 serum, prior to incubation with cells (Krzystyniak and Dupuy, 1981). Control of nonspecific uptake by macrophages was also performed by adding the appropriate amount of 3H-labeled material (equivalent in dpm/106 cells), obtained from noninfected L-cell cultures, and processing them in the same fashion as radiolabeled virus (Krzystyniak and Dupuy, 1981).

Phagocytosis assay. The phagocytic activity of macrophages was determined by incubation of cell monolayers with 3H-labeled carboxylated latex beads, as described by Ito et al. (1979). Latex beads (diameter 0.86 μm, 0.25 meq CO2/g) were labeled covalently with [3H]tyramine (New England Nuclear, Boston, Mass.) in the presence of 1 mM 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-p-toluene sulfonate (Aldrich Chemical Co., Milwaukee, Wisc.). Fifty microliters of dialyzed 3H-latex beads, containing approximately 3 × 104 dpm, was added onto macrophage monolayers (ratio: 102 particles/cell), preincubated at 37°C for 24 hr with or without 1 μg/ml lipopolysaccharide (LPS; Difco, Detroit, Mich.) or 1 hr with 5 μg/ml cytochalasin B (Sigma, St. Louis, Mo.). Monolayers were then incubated at 37 or 0°C for 3 hr, washed several times with MEM containing 10% fetal calf serum, incubated for 10 min at 37°C with trypsin–EDTA solution, and washed again to remove uningested beads (Ito et al., 1979).

Dot-blot immunoperoxidase assay and enzyme-linked immunosorbent assay (ELISA). MHV3 antigen in peritoneal macrophages originating from vehicle-treated controls and dieldrin-exposed animals, infected in vivo with the virus, was quantified by the dot-blot immunoperoxidase dilution test as described by Hawkes et al. (1982). Briefly, appropriate dilutions of cellular homogenates were applied as spots onto nitrocellulose papers (Krzystyniak and Dupuy, 1983). The papers were incubated first with the anti-MHV3 serum obtained from the A/J mouse strain immunized twice with MHV3 (Krzystyniak and Dupuy, 1981) and then with the peroxidase-conjugated anti-mouse Ig antibody (Boehringer). After the development of peroxidase with 4-chloro-1-naphthol (Sigma) and H2O2 (Sigma), a positive reaction was detected as a colored dot against the white nitrocellulose paper background. The results are expressed as the minimal cell number giving positive immunoperoxidase reaction.

The humoral response to MHV3 infection was determined in untreated controls and dieldrin-exposed and vehicle-treated C57Bl/6 mice. Four days after ip injection of 0 to 36 mg/kg body wt of dieldrin, the animals were infected with 103 LDSO YAC-MHV3 variant of reduced in vivo pathogenicity (Lamontagne and Dupuy, 1984). Seven days after infection, the animals were killed, and spleen cells and blood samples were collected. Anti-MHV3 IgG antibodies were determined in mouse sera and in supernatant fractions of 48-hr spleen cell cultures by ELISA, a modification of the method of Engvall and Perlmann (1972). Briefly, equal quantities of homogenates of L cells infected with MHV3, 18 hr prior homogenization, were used as the MHV3 antigen (Krzystyniak and Dupuy, 1983). Appropriate controls were performed with noninfected L-cell homogenates. The antigen was incubated in flat-bottomed Linbro microplates and washed, and the plates were saturated with 3% bovine serum albumin (Sigma). Subsequently, several dilutions of mouse sera and culture supernatant fractions were incubated overnight with the antigen and then washed several times. As a second antibody, peroxidase-conjugated anti-mouse IgG antibody was used at concentration 1:3000. The substrate for peroxidase development was 2,3-azino-di-(3-ethylbenzthiazoline) sulfonate (Kirkegaard & Perry Laboratories, Gaitherburg, Md.) and H2O2,
and the colored product was determined photometrically at OD$_{405}$.

RESULTS

In Vivo Effects of Dieldrin on Acute MHV3-Disease

Infection of susceptible C57Bl/6 mice with a lethal dose of wild MHV3 resulted in acute disease and subsequent death, within 6 days, of untreated controls (not shown) and animals treated with vehicle only (Table 1). Exposure of C57Bl/6 mice to a single ip dose of dieldrin, 4 days prior to virus infection, resulted in more rapid virus spread in peritoneal macrophages and more rapid death of animals (Table 1). Some deaths occurred only 48 hr postinfection in the dieldrin-treated group and virus replication was relatively high as shown by quantification of MHV3 antigens and virus titer in cultures of peritoneal macrophages (Table 1). No deaths occurred 48 hr postinfection in vehicle-treated animals and untreated controls, and less than 15% death occurred in these animals 72 hr postinfection, whereas over 90% of dieldrin-exposed animals were dead at that time (Table 1). The mean day of death (MDD) for the vehicle group was significantly higher than that for the dieldrin group ($4.13 \pm 0.74$ compared to $2.93 \pm 0.45$, respectively), which was found by the Student $t$ test ($p < 0.001$) and by the nonparametric Mann–Whitney $U$ test ($p < 0.02$). No significant differences in MDD between MHV3-infected untreated controls and controls treated with corn oil (ip or po, Table 2) were found by the Mann–Whitney $U$ test. However, po administration of 18 or 30 mg/kg body wt of dieldrin resulted in a significant decrease in the MDD between vehicle-treated animals and dieldrin-exposed animals, subsequently infected with the virus ($p < 0.05$ and $p < 0.005$, respectively, by the Student $t$ test and $p < 0.02$ by the Mann–Whitney $U$ test). This effect of dieldrin was observed clearly upon infection of susceptible C57Bl/6 and semisusceptible (C57Bl/6 $\times$ A/J)F$_1$ mice with a relatively low dose of a less pathogenic, L-cloned substrain of MHV3 (Fig. 1). All C57Bl/6 mice (15 animals per group) died after infection with

### TABLE 1

| Day after infection | Control (vehicle) | Dieldrin |
|--------------------|-------------------|----------|
| Acute disease$^a$  | Peritoneal macrophages$^b$ | Acute disease$^a$  | Peritoneal macrophages$^b$ |
| No. dead/No. tested | MHV3 titer | MHV3 Ag | No. dead/No. tested | MHV3 titer | MHV3 Ag |
| 1 0/15 0 0 | $10^2$ | 5.0 x $10^3$ | 0/15 0 | 0.5 x $10^2$ | 5.0 x $10^3$ |
| 2 0/15 0 | $10^2$ | 2.5 x $10^3$ | 2/15 13.3 | $10^3$ | 1.5 x $10^4$ |
| 3 2/15 13.3 | 5.0 x $10^3$ | 1.5 x $10^4$ | 14/15 93.3 | 5.0 x $10^4$ | 7.5 x $10^3$ |
| 4 12/15 80.0 | $5.0 \times 10^{2}$ | $5.0 \times 10^{3}$ | 15/15 100.0 | — | — |
| 5 14/15 93.3 | $5.0 \times 10^{2}$ | $5.0 \times 10^{3}$ | — | — | — |
| 6 15/15 100.0 | — | — | — | — | — |

$^a$Animals were infected ip with $10^7$ LD50/mouse of MHV3 4 days after dieldrin exposure.

$^b$Peritoneal macrophages were collected 1 to 6 days after infection with MHV3. The cells were incubated in vitro for 24 hr at 37°C. The MHV3 titer was determined in culture supernatant fractions and expressed as pfu/ml. MHV3 antigens in collected macrophages were determined by immunoperoxidase dot-blot assay, and the results are expressed as minimal number of cells giving positive immune reaction on nitrocellulose blots. The results are the mean of triplicates; SD did not exceed 15%. 

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| 2 0/15 0 | $10^2$ | 2.5 x $10^3$ | 2/15 13.3 | $10^3$ | 1.5 x $10^4$ |
| 3 2/15 13.3 | 5.0 x $10^3$ | 1.5 x $10^4$ | 14/15 93.3 | 5.0 x $10^4$ | 7.5 x $10^3$ |
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$^a$Animals were infected ip with $10^7$ LD50/mouse of MHV3 4 days after dieldrin exposure.

$^b$Peritoneal macrophages were collected 1 to 6 days after infection with MHV3. The cells were incubated in vitro for 24 hr at 37°C. The MHV3 titer was determined in culture supernatant fractions and expressed as pfu/ml. MHV3 antigens in collected macrophages were determined by immunoperoxidase dot-blot assay, and the results are expressed as minimal number of cells giving positive immune reaction on nitrocellulose blots. The results are the mean of triplicates; SD did not exceed 15%.
MHV3 susceptibility of macrophages and dieldrin

TABLE 2

DOSE-DEPENDENT EFFECTS OF PO ADMINISTERED DIELDRIN ON MHV3-INDUCED ACUTE DISEASE IN C57BI/6 MICE

| Dose  | Cumulative mortality (No. dead/No. tested) | Mean day of death ± SD |
|-------|-------------------------------------------|------------------------|
|       | 2 | 3 | 4 | 5 |                  |
| (Untreated) | 0/10 | 2/10 | 9/10 | 10/10 | 3.9 ± 0.5 |
| (Oil) | 0/10 | 2/10 | 8/10 | 10/10 | 3.9 ± 0.5 |
| 4.0 | 0/10 | 2/10 | 9/10 | 10/10 | 4.0 ± 0.6 |
| 12.0 | 0/10 | 2/10 | 8/10 | 10/10 | 4.0 ± 0.6 |
| 18.0 | 1/10 | 6/10 | 10/10 | — | 3.3 ± 0.6** |
| 30.0 | 0/10 | 8/10 | 10/10 | — | 3.3 ± 0.4*** |

a Dieldrin was administered by gavage as a single dose of the pesticide dissolved in 0.1 ml corn oil.

b Animals were infected with 10^3 LD50 of wild MHV3 5 days after administration of dieldrin.

** p < 0.05 by the Student’s t test.

*** p < 0.005.

L-MHV3, and significant differences in MDD between vehicle-treated and dieldrin-treated mice were found (9.7 ± 2.6 compared to 5.4 ± 1.1, respectively, p < 0.001 by the Student t test and p < 0.02 by the Mann–Whitney U test). Mortality in semisusceptible (C57BI/6 × A/J)F1 mice (25 animals per group) reached 26% within 3 weeks after infection, and no death occurred during that time in vehicle-treated controls infected with the same, low dose of virus (Fig. 1). It appears from these data that exposure to dieldrin shortened the survival time in acute MHV3 disease of the susceptible C57BI/6 strain and increased susceptibility to the acute disease in the semisusceptible (C57BI/6 × A/J)F1 strain.

In the next experiment we examined the effect of dieldrin on the humoral immune response of C57BI/6 mice to the infection with YAC-MHV3 variant of reduced in vivo pathogenicity. Untreated controls, vehicle-treated animals, and mice receiving a single ip dose of 4.0, 9.0, 18, and 36 mg/kg body wt of dieldrin were infected with the virus 4 days after the treatment. All animals (5 mice per group) survived a 7-day period without any signs of acute MHV3 disease. The animals were then killed, and anti-MHV3 IgG antibodies were quantified in blood samples and in culture supernatant fractions of spleen cells incubated in vitro for 48 hr at 37°C. As shown in Fig. 2, a marked decrease of anti-MHV3 IgG antibody, determined by ELISA, was observed in animals treated with 18 and 36 mg/kg body wt of dieldrin. A slight stimulatory effect of the vehicle on anti-MHV3 IgG antibody was observed, as compared to virus-infected, untreated controls (Fig. 2). Interestingly, a decrease of anti-MHV3 IgG antibody by dieldrin was observed simultaneously in blood samples and in supernatant fractions of in vitro cultured antibody-producing spleen cells, originating from virus-infected, dieldrin-exposed animals (Fig. 2).

The effect of dieldrin on the acute MHV3 disease in the resistant A/J strain was examined in animals receiving a single, subchronic dose of the pesticide. All three groups, A/J strain, untreated controls, vehicle-treated controls, and mice exposed ip to 36 mg/kg body wt of dieldrin, 4 days prior to virus infection, were resistant to a lethal dose (10^3 LD50/animal of the wild MHV3). It appeared, therefore, that the genetic factor of natural resistance to MHV3 acute disease in
Fig. 1. Acute MHV3-disease, expressed as a percentage of cumulative mortality, in C57Bl/6 mice exposed to vehicle only (Δ) or to a single ip dose of 36 mg/kg body wt dieldrin (▲) and in (C57Bl/6 × A/J)F₁, vehicle-treated (□) or dieldrin-exposed (■) mice, infected with 20 LD₅₀ of L-cloned substrain of MHV3.

A/J strain was not affected by exposure to dieldrin administered at the same dose which aggravated acute MHV3 disease in susceptible C57Bl/6 strain. The suppressive effect of dieldrin was observed in the resistant A/J strain only after two subsequent injections of 36 mg/kg body wt of the pesticide followed by infection with extreme, lethal doses of wild MHV3. Groups of the 10 animals per infectious dose were exposed to dieldrin at Days 0 and 7 and, 4 days after the second exposure, infected with $10^4$ to $10^6$ LD₅₀ MHV3. A significant increase in cumulative mortality was found by nonparametric Mann–Whitney U test ($p < 0.02$), when compared the dieldrin-exposed animals infected with $10^4$ LD₅₀ MHV3 (100% mortality) to untreated controls and vehicle-treated controls infected with the same dose of MHV3 (20% mortality in both these groups). These results, however, should be interpreted with precaution as the dieldrin dose and the virus dose were extremely high. Generally, the in vivo data showed that exposure to dieldrin induced increased susceptibility to MHV3 infection in animals of different genetic resistance to the virus. Dieldrin exposure increased the spread of the virus, shortened survival time, suppressed the humoral antiviral response in susceptible C57Bl/6 mice, and induced mortality in semisusceptible (C57Bl/6 × A/J)F₁ hybrids infected with low dose of the less pathogenic, cloned L-MHV3 substrain. In addition, dieldrin increased susceptibility to high lethal doses of MHV3 in the resistant A/J strain.
MHV3 SUSCEPTIBILITY OF MACROPHAGES AND DIELDRIN

FIG. 2. Effect of single, ip doses of dieldrin (0 to 36 mg/kg body wt in 0.1 ml corn oil) on anti-MHV3 IgG antibody response, determined by ELISA in C57Bl/6 mouse serum (○) and in culture supernatant fractions of in vitro incubated spleen cells (), 7 days after infection with the YAC-MHV3 virus 11 C; untreated controls. Serum dilutions and supernatant dilutions used for the ELISA are indicated above each curve. Bars represent SD of triplicate samples.

Effects of Dieldrin on the in Vitro Activities of Peritoneal Macrophages

As shown in Table 1, increased virus titer and increased virus antigen were detected during the early phase of acute MHV3 disease in peritoneal macrophages derived from dieldrin-exposed C57Bl/6 mice, subsequently infected with the virus. These results suggested possible dieldrin–macrophage interactions, affecting therefore replication and spread of the virus in these host cells. In our next experiments we examined the effect of dieldrin on the cellular effector activity, i.e., macrophage adherence, phagocytosis, uptake of ³H-radiolabeled MHV3 virus particles, and on the intrinsic resistance of macrophages to MHV3 infection. The control cells were macrophages from vehicle-treated animals. We observed a marked, over threefold, increase in the number of peritoneal exudates in C57Bl/6 mice treated with either oil or
oil-dissolved dieldrin. No significant difference in the number of collected exudates per mouse, however, was found between the latter two groups, 4 days after treatment (6.3 x 10^6 ± 1.2 x 10^6 cells/mouse and 5.8 x 10^6 ± 0.9 x 10^6 cells/mouse, respectively, p > 0.5 in the Student t test). Number of differentials, cell viability, provided by trypan blue exclusion test, and incorporation of [3H]thymidine (5 μCi/culture, 48 hr at 37°C, 5% CO2) were similar in macrophage cultures originating from vehicle-treated and dieldrin-exposed C57Bl/6 animals.

Attachment of macrophages to plastic, determined after a 2-hr incubation at 37°C, 5% CO2, of peritoneal exudates collected from untreated control animals, from vehicle-treated mice, and from animals exposed to 36 mg/kg body wt dieldrin, was similar in all three groups. A slight, but not significant, decrease in the adherence capacity of macrophages from dieldrin-exposed animals was observed in the presence of an inhibitor of cell adherence, 5 to 500 μM dansycadaverine.

A significant decrease, however, of phagocytosis of 3H-labeled latex beads in untreated and LPS-stimulated macrophage cultures was observed, but not in cytochalasin B-treated peritoneal macrophages originating from animals exposed to dieldrin (Fig. 3). At 3 hr of incubation of cells with 3H-latex beads, 22% inhibition of phagocytosis was observed in C57Bl/6 cells derived from dieldrin-exposed animals (p < 0.001) and 24% inhibition of

![Graph](image-url)  
**Fig. 3.** Phagocytosis of 3H-latex beads by peritoneal macrophages from C57Bl/6 vehicle-treated mice, expressed as the cell-associated radioactivity after incubation at 4°C (∇) and at 37°C of in vitro untreated (○), cytochalasin B-inhibited (△), and LPS-stimulated cells (○), or from dieldrin-exposed mice, incubated at 4°C (●) and at 37°C of in vitro untreated (▴), cytochalasin B-inhibited (●), and LPS-stimulated cells (●). Bars represent SD of 4 replications.
phagocytosis was observed in the latter cells stimulated subsequently in vitro with LPS ($p < 0.001$) (Fig. 3). These data showed inhibition of phagocytic activity of macrophages by a single, sublethal dose of dieldrin 4 days after in vivo challenge of C57Bl/6 animals with the pesticide.

Attachment and uptake of the $^3$H-labeled virus by peritoneal macrophages originating from dieldrin-exposed C57Bl/6 mice and from vehicle-treated controls showed no significant differences between these two groups; the attachment of $^3$H-MHV3 after 60-min incubation at $0^\circ$C was, respectively, 5526 ± 321 dpm compared to 5483 ± 281 dpm ($p > 0.5$) and the uptake of $^3$H-MHV3 after 20-min incubation at $37^\circ$C and subsequent 90-min incubation at $0^\circ$C with 5 mg/ml proteinase K was, respectively, 3260 ± 188 dpm compared to 3340 ± 126 ($p > 0.5$). The data showed that in vivo treatment with a single, sublethal dose of dieldrin did not affect the attachment and internalization of virus particles into C57Bl/6 peritoneal macrophages.

The effector activity of virus restriction by peritoneal macrophages originating from either susceptible C57Bl/6 animals or resistant A/J mice was examined after in vivo exposure to the pesticide or upon in vitro incubation of cells with dieldrin. Figure 4A shows increased MHV3-induced cell lysis, observed 24 and 48 hr after in vitro infection of C57Bl/6 peritoneal macrophages derived from dieldrin-exposed animals. No increase of MHV3 titer, however, was found in macrophage cultures, suggesting therefore that virus replication itself was not augmented by the pesticide (Fig. 4B). Paradoxically, decreased virus titer, at higher infectious doses, was observed in culture supernatant fractions of dieldrin-exposed macrophages (Fig. 4B). This decrease might be explained by more rapid cell lysis and earlier terminating of virus production in dieldrin-exposed macrophage cultures, where apparently no cells were left after multicycle virus replication (24 to 48 hr) as compared to oil-treated controls (Fig. 4). In addition, in vitro treatment of C57Bl/6 peritoneal macrophages with 0 to 10 μg/ml dieldrin showed the pesticide-dose dependent increase of MHV3-induced cytopathic effects and decrease of the virus titer at concentrations of the pesticide > 1 μg/ml (Fig. 5). Uptake of $^3$H-MHV3 into C57Bl/6 peritoneal macrophages treated for 48 hr in vitro with dieldrin at a concentration > 1 μg/ml was significantly decreased (Fig. 5A), and virus replication appeared to be slightly inhibited at different infectious doses (0.001 to 0.1 moi) (Fig. 5B). These results suggest that at low in vitro doses of dieldrin (less than 1 μg/ml) there was little effect of the pesticide on virus growth and/or virus-induced cytopathic effects, and that at higher doses of
dieldrin there was increased susceptibility of the macrophages to MHV3-induced cytolysis. This probably secondarily accounts for the reduced plaque-forming units observed.

**DISCUSSION**

We present the evidence that in vivo exposure to a single dose of the organochlorine pesticide dieldrin resulted in increased susceptibility of C57Bl/6 murine macrophages to MHV3 infection. This increased susceptibility was manifested as an accelerated and increased in vivo appearance of MHV3 antigens in macrophages and increased cytolysis of these cells infected in vitro with the virus. Since peritoneal macrophages are the primary target cells for MHV3 infection (Bang and Warwick, 1960), the effects of the pesticide seemed to be related with the virus–macrophage interaction during MHV3 acute disease (Krzystyniak et al., 1984). The MHV3 replication cycle itself, however, seemed to be unmodified in peritoneal macrophages from dieldrin-exposed animals: (1) Viral adsorption and (2) viral penetration seemed to be similar as in control cells. (3) Inhibition of phagocytic activities of macrophages originating from dieldrin-exposed animals seemed to be without any effect on MHV3 uptake, since penetration of MHV3 was shown to be a phagocytosis-independent process (Krzystyniak and Dupuy, 1981). (4) Furthermore, replication and assembly of infectious MHV3 particles in macrophages from dieldrin-exposed animals were shown in in vitro experiments to be equal or decreased, as compared to MHV3 replication in control cultures. (5) In addition, in vitro treatment of cells with 1 to 10 μg/ml dieldrin clearly showed inhibition of virus titer, suggesting therefore a mechanism different from the direct augmenting of the virus yield by the pesticide.

MHV3 infection of macrophages results in cytolysis of the susceptible cells (Dupuy et al., 1980). Resistance to MHV3-induced cytopathic effects is an intrinsic factor, possibly genetically controlled (Dupuy et al., 1980; Arnheiter et al., 1982). Induction of viral cytopathic effects may be related to modifications of lysosomal membranes (Allison, 1967) or to a depletion of surface membrane resources following emergence of enveloped virus without compensatory membrane production (Quigley et al., 1972). Because organochlorine pesticides interact primarily with cell membranes (Omann and Lakowicz, 1982; Desaih, 1981; Antunes-Madeira and Madeira, 1979), we can assume that the...
dieldrin-exposed macrophages were more susceptible to MHV3-induced cytopathic effects due to membrane modifications by the pesticide. The nature of the interaction of dieldrin with MHV3-induced cytolysis, however, requires further studies. Dieldrin is a potent toxic agent, affecting the population of peritoneal macrophages (Loose et al., 1981; Kaminski et al., 1982), i.e., decreasing cell viability and decreasing phagocytic activities of cells.

It has been reported that exposure of macrophages to pesticides impair effector macrophage activity, i.e., antigen processing, tumor cell killing, induction of T-suppressor cells, and not the effector macrophage parameters, like cellular respiration, phagocytic activity, and chemotaxis (Loose et al., 1981; Loose, 1982). Our studies similarly revealed slight or no alteration of the effector macrophage activities by dieldrin in C57Bl/6 mice. Further studies, however, are necessary to establish the effects of dieldrin on macrophage effector activity in MHV3 infection. We cannot exclude the interaction of dieldrin with cellular host defense mechanisms other than macrophages and affecting in vivo macrophage–lymphocyte cooperation in MIIV3 acute disease (Dupuy et al., 1980; Siddel et al., 1981). Our preliminary studies showed the pesticide effects on other cells involved in MHV3 restriction; for instance, the viral abrogation of lymphoproliferative activity of T cells was markedly aggravated by the exposure of C57Bl/6 animals to dieldrin (Krzystyniak et al., 1984).

Increased MHV3 susceptibility observed in the dieldrin-exposed C57Bl/6 strain and no analogous effects in the resistant A/J strain exposed to a single, subchronic dose of dieldrin can be possibly related to the strain-specific susceptibility or resistance to MHV3 infection. This susceptibility is controlled by at least two major genes, or gene complex, e.g., one for the acute disease and the other, H-2 linked, for the chronic disease (Levy-Leblond et al., 1979). However, we cannot exclude the strain-specific differences in dieldrin toxicity and pharmacokinetics of dieldrin between C57Bl/6 and A/J strains. Such differences have been shown for other mouse strains and other xenobiotics, like TCDD, which induce toxicity through an Ah locus-dependent mechanism in mice (Poland and Glover, 1980; Nagarkatti et al., 1984).

In conclusion exposure of C57Bl/6 mice to dieldrin induces increased in vivo macrophage susceptibility to MHV3 infection and results in increased in vitro MHV3-induced cytopathic effects in these cells. This phenomenon possibly results in augmented virus spread in peritoneal macrophages, increasing therefore the susceptibility of C57Bl/6 animals to acute MHV3 disease.

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