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SUPPRESSION OF MHV3 VIRUS-ACTIVATED MACROPHAGES BY DIELDRIN

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Abstract—Dieldrin (36 mg/kg body weight) administered intraperitoneally prolonged recovery from infection with mouse hepatitis virus 3 (MHV3) in the genetically-resistant A/J strain, affected the humoral anti-MHV3 IgG immune response, and inhibited the intrinsic antiviral activity of peritoneal macrophages upon *in vitro* rechallenge with the virus. Infection of untreated A/J animals and vehicle controls with MHV3 resulted in marked and reproducible activation of peritoneal macrophages, observed *in vitro* as resistance to MHV3-cytopathic effects 48 hr after rechallenge with the virus, whereas exposure to dieldrin resulted in apparent loss of the intrinsic capacity of cells to restrict replication of MHV3 and to protect them from cytolysis. In addition, *in vitro* treatment of MHV3 virus-activated macrophages with dieldrin, mitomycin C and X-irradiation, inhibited the intrinsic capacity of cells to restrict MHV3 replication. This mechanism of cellular restriction of the virus by MHV3-activated macrophages from the resistant A/J strain appeared to be one of the sensitive targets for the suppressive action of dieldrin on host resistance, as (i) no major changes in macrophage cellular parameters were observed in *in vitro* studies of cell viability, adherence to plastic, and superoxide anion generation; (ii) the increased cell yield in the peritoneal exudates during MHV3 virus infection was not affected by dieldrin exposure; and (iii) the attachment and uptake of [³H]MHV3 by virus-activated macrophages was shown to be unchanged by dieldrin exposure.

Restriction of virus replication by macrophages is the primary defense mechanism in several virus infections, including that with MHV3 [1-5]. It is generally accepted that the most important factors determining whether the infected host recovers or not are the virulence of the virus and the ability of the defense mechanisms of the host to control the viral infection. It is also known that immune deficiencies, including xenobiotic-induced suppression of the host defense mechanism, can result in severe viral disease, in many cases leading to death [6-8].

In preliminary studies, we found an increased susceptibility to acute disease induced by MHV3 infection in the genetically MHV3-susceptible C57B1/6 strain and semisusceptible (C57B1/6 × A/J)F₁ hybrids challenged with sublethal doses of the organochlorine pesticide, dieldrin [9]. *In vitro* experiments showed decreased phagocytosis and decreased resistance to MHV3-cytolysis by peritoneal macrophages after exposure to dieldrin [9]. This pesticide, which is a potent health hazard for humans [10], has been shown to be a potent toxic agent affecting the population of peritoneal macrophages [11, 12]. In this study, we describe the interaction of dieldrin with mouse peritoneal macrophages, which are the primary target cells in MHV3 infection and which can be antivirally activated in the genetically MHV3-resistant A/J mouse strain. Further evidence of the suppressive effect of dieldrin on macrophage functions is provided here.

MATERIALS AND METHODS

Inbred female A/J mice, 8- to 10-weeks-old, were obtained from the Jackson Laboratory, Bar Harbor, ME. The animals received one intraperitoneal dose of 36 mg/kg body weight of dieldrin (99.9% purity, Supelco Inc., Bellefonte, PA) dissolved in 0.1 ml corn oil, which was 0.6 LD₅₀ i.p. [9]. MHV3 virus was injected i.p. 4 days later into dieldrin-exposed animals, untreated controls, and animals receiving 0.1 ml corn oil.

Peritoneal exudate macrophages were obtained and cultured as described elsewhere [13]. Briefly, mice were killed 7 days after MHV3 infection, and peritoneal exudates were obtained by washing the peritoneal cavity of mice with a total volume of 8 ml of heparinized Eagle's minimum essential medium (MEM). Nonadherent cells were removed after 2 hr of incubation at 37° in Petri dishes by three washings with MEM. Adherent cells were shown to meet the morphological, functional, and biochemical criteria of macrophages, as identified earlier [13].

Adherence of macrophages to plastic surface, cell viability (determined by the trypan blue exclusion test), and phagocytosis of ⁵¹Cr-labelled sheep erythrocytes by macrophage cultures have been described before [9, 13]. Generation of superoxide anion by macrophages during phagocytosis of opsonized zymosan was determined by the reduction of ferricytochrome *c* (80 μM) (Sigma Chemical Co., St. Louis, MO) in Krebs-Ringer phosphate (pH 7.35) containing 2 mg/ml dextrose (Sigma), according to the technique of Johnston [14]. Specific reduction of

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cytochrome *c* by superoxide anion was calculated by subtracting the nonspecific reduction in the presence of 30 $\mu\text{g/ml}$ superoxide dismutase (SOD) (Calbiochem, San Diego, CA). The SOD-inhibitable reduction of cytochrome *c* at 550 nm was converted to nanomoles of cytochrome *c* reduced using the extinction coefficient $E_{550} 21.0 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ [15].

In vitro infection of cells with MHV3 produces virus-induced cytopathic effects (CPE) and virus-induced foci formation in macrophage monolayers and are described elsewhere [4, 9]. Briefly, virus-induced damage of macrophages was determined microscopically 24–48 hr postinfection as the percentage of the monolayer cell lysis (CPE) [9] or as the number of multinuclear fused giant cells per culture (foci formation) [4] 48 hr after collection and culture of peritoneal macrophages from *in vivo* virus-infected animals.

Determination of the attachment and uptake of MHV3 into cells was performed with ^3H -radio-labelled virus, as described previously [13]. Cell monolayers were infected with [^3H]MHV3 for 60 min at 0° , washed several times in ice-cold MEM, and dissolved with 2% sodium dodecyl sulfate (SDS) (Sigma); the remaining radioactivity was counted and calculated as the surface-bound virus [16]. Uptake of [^3H]MHV3 was determined by infection of macrophage monolayers with the virus at 37° for 20 min, followed by several washings with ice-cold MEM and incubation at 0° for 90 min with 5 mg/ml of proteinase K (Sigma), to distinguish surface-bound from internalized virus [16]. Negative controls were performed by pretreatment of [^3H]MHV3 for 3 hr at 4° with anti-MHV3 serum, prior to incubation with cells [13].

Incorporation of [5,6- ^3H]uridine (Amersham, Arlington Heights, IL; sp. act. 43 Ci/mmol) by virus-activated macrophages, 7 days after *in vivo* infection with 10^3 LD_{50} MHV3, was determined in 24-hr microcultures of cell monolayers by autoradiography. This was performed by exposure to Kodak NTB-3 nuclear track emulsion, development with Kodak D-19 developer, coloration with Giemsa, and subsequent microscopic counting of cell-associated radioactivity grains, as described by Brahic and Haase [17].

MHV3 titer in macrophage cultures was determined by transferring of several dilutions of 48-hr culture supernatant fractions onto L cell cultures and observing the CPE; the MHV3 titer was then calculated in plaque-forming units (PFU) [18]. L cells were grown as monolayers in MEM medium supplemented with 10% fetal calf serum and antibiotics, as described elsewhere [9]. For *in vitro* studies of dieldrin effects on proliferating cell cultures, L cell monolayers were incubated with ethanol solutions of dieldrin (0–10 $\mu\text{g/ml}$ dieldrin in ethanol, the proportion of ethanol never exceeding 2%), and pulse-labelled with [^3H]thymidine (Amersham; sp. act. 18.2 Ci/mmol). Incorporation of [^3H]thymidine into cellular DNA was determined by liquid scintillation, as described before [19].

The humoral immune response to MHV3 infection was determined in mouse sera and supernatant fractions of 24-hr spleen cell cultures, by enzyme-linked immunosorbent assay (ELISA) [20] as described

before [9]. Briefly, spleen cells were incubated at 37° in RPMI-1640 medium (Grand Island Biological Co., Grand Island, NY) supplemented with 10% fetal calf serum and antibiotics. Appropriate dilutions of culture supernatant fractions and sera were incubated with MHV3 antigen prepared as homogenate of MHV3-infected L cells [19] and incubated in flat-bottom Linbro microplates (Flow Laboratories, McLean, VA), followed by saturation with 1.5% bovine serum albumin (Sigma) in 20 mM Tris-HCl buffer and 200 mM NaCl, final pH 8.0. Appropriate controls were performed with non-infected L-cell homogenates. As a second antibody, peroxidase-conjugated anti-mouse IgG antibody (Sigma) was used at a dilution of 1:3000. The substrate for peroxidase development was 2,3-azino-di(3-ethylbenzothiazoline) sulfonate and hydrogen peroxide (Kirkegaard & Perry Laboratories, Gaithersburg, MD), and the colored product was determined spectrophotometrically at O.D._{405nm}.

MHV3 antigen in peritoneal exudates originating from virus-infected and dieldrin-exposed and control animals was quantified by the dot-blot immunoperoxidase dilution test [21] as described previously [9]. Briefly, appropriate dilutions of cellular homogenates were applied as spots onto nitrocellulose papers, saturated with 1.5% bovine serum albumin in 20 mM Tris-HCl buffer and 200 mM NaCl, final pH 8.0, and incubated with anti-MHV3 mouse serum [13], followed by reaction with peroxidase-conjugated anti-mouse IgG antibody (Sigma) and coloration with hydrogen peroxide and 4-chloro-1-naphthol (Sigma). A positive reaction was detected as a colored dot against the white nitrocellulose background. The results are expressed as minimal cell number giving positive immunoperoxidase reaction.

RESULTS

Infection of the genetically-resistant A/J strain with MHV3 virus resulted in rapid recovery of animals from the infection and in marked and reproducible activation of peritoneal macrophages, as observed as the restriction of MHV3 replication by the cells rechallenged by the virus *in vitro*. In the first experiment, we followed the recovery from MHV3 infection in control or vehicle-treated A/J mice, or in animals exposed 4 days prior to infection to a single sublethal i.p. dose of the organochlorine pesticide dieldrin (36 mg/kg body weight). On each day following virus infection, virus clearance by the primary target cells for MHV3 infection, i.e. peritoneal exudate macrophages, was examined. This was determined by immunochemical dot-blot quantification of cell-associated MHV3 antigens, virus titer, and virus-induced foci formation in cultures of peritoneal macrophages incubated *in vitro* at 37° , 5% CO_2 , for 48 hr (Fig. 1). In untreated controls and vehicle-exposed animals, recovery from acute MHV3 disease was completed within 4 days, as (i) no virulent MHV3 was detectable at days 5–7 postinfection in cultures of peritoneal macrophages collected from infected animals, (ii) relatively low foci formation was observed in these cell cultures 48 hr after *in vitro* incubation, and (iii) only traces of MHV3-antigen

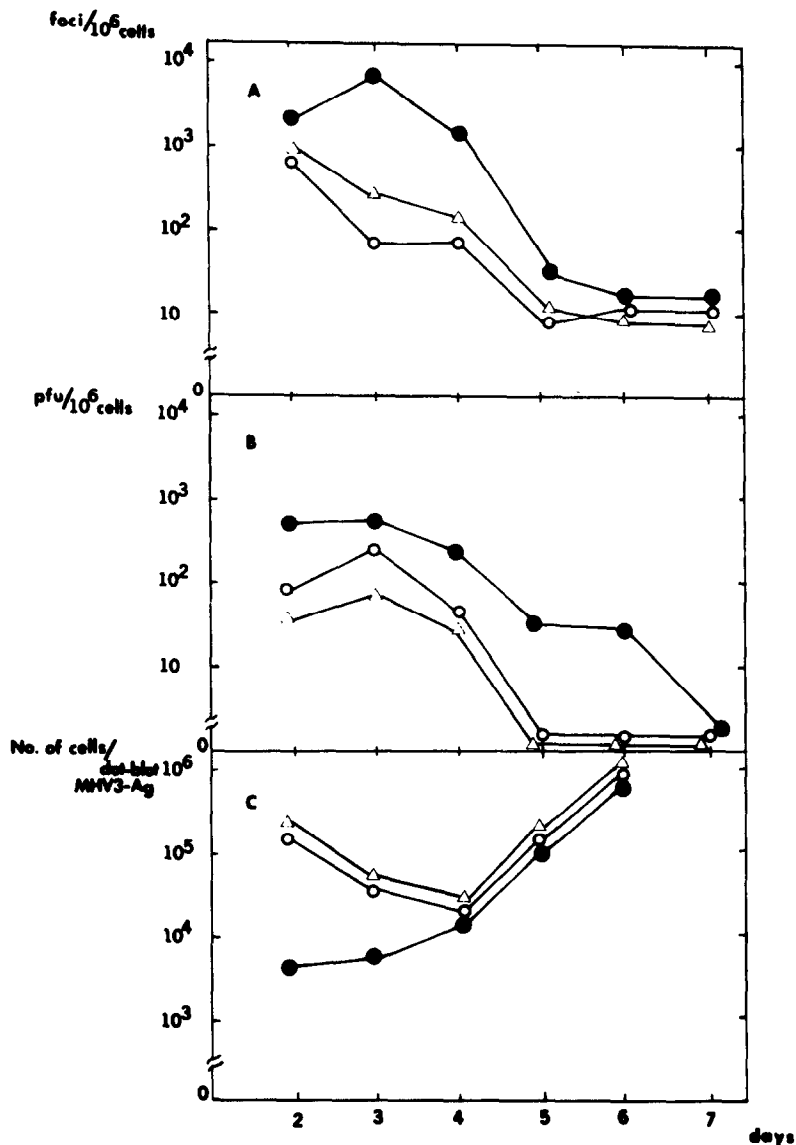


Fig. 1. Macrophage recovery from *in vivo* MHV3 infection of A/J resistant mice. Key: unexposed controls (O), exposed to vehicle only (Δ), or exposed to a single, sublethal dose of dieldrin (\bullet). (A) virus-induced foci formation in cultures of peritoneal macrophages collected at 2-7 days postinfection and incubated *in vitro* for 48 hr; (B) virus titer in supernatant fractions of 48-hr cultures of peritoneal macrophages collected 2-7 days postinfection; (C) MHV3-antigen associated with peritoneal exudates collected from infected animals 2-7 days postinfection, expressed as limiting cell number yielding a positive immunochemical dot-blot reaction. Results are expressed as the mean of triplicates (four individuals per group per day). S.D. did not exceed 25%.

were detectable in 10^6 cells by the immunochemical dot-blot dilution test (Fig. 1). Macrophages from dieldrin-exposed animals displayed high foci formation at days 3 and 4 postinfection, a high amount of MHV3-antigen was already detectable at days 2 and 3 postinfection, and virulent MHV3 was found in supernatant fractions of 48-hr macrophage cultures at 2-6 days postinfection (Fig. 1). It appeared, therefore, that recovery from MHV3-acute disease in dieldrin-exposed animals was suppressed and took longer than in vehicle and unexposed controls. The spread of the virus in dieldrin-exposed animals was

also more rapid, as shown by high amounts of MHV3-antigen in peritoneal exudates at 2 and 3 days postinfection and by markedly elevated virus-induced CPE. There was, however, full recovery from the disease at day 7 in all three groups of animals as no virus was detectable by either immunochemical dot-blot MHV3 antigen coloration, or virus titration. Foci formation in macrophage cultures was extremely low and comparable with the formation of spontaneous foci. The viability of cells was over 95% in all three groups of macrophage cultures, at day 7 postinfection, as determined by

Table 1. Cellular and functional variables of virus-activated peritoneal macrophages of A/J mice exposed to the vehicle and dieldrin and subsequently infected with MHV3

	No. of peritoneal exudate cells/animals*	% of viable cells in 24-hr cultures†	% of inhibition of cell adherence by dansylcadaverine‡	Phagocytosis of ⁵¹ Cr-SRBC§	Macrophage generation of superoxide anion (O ₂ ⁻)
Untreated controls	5.1 ± 1.8 × 10 ⁶	96.5 ± 5.0	75.0 ± 12.0	5782.6 ± 680	191.6 ± 23
Vehicle controls	6.1 ± 1.2 × 10 ⁶	98.0 ± 5.5	78.0 ± 9.0	6200.5 ± 668	186.6 ± 18
Dieldrin, 36 mg/kg body wt	6.3 ± 1.0 × 10 ⁶	95.5 ± 6.0	90.0 ± 5.0	4869.9 ± 765	214.0 ± 18

Animals were infected with 10³ LD₅₀ MHV3 at 4 days after exposure to dieldrin or to the vehicle only, and the cells were collected at 7 days after infection with the virus. Values are means ± S.D. All differences had P values of >0.5, indicating non-significance.

* Mean of five individuals.

† Trypan blue exclusion test, mean of triplicates.

‡ Cells were preincubated with 250 μM dansylcadaverine, 30 min, 37°; mean of triplicates.

§ Expressed as cpm/10⁶ cells; incubation for 1 hr, 37°, with ⁵¹Cr-SRBC (ratio 1:20); mean of quadruplicates.

|| Expressed as nmoles O₂⁻/mg protein, capable of reducing ferricytochrome *c* (reduction eliminated by 33 μg/ml superoxide dismutase); mean of quadruplicates.

the trypan blue exclusion test (Table 1). Phagocytosis of ⁵¹Cr-labelled sheep erythrocytes by macrophages collected at day 7 after infection with MHV3 showed no significant differences between dieldrin group and control groups (Table 1). Formation of superoxide anion was elevated in all three MHV3-infected groups (Table 1), as compared with generation of superoxide anion by uninfected controls (87.0 ± 16.2 nmoles O₂⁻/mg protein, *P* < 0.01). This was determined by incubation of macrophages with opsonized zymosan, with or without superoxide dismutase, and the cytochrome *c* reduction assay. In other experiments, we determined the generation of superoxide anion by peritoneal macrophages from A/J resistant mice exposed to different sublethal dieldrin doses, 0–36 mg/kg body wt and subsequently infected with 10³ LD₅₀ MHV3, 4 days after exposure. Similar to previous results, macrophages collected 7 days after MHV3 infection, generated from 182.6 ± 12.0 nmoles O₂⁻/mg protein to 219.2 ± 20.1 nmoles O₂⁻/mg protein after exposure to single dieldrin doses (4.5, 9, 18 and 36 mg/kg body wt), which was not statistically significant, as compared to vehicle and untreated controls (*P* > 0.5).

As shown in Table 1, the peritoneal exudate cell yield was unaffected by sublethal dieldrin exposure. In addition, the population of peritoneal exudates in virus-infected animals was elevated, as compared to uninfected controls, despite dieldrin or vehicle exposure, showing therefore that exposure to the pesticide did not affect the cell yield or the virus-induced augmentation of peritoneal exudate number. Furthermore, autoradiography of [³H]uridine-labelled microcultures of peritoneal macrophages showed similar, low numbers of metabolically-active, radioactivity grain-positive macrophages in cultures from dieldrin-exposed, vehicle-exposed, and control animals at 7 days after *in vivo* infection with MHV3 (not shown). In addition, all

these three groups of cells displayed similar capacities to adhere to plastic surface at 37° during a 2-hr incubation period. Inhibition of the cell adherence by dansylcadaverine in dieldrin-exposed group and the control groups was shown to be similar (Table 1).

Taken together, these results showed no major changes in macrophage effect or activities upon exposure to single, sublethal doses of dieldrin. However, virus-macrophage interactions were altered markedly by the exposure to pesticide. This was further examined *in vitro* by analysis of antiviral activation of cells upon reinfection with MHV3. Anti-MHV3 activation of peritoneal macrophages was observed at 7 days after *in vivo* infection of A/J mice with MHV3 (Fig. 2). Slight virus-induced CPE and low virus titer in cultures of these MHV3-activated macrophages were observed only at a multiplicity of infection (MOI) over 0.5 at 48 hr post-infection (Fig. 2), whereas cultures of resident macrophages from non-immunized A/J controls, infected *in vitro* with 0.1 MOI of MHV3, showed at the same time over 80% of CPE and high virus titer (10⁵ PFU/ml). It should be noted that oil-elicited peritoneal macrophages from naive animals did not show any restriction capacity upon *in vitro* infection with MHV3, similar to the resident macrophages. In addition, no interaction of the vehicle was observed with the MHV3-restriction capacities of virus-activated macrophages from MHV3-immunized A/J mice.

Administration of a single, sublethal dose of dieldrin (36 mg/kg body wt) almost totally inhibited the capacity of virus-activated macrophages to restrict MHV3 replication after *in vitro* reinfection (Fig. 2). Increases in virus titer and CPE in MHV3-infected macrophage cultures originating from dieldrin-exposed animals were observed at infectious doses as low as 0.5 × 10⁻³ MOI, at 24 hr postinfection, as compared to oil-treated controls (Fig. 2A). Almost

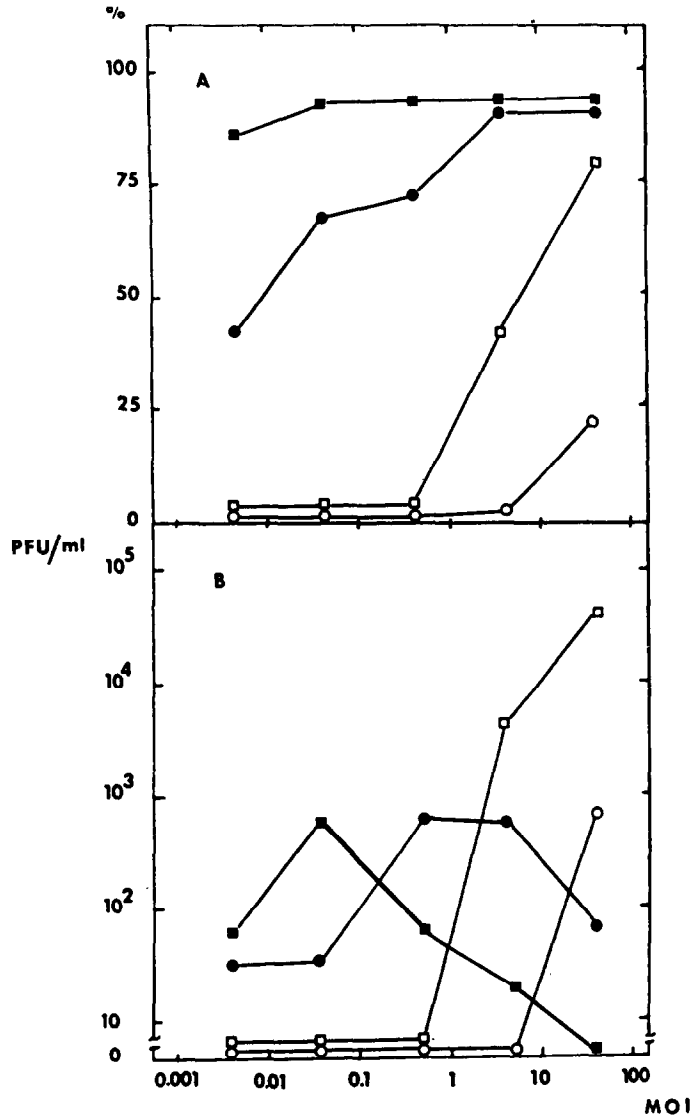


Fig. 2. Virus-induced cytopathic effects (A) and virus titer (B) in cultures of peritoneal macrophages from MHV3-immunized A/J mice exposed 4 days prior to immunization to dieldrin or to the vehicle. Cultures were reinfected *in vitro* with different doses of MHV3: vehicle, 24 hr postinfection (○) and 48 hr postinfection (□); dieldrin, 24 hr postinfection (●) and 48 hr postinfection (■).

no cells were left in macrophage cultures from dieldrin-exposed animals after *in vitro* reinfection with MHV3 at MOI 0.1 (Fig. 2B). In the vehicle control group, the cells infected at MOI > 0.5 showed time- and virus dose-dependent CPE (Fig. 2A) and increased virus titer in culture supernatant fractions (Fig. 2B).

Macrophages from immunized A/J mice showed dieldrin dose-dependent increased susceptibility to MHV3 infection after *in vitro* treatment of cells with 0–10 $\mu\text{g}/\text{ml}$ dieldrin (Fig. 3). At MOI 0.1, the virus-induced CPE and MHV3 titer were detectable at 24 hr after *in vitro* MHV3 infection (not shown) and were increased markedly at 48 hr postinfection, as compared to the untreated, MHV3-infected cell cultures (Fig. 3). Interestingly, the maximal MOI, at which the cells were still able to restrict the virus

replication, depended on dieldrin concentration (Fig. 3).

To exclude direct virus–dieldrin interactions, several controls were performed, including virus attachment by, and uptake to, dieldrin-exposed cells (Fig. 3), and the effect of dieldrin on MHV3 replication in highly proliferative mouse lymphoma L cells (Fig. 4). No significant decrease of proteinase K-sensitive radioactivity was found in macrophage cultures preincubated for 48 hr with 0–1 $\mu\text{g}/\text{ml}$ dieldrin and subsequently incubated for 30 min at 0° with ^3H -radio-labelled MHV3, clearly indicating that attachment of the virus was not altered by the pesticide (not shown). Similarly, no significant changes in [^3H]MHV3 uptake by macrophages pretreated with 0–1 $\mu\text{g}/\text{ml}$ dieldrin were observed (Fig. 3), indicating that uptake of the virus by activated A/J macro-

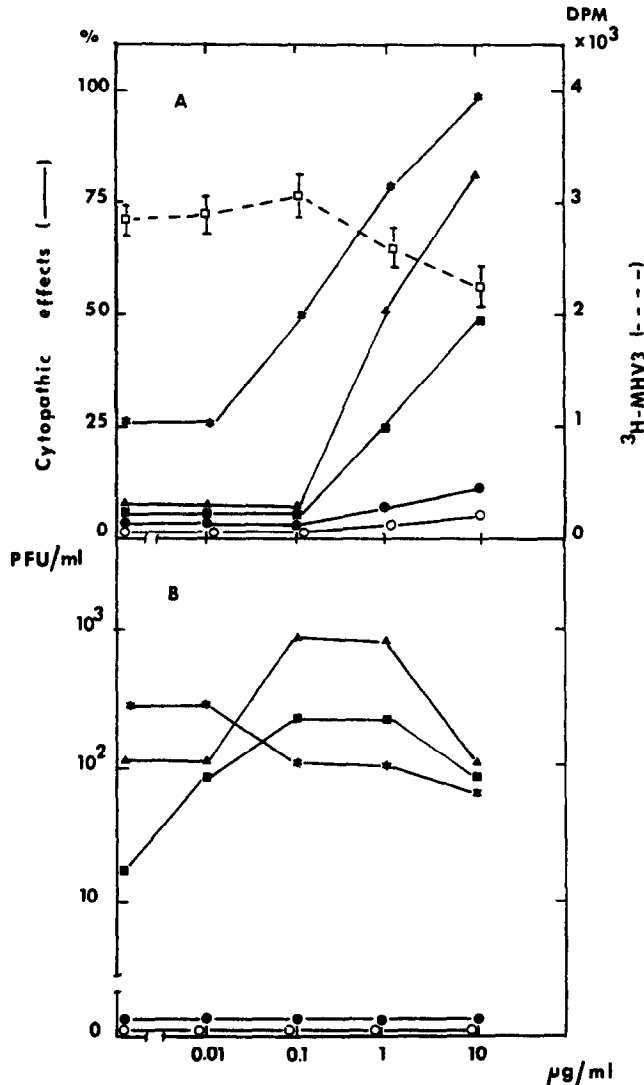


Fig. 3. (A) Uptake of [³H]MHV3 (□) and virus-induced cytopathic effects and (B) virus titer in cultures of MHV3-activated A/J peritoneal macrophages, incubated *in vitro* with 0–10 µg/ml dieldrin for 48 hr and subsequently infected with different MHV3 doses, 24 hr postinfection. Key: (○) 0.0005, (●) 0.005, (■) 0.5, (▲), 5 and (★) 50 MOI.

phages was unchanged by these concentrations of dieldrin. As shown in Fig. 4, preincubation of highly proliferating L cell cultures with 0 to 0.1 µg/ml dieldrin did not alter MHV3 replication in these cells, as determined by virus titer in culture supernatant fractions. At concentrations > 1 µg/ml of dieldrin, about 50% of [³H]thymidine incorporation into cellular DNA was inhibited and MHV3 titer was increased, indicating direct effect of dieldrin on cellular metabolism and virus production at this level of the pesticide. It should be noted that changes in cellular viability were observed neither in cultures preincubated *in vitro* with 0–10 µg/ml dieldrin, nor in cultures of activated macrophages originating from dieldrin-exposed mice.

Generally, dieldrin inhibited the capacity of peritoneal macrophages activated *in vivo* with MHV3

to restrict virus production after reinfection. This action of dieldrin was shown to be possibly related to the inhibition of the intrinsic resistance mechanism(s) of MHV3-activated macrophages, but not to any virus-pesticide interactions. This possibility was further examined in the next experiment, in which dieldrin effects were compared to the non-specific inhibition of MHV3 restriction in virus-activated macrophages by X-irradiation and mitomycin C treatment (Table 2). Pretreatment of virus-activated macrophages with dieldrin, mitomycin C or irradiation of cells resulted in almost total inhibition of virus restriction, as evidenced by high CPE and high virus titer, 48 hr after infection with 0.1 MOI of MHV3 (Table 2).

In the next experiment we wanted to determine whether this dieldrin-induced inhibition of MHV3

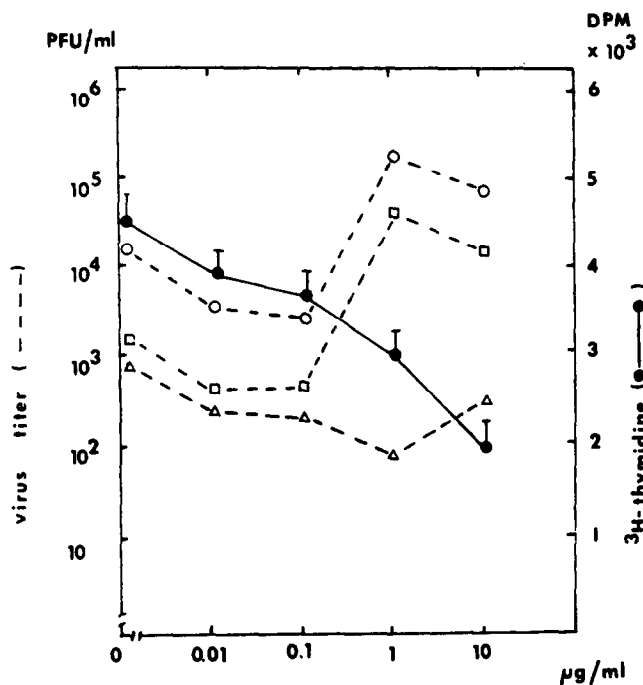


Fig. 4. Uptake of [^3H]thymidine (●) and virus titer (---) in L cell cultures pretreated *in vitro* with 0–10 $\mu\text{g}/\text{ml}$ dieldrin and subsequently infected with 10^2 (Δ), 10^3 (\square), and 10^4 (\circ) PFU/culture of MHV3, 24 hr postinfection.

restriction by activated A/J macrophages lasted for a longer time than the life-time of macrophages. Therefore, the A/J mice were exposed three times, with 2-week intervals, to 30 mg/kg body wt dieldrin or to the vehicle only, and then the animals were left for 3 months after the last treatment. Subsequently, these dieldrin-exposed, vehicle-treated, and untreated animals of appropriate age were immunized by i.p. injection of 10^3 MHV3 and, 7 days later, peritoneal macrophages, blood sera and spleens were collected. Humoral immune response to MHV3 was examined by determination of anti-MHV3 IgG antibodies in sera and culture super-

natant fractions of spleen cells cultured for 24 hr and compared to the humoral response shortly after exposure to dieldrin (Table 3). Furthermore, macrophage restriction of virus replication was examined after *in vitro* infection with MHV3 (Table 3). The results showed inhibition of MHV3 restriction by macrophages and inhibition of anti-MHV3 IgG antibody titer in sera and supernatant fractions of spleen cultures shortly after exposure to dieldrin, but not after a 3-month interval (Table 3). It appears, therefore, that despite heavy intoxication of animals with dieldrin, after an interval longer than the life-time of macrophages, there was no suppression of MHV3

Table 2. Inhibition of macrophage restriction of MHV3 replication by X-irradiation, mitomycin C and dieldrin

<i>In vitro</i> treatment*	Resistance of virus-activated macrophages to reinfection with MHV3†	
	CPE (% \pm S.D.)	Virus titer (PFU/ml)
None	0	0
X-irradiation, 1000 rads	75 \pm 12	5 \times 10 ⁴
Mitomycin C, 0.25 $\mu\text{g}/\text{ml}$, 1 hr	80 \pm 9	2 \times 10 ⁴
Dieldrin, 10 $\mu\text{g}/\text{ml}$, 48 hr	75 \pm 11	5 \times 10 ⁴

* Cell viability in cultures of macrophages, collected from A/J mice immunized 7 days previously with 10^3 LD₅₀ MHV3, was determined by the trypan blue exclusion test at the end of *in vitro* treatment and was shown to be unaffected by the treatment, as compared to the untreated controls.

† Cells were infected *in vitro* with 0.1 MOI of MHV3, and CPE and virus titer were determined 48 hr postinfection.

Table 3. Effect of dieldrin on humoral immune response to MHV3 and on the virus restriction by peritoneal macrophages, 4 days and 3 months after exposure to the pesticide

<i>In vivo</i> immunization with MHV3 after exposure to dieldrin*	Group	Anti-MHV3 IgG antibodies ELISA†		Resistance of peritoneal macrophages to MHV3 infection	
		Serum O.D. ₄₀₅	Culture supernatant O.D. ₄₀₅	CPE (%)	Virus titer (PFU/ml)
4 Days	Untreated controls	1.26 ± 0.06	0.76 ± 0.03	0	0
	Vehicle	1.42 ± 0.06	0.89 ± 0.04	0	0
	Dieldrin	0.32 ± 0.09‡	0.32 ± 0.06‡	85	10 ²
3 Months	Untreated controls	1.02 ± 0.19	0.62 ± 0.15	0	0
	Vehicle	0.99 ± 0.17	0.86 ± 0.15	0	0
	Dieldrin	1.16 ± 0.28	0.74 ± 0.10	0	0

* A/J mice were immunized with 10³ LD₅₀ MHV3, 4 days after a single exposure to 36 mg/kg body wt dieldrin or 3 months after three subsequent doses of 30 mg/kg body wt dieldrin. Peritoneal macrophages, spleen cells and blood samples were collected 7 days after immunization.

† The results of ELISA (serum dilution 1:100, supernatant dilution 1:10) are expressed as the mean from five individuals ± S.D.

‡ P < 0.01 (Student's *t*-test).

restriction by the virus-activated macrophages. Similarly, no inhibition of the humoral immune response to MHV3 was observed after that time, despite the very high dose (3 × 30 mg/kg body wt) of the pesticide.

Thus, exposure to a single, sublethal dose of dieldrin inhibited cellular capacities of virus restriction by MHV3-activated peritoneal macrophages in the genetically resistant A/J strain in acute MHV3-disease. The humoral immune response to MHV3 was also inhibited by dieldrin exposure. These results indicate, therefore, that both the immune response and natural resistance mechanisms, such as antiviral macrophage activation, can be suppressed by the exposure to dieldrin.

DISCUSSION

The organochlorine pesticide, dieldrin, suppressed the antiviral response to MHV3 in mouse strains of genetic susceptibility and semisusceptibility to the virus, leading to acute MHV3 disease and death of animals, and in the resistant A/J strain exposed to extreme doses of the pesticide and high virus infection doses [9]. We present evidence that antiviral activation of peritoneal macrophages of MHV3-challenged, resistant A/J mice can be at least one of the targets for interaction of sublethal doses of dieldrin with the host's defense mechanisms to control viral infection. This intrinsic capacity of MHV3-activated macrophages to restrict the virus appeared to be highly sensitive to sublethal dieldrin exposure since *in vitro* studies revealed no major changes in other cellular variables, such as viability, adherence to plastic, generation of superoxide anion, uptake of ⁵¹Cr-labelled sheep erythrocytes, and incorporation of [³H]uridine. It must be emphasized, however, that dieldrin, depending on the dose and the time of exposure, can be a potent toxic agent affecting

macrophage phagocytosis [9, 11, 12], resistance to MHV3-cytolysis [9], and cell yield and viability [12].

Intrinsic antiviral activity has been used to describe *in vitro* restriction of virus replication in macrophages from a host resistant to viral infection [2]. Stohlman *et al.* [3] found that this expression of intrinsic antiviral activity of macrophages against the mouse coronavirus JHM (MHV4) correlated with the ability of the host to resist virus challenge, and possibly constituted a dominant mechanism for determining *in vivo* resistance to this virus. Generally, intrinsic antiviral activity of macrophages is related to the ability to either phagocytose and degrade virus, thereby rendering it non-infectious, or to adsorb virus at the cell surface and restrict replication within the cellular cytoplasm [22]. Restriction of MHV3 by activated A/J macrophages seems to be related to the latter mechanism since penetration of MHV3 was shown to be a phagocytosis-independent process [13]. In addition, uptake of [³H]MHV3 by virus-activated A/J macrophages was not affected markedly by dieldrin exposure, similar to previous data of [³H]MHV3 uptake by naive, dieldrin-exposed C57B1/6 macrophages [13].

More specifically, macrophage restriction of MHV3 was shown to be related to at least three factors: (i) *in vivo* activation of macrophages with the virus, (ii) genetic resistance factor(s), and (iii) intact cell metabolism [4]. Since peritoneal macrophages from dieldrin-exposed, MHV3-immunized animals, or challenged *in vitro* with the pesticide, showed inhibition of intrinsic MHV3 restriction, at least one of these three factors must be altered by pesticide exposure. The data suggest alteration of macrophage metabolism by dieldrin, which can be comparable to the effects of X-irradiation or mitomycin C treatment. As the incorporation of [³H]thymidine into the cell genome of peritoneal macrophages was extremely low due to the low frequency of dividing

cells, we could only measure the effect of dieldrin on the number of proliferating cells in the macrophage population by the autoradiography technique. However, since the interaction of dieldrin with the genome of proliferating L cells was examined, and resulted in the inhibition of incorporation of [³H]thymidine into cellular DNA, we cannot exclude the possibility of dieldrin-induced inhibition of the synthesis and functioning of the resistance gene(s) product(s) in activated A/J macrophages. The involvement of resistance gene product(s) in the mechanism of intracellular restriction of influenza virus was reported by Haller [23].

The genetic resistance factor is of primary importance in *in vivo* infection with MHV3 [24]. *In vivo* data suggest that dieldrin does not influence the effect of genetic resistance factor(s), as dieldrin-exposed A/J mice survived MHV3 infection, and only extreme virus and pesticide doses change recovery from MHV3 infection to severe disease and death [9]. Similarly, when the cellular or the humoral immune systems are crippled by such non-selective methods as whole body irradiation, treatment with anti-lymphocyte serum, or induction of graft-vs-host reaction, resistance can also be abolished [25]. Arnheiter *et al.* [26], in their hypothesis, emphasized the role of neutralising antibodies as a crucial factor for the increased virus spread in MHV3-infected mice of different genetic susceptibility. Dieldrin is a potent inhibitor of the humoral response, as shown by Wasserman *et al.* [27]. Sublethal dieldrin exposure was shown previously to inhibit anti-MHV3 IgG antibody both in serum and splenocyte culture supernatant fractions of susceptible C57B1/6 animals infected with the YAC-MHV3 virus substrain of reduced *in vivo* pathogenicity [9]. A marked decrease of anti-MHV3 IgG antibody was also observed in resistant A/J strain after exposure to sublethal doses of dieldrin. This might be due to depression of immunoglobulin production by secreting B cells and/or to the formation of circulating virus-antibody complexes under prolonged presence with virus. Our preliminary studies* revealed the presence of these complexes in both control- and dieldrin-exposed A/J animals and, after dissociation with NaSCN, the hidden viral antigens were found by immune reaction. These results are similar to experiments described by Neurath *et al.* [28]. Quantification of these immune complexes, however, showed no significant differences between dieldrin and vehicle groups. In addition, the marked decrease of anti-MHV3 IgG antibody in splenocyte culture supernatant fractions after dieldrin exposure suggests rather direct inhibition of antibody-producing cell number by the pesticide. This assumption is also based upon the observation that the ratio of antibody-forming cells/ELISA in spleen cell supernatant fractions (expressing a direct relationship between the number of antibody-producing cells and the amount of secreted antibodies specific to the priming antigen) was shown to be stable in animals

exposed to selected pesticides, including dieldrin [29].

In conclusion, exposure to dieldrin of the A/J mouse strain resistant to MHV3 infection decreased anti-MHV3 humoral immune response and inhibited intrinsic antiviral activity of peritoneal macrophages. This antiviral activation of macrophages does not seem to be a specific target for dieldrin action; however, it appears to be quite sensitive to the pesticide. Therefore, the dieldrin-induced inhibition of antiviral restriction by MHV3-activated macrophages can be one of the important mechanisms by which the pesticide suppresses the resistance of the host to the virus. This inhibition resulted in increased MHV3 antigen, increased damage of peritoneal macrophages and prolonged recovery from MHV3 infection in the genetically-resistant A/J mouse strain.

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