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IMMUNOLOGIC EFFECTS OF VINYL CHLORIDE IN MICE

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IMMUNOLOGIC EFFECTS OF VINYL CHLORIDE IN MICE

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Exposure to vinyl chloride (VC) has been associated with various disease syndromes in man and animals. In workers exposed to high levels of VC, hepatic fibrosis associated with or leading to portal hypertension and angiosarcoma of the liver has been described.[1] Vinyl chloride disease has also been associated with dermal induration, acro-osteolysis, splenomegaly and thrombocytopenia.[2,3] In animals, VC exposure has been reported to cause several types of tumors.[4]

In a recent report, Ward and coworkers[5] suggested the presence of aberrant immune complexes in workers exposed to vinyl chloride. The incidence and the level of these complexes were correlated with the level of vinyl chloride exposure as well as other manifestations of toxicity. An increase in immunoglobulins and a corresponding reduction in peripheral T-lymphocytes was suggested. In addition, the presence of circulating immune complexes (inferred by the presence of mixed cryoglobulins and decreased values of complement) and autoantibodies against various tissue components were demonstrated in exposed workers. On the basis of their findings, Ward *et al.*[5] suggested that the vinyl chloride disease may be an immune complex disorder and the immune response may be initiated by the adsorption of vinyl chloride or its metabolite on the tissue or plasma proteins.

In another related report, Page *et al.*[6] demonstrated elevated levels of carcinoembryonic antigen (CEA) in the plasma of workers exposed to vinyl chloride. Lange *et al.*[3] have reported both increases and decreases of different fractions of immunoglobulins in 10 patients suffering from VC disorders and on the basis of their findings excluded the possibility of an autoimmune phenomenon in this syndrome.

On the basis of these contradictory reports, we investigated the effects of vinyl chloride exposure on the immune system of mice. For the evaluation of immune reactivity in individuals and animals, the response of lymphocytes in culture, particularly blast formation in the presence of antigenic substances, has been suggested as a useful index.[7] Sensitized lymphocytes have been shown to play a very significant role in the production of autoimmune diseases.[8] The findings that T- and B-lymphocytes can be selectively transformed *in vitro* by different lectins[9-11] have provided a working model to evaluate the responsiveness of these lymphocytes in cell cultures. Our results, described in this report, suggest that VC exposure caused stimulation of lymphocyte transformation in cultures. Direct exposure of splenic cultures to VC *in vitro* did not have such an effect. Metabolite(s) of VC rather than the parent chemical itself appear to be involved in the process of this stimulation.

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METHODS

Vinyl chloride monomer (obtained from Matheson Gas Products, Joliet, Ill.) of 99.9% minimum purity was used for exposure of mice. The exposures were conducted in large (6′ × 6′ × 8′) inhalation chambers. The concentration of VC was controlled to provide atmospheric concentrations of 0 (control), 10, 100, and 1000 ppm, 6 hr/day, 5 days/week, for durations of up to 8 weeks. The time-weighted averages of vinyl chloride concentrations in the chamber, as determined by a gas chromatograph are described in TABLE 1.

Male, CD-1 mice (approximately 7 weeks old and weighing 35 g each at the beginning of exposure) were obtained from Charles River (Wilmington, Mass.) and acclimated for 2 weeks with the surroundings. The animals were housed 4 to a cage, identified by ear punch, and were allowed free access to food and water except during the exposure period. No food or water was allowed during the exposure. The animals were weighed once a week. Groups of mice (4 animals/group/VC level) were sacrificed at 2, 4, and 8 weeks of exposure and blood samples obtained. Liver, kidney,

TABLE 1

EXPOSURE CHAMBER ANALYSES FOR VINYL CHLORIDE*

	VC Concentration, ppm		
Desired Concentration	10	100	1000
Concentration determined (means ± S.D.)	9.9 ± 0.64	101.3 ± 4.8	982.8 ± 34.9
Percent of exposure days within 10% of mean analytic concentration	87	98	100
Number of analyses (days × analytic determinations per day)	45 × 4	45 × 4	45 × 4

*VC concentrations were determined on a Varian 2400 gas chromatograph equipped with a flame ionization detector. The column used was 3′ × ⅛″ stainless steel, Porpak QS, 80/100 mesh, maintained at a temperature of 120°C (injector and detector at 260°C and 225°C, respectively). Carrier gas was He, 30 ml/min, and samples of 1 ml analyzed in several replicates and compared with an appropriate standard curve prepared simultaneously.

spleen, and thymus were weighed and these organs, except the spleen, were fixed and processed for histologic examination. The spleen was saved in sterilized saline for lymphocyte cultures.

Hematology and Serum Immunoelectrophoresis

Total erythrocyte and leukocyte counts and differential leukocyte counts were performed on all blood samples by routine methods. The relative concentrations of total Ig were determined by immunoelectrophoretic techniques modified from Bjerrum *et al.*[12]

Splenic Lymphocyte Cultures

The cultures were performed as described elsewhere in detail.[13] The spleens were washed with sterilized saline and mashed in saline with a forceps. Subsequently, the

agglutinated cells and large particles were removed by passing the cells through a series of hypodermic needles and the resulting cell suspension was transferred to a glass tube. After separating the cells by centrifugation, the cells were suspended in culture medium (RPMI-1640 supplemented with glutamine, penicillin, and streptomycin), an aliquot counted electronically, and the concentration of cells adjusted to approximately 8×10^6 cells/ml. Aliquots of this suspension were transferred to microculture plates, more medium with or without mitogenic lectins was added, and the system incubated at 37°C in humidified air containing 5% carbon dioxide. Optimal concentrations of phytohemagglutinin (PHA) and pokeweed mitogen (PWM) were used. After 50–52 hours of incubation, 0.5 μCi of ^{3}H-thymidine (New England Nuclear, Boston, Mass.) was added to each culture and incubation continued for an additional 16 hours. The cells were then harvested and the radioactivity counted using a liquid scintillation spectrometer. Cells from each animal were cultured in triplicate and the three values were averaged after corrections for quenching. Stimulation indices for PHA and PWM were determined for each spleen by dividing the disintegrations in the presence of the mitogen by those in the system containing no mitogen.

When splenic lymphocyte cultures were incubated in the presence of VC, these were held in specially designed humidified glass containers which were well purged with either 5% CO_2 in air or with 1000 ppm VC in 5% CO_2 in air mixture. These containers were kept in a constant temperature incubator at 37°C for the incubation as described above.

Alteration of VC Metabolism

Groups of mice were injected i.p. with either saline, phenobarbital sodium (100 mg/kg), or ethanol (3.2 g/kg as 30% solution) 30 minutes prior to VC exposure. The concentration of phenobarbital and ethanol were adjusted so that all animals (including those receiving saline) were given 10 ml solution/kg (0.3 ml/30 g mouse). These were divided into equal subgroups and were exposed either to normal air atmosphere or to 1000 ppm vinyl chloride in 1 meter^3 inhalation chambers for 5 days. Two days after the last exposure, their spleens were obtained and cultured as mentioned above.

Statistical

The results are expressed as means and their standard errors. Comparisons were made with the respective control group using a t-test[14] and a predetermined value of $p = 0.05$.

Results

In mice, vinyl chloride exposure for 8 weeks produced no consistent effects on growth, or weights of liver, kidney or thymus (Table 2). Spleen weights were increased at the highest level of exposure, 1000 ppm. This effect was noticed after 2 weeks of exposure and persisted throughout the exposure. A slight reduction in liver/body weight ratios after 8 weeks of exposure to 1000 ppm VC may be attributable to greater body weights in mice as compared to controls. The body weights of mice exposed to 1000 ppm VC were consistently higher than controls after

TABLE 2
ORGAN AND BODY WEIGHTS OF MICE EXPOSED TO VINYL CHLORIDE FOR DIFFERENT DURATIONS

Duration of Exposure	Level of Exposure (ppm)	Body Weight (g)	Organ Weights and Their Relation to Body Weight‡							
			Thymus		Spleen		Liver		Kidney	
			(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)	(g)	(g/100g)
Preexposure		035 ± 20.05 ± 0.010.14 ± 0.010.10 ± 0.01			0.27 ± 0.01	1.91 ± 0.07	5.42 ± 0.28	0.54 ± 0.051.53 ± 0.20		
2 weeks	0	36 ± 3	0.04 ± 0.02	0.12 ± 0.03	0.09 ± 0.01	0.24 ± 0.00	1.98 ± 0.30	5.46 ± 0.46	0.59 ± 0.09	1.64 ± 0.18
	10	35 ± 5	0.05 ± 0.01	0.14 ± 0.03	0.08 ± 0.02	0.24 ± 0.03	1.93 ± 0.41	5.48 ± 0.47	0.58 ± 0.09	1.67 ± 0.20
	100	36 ± 2	0.04 ± 0.01	0.12 ± 0.02	0.09 ± 0.02	0.23 ± 0.04	1.99 ± 0.23	5.49 ± 0.35	0.57 ± 0.06	1.58 ± 0.16
	1000	35 ± 4	0.04 ± 0.01	0.10 ± 0.02	0.13 ± 0.01*	0.38 ± 0.07*	2.09 ± 0.29	5.92 ± 0.29	0.64 ± 0.08	1.83 ± 0.05
4 weeks	0	34 ± 2	0.05 ± 0.01	0.15 ± 0.04	0.07 ± 0.02	0.21 ± 0.05	1.75 ± 0.15	5.15 ± 0.23	0.55 ± 0.07	1.63 ± 0.21
	10	38 ± 4	0.05 ± 0.02	0.14 ± 0.03	0.10 ± 0.03	0.25 ± 0.05	2.29 ± 0.22*	5.98 ± 0.45*	0.66 ± 0.08	1.74 ± 0.29
	100	35 ± 1	0.05 ± 0.01	0.13 ± 0.04	0.08 ± 0.01	0.22 ± 0.02	2.06 ± 0.19	5.92 ± 0.45*	0.58 ± 0.06	1.66 ± 0.15
	1000	37 ± 2	0.05 ± 0.01	0.14 ± 0.03	0.11 ± 0.01*	0.30 ± 0.04*	2.11 ± 0.11*	5.69 ± 0.29	0.64 ± 0.05	1.73 ± 0.12
8 weeks	0	38 ± 4	0.04 ± 0.01	0.11 ± 0.03	0.09 ± 0.03	0.23 ± 0.06	2.09 ± 0.33	5.57 ± 0.37	0.67 ± 0.11	1.78 ± 0.18
	10	42 ± 2	0.03 ± 0.01	0.07 ± 0.02	0.10 ± 0.01	0.23 ± 0.03	2.46 ± 0.21	5.90 ± 0.42	0.76 ± 0.04	1.83 ± 0.10
	100	42 ± 3	0.05 ± 0.01	0.13 ± 0.04	0.10 ± 0.01	0.23 ± 0.05	2.30 ± 0.08	5.51 ± 0.30	0.70 ± 0.06	1.67 ± 0.12
	1000	42 ± 1	0.05 ± 0.02	0.11 ± 0.05	0.11 ± 0.01	0.26 ± 0.03	2.02 ± 0.15	4.82 ± 0.26*	0.64 ± 0.06	1.53 ± 0.14

‡Mean ± S.D. of 4 animals per group.
*Indicates significant deviation from control ($p < .05$, Dunnett's test).

4 weeks of exposure when the body weights of all animals in that group were considered (data not shown in the table). No differences were seen in the hematologic parameters of any group exposed to VC (data not shown). At the time of sacrifice, there were no gross lesions observed in any of the organs that can be related to the treatment. Subsequent histologic examination of selected tissues revealed no differences.

Vinyl chloride exposure caused stimulation of spontaneous lymphocyte transformation in mouse splenic cultures. This effect was observed at the highest exposure level of 1000 ppm of VC after 2 weeks of exposure. After 4 and 8 weeks of exposure, an increased transformation of lymphocytes prepared from the exposed animals was observed at all levels of VC (FIGURES 1–3). The stimulation indices for PHA were

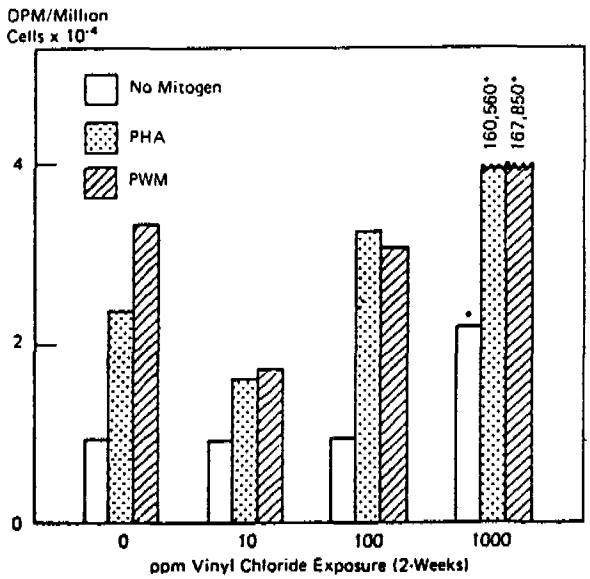

FIGURE 1. Thymidine uptake by lymphocyte cultures prepared from mice exposed for 2 weeks to vinyl chloride. Average of 4 animals, the cells cultured in triplicate. Values significantly different from controls are marked with an asterisk ($p < 0.05$).

significantly increased in mice exposed to 1000 ppm VC for 2 weeks (FIGURE 4). At subsequent time intervals, however, there was no definite dose-response, since in the mice exposed to 1000 ppm VC the indices were lower than for those exposed to the two lower levels. The stimulation indices at these points were, however, consistently greater than those observed for controls (FIGURES 5 and 6).

For all groups of mice, the DNA synthesis by lymphocytes in culture decreased with time. Such findings were consistent with those generally observed in growing animals. This was consistent in all exposure groups including control, the latter showing a considerable drop in the stimulating indices by PHA and PWM.

TABLE 3 shows the relative amounts of immunoglobulins in mouse serum at different intervals of VC exposure. After 2 and 4 weeks of exposure, an increase was

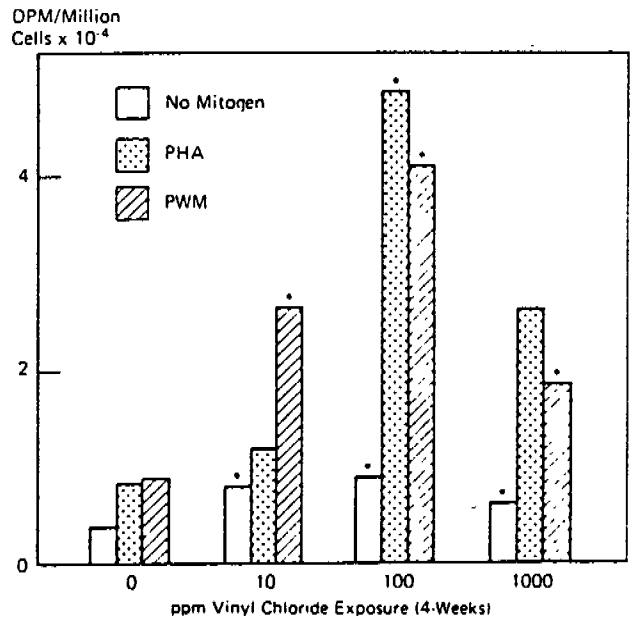

FIGURE 2. Thymidine uptake by lymphocyte cultures prepared from mice exposed for 4 weeks to vinyl chloride. Average of 4 animals, the cells cultured in triplicate. *Indicates value significantly different from respective control ($p < 0.05$).

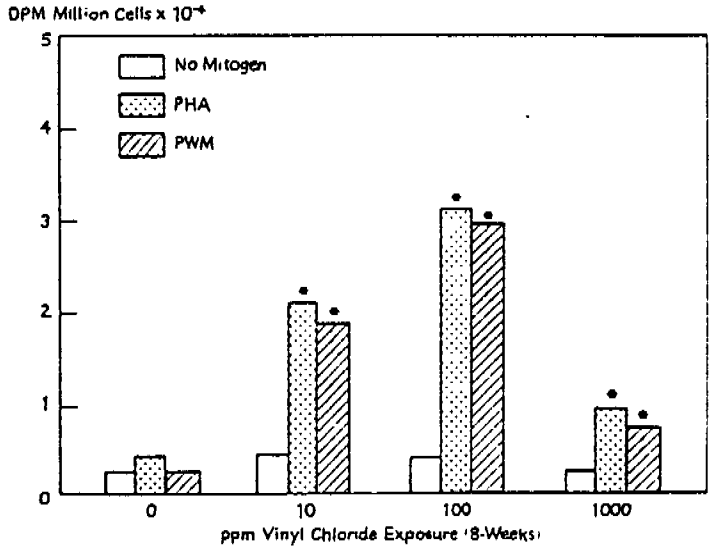

FIGURE 3. Thymidine uptake by lymphocyte cultures prepared from mice exposed for 8 weeks to vinyl chloride. Average of 4 animals, the cultures performed in triplicate. *Indicates value significantly different from control at $p < 0.05$.

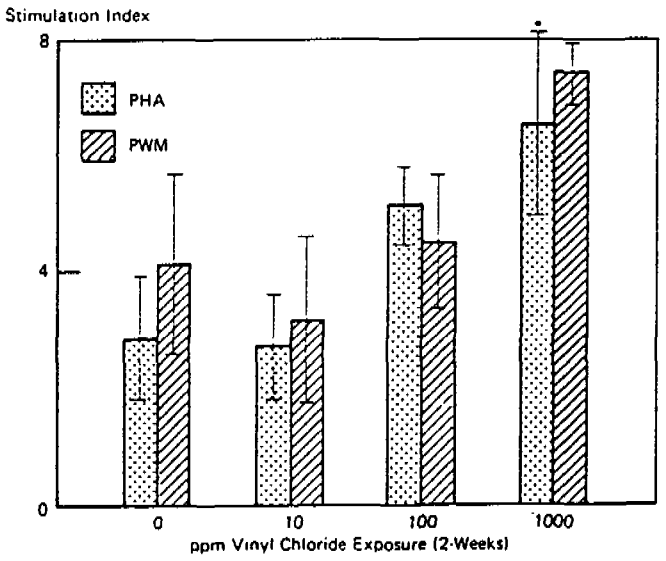

FIGURE 4. Response of mouse splenic lymphocytes to PHA and PWM after 2-week exposure to vinyl chloride. All values are average of 4 animals, the cells cultured in triplicate. Vertical bars represent standard error of mean and * indicates value significantly different from control ($p < 0.05$). Stimulation index = dpm in the presence of a mitogen divided by the dpm without any mitogen.

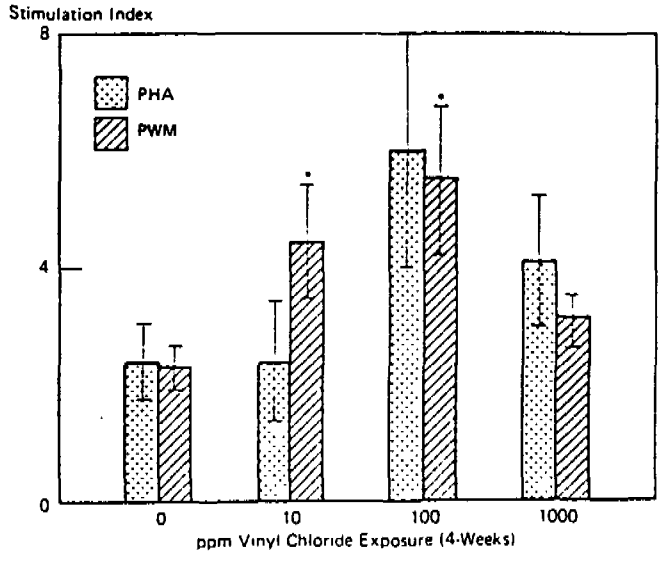

FIGURE 5. Response of mouse splenic lymphocytes to PHA and PWM after 4-week exposure to vinyl chloride. All values are average of 4 animals, the cells cultured in triplicate. Vertical bars represent standard error of mean and * indicates value significantly different from control at $p < 0.05$. Stimulation index = dpm in the presence of a mitogen divided by the dpm without any mitogen.

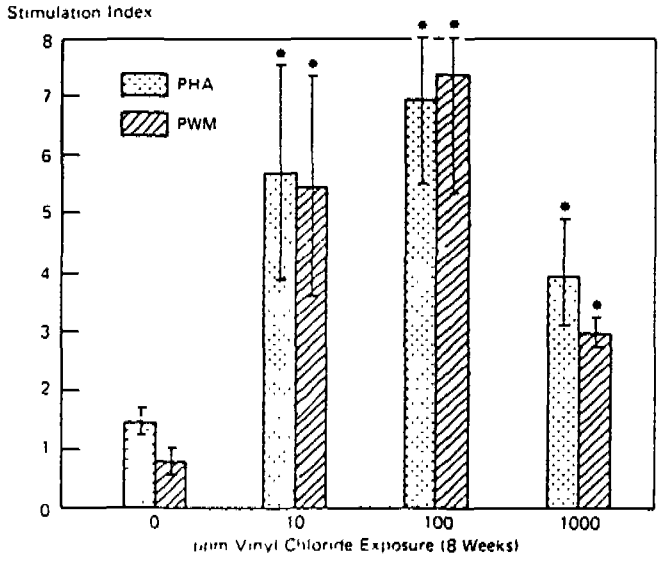

FIGURE 6. Response of mouse splenic lymphocytes to PHA and PWM after 8-week exposure to vinyl chloride. All values are average of 4 animals, the cells cultured in triplicate. Vertical bars represent standard error of mean and * indicates value significantly different from control at $p < 0.05$. Stimulation index = dpm in the presence of a mitogen divided by dpm without mitogen.

noticed in VC-exposed animals as compared to the respective control group. A significant increase was noted in 10 ppm VC level at 4 weeks of exposure.

The presence of vinyl chloride *in vitro* in the incubating atmosphere of lymphocyte cultures had no stimulating effect on mouse splenic lymphocyte transformation. The results of one such experiment are shown in TABLE 4. When animals were exposed *in vivo* to 1000 ppm of VC for a period of 8 weeks, the lymphocytes obtained from their spleens had an increased spontaneous transformation when incubated in normal (CO_2:air) atmosphere, as compared to control animals. The presence of 1000 ppm VC in the incubation environment, in fact, slightly decreased the blast formation of lymphocytes enhanced by exposure of mice to 1000 ppm VC for 8 weeks and the

TABLE 3

SERUM IMMUNOGLOBULIN IN MICE EXPOSED TO VINYL CHLORIDE

Vinyl Chloride Concentration ppm	Serum Ig at*			
	Preexposure	2 weeks	4 weeks	8 weeks
0	0.8 ± 0.1	1.2 ± 0.1	0.9 ± 0.1	2.1 ± 0.2
10	—	1.2 ± 0.1	1.6 ± 0.3**	1.2 ± 0.2**
100	—	1.2 ± 0.1	1.2 ± 0.2	1.7 ± 0.3
1000	—	1.8 ± 0.5	1.4 ± 0.3	1.7 ± 0.1**

*Values indicated are the ratios of total serum Ig to that in normal pooled mouse serum. Mean ± S.E. of 4 animals per group.

**Indicates significant deviation from control at $p < .05$.

values were comparable to those obtained for lymphocytes cultures prepared from mice with no VC exposure. The stimulating indices by PHA and PWM were likewise unaltered.

Experiments similar to those shown in TABLE 4 were also conducted in the presence of hepatic metabolizing systems. Addition of liver supernatant supplemented with necessary cofactors (required for microsomal oxidation system)[15] inhibited the mitotic activity of lymphocytes considerably. Approximately 30% of normal thymidine uptake was seen in the presence of such a metabolizing system and the results were inconclusive (data not shown). The inhibitory effect of VC *in vitro*, as shown in TABLE 4, for splenic lymphocytes from VC-exposed animals, however, was not observed under such conditions.

Culturing of lymphocytes from animals exposed to vinyl chloride and treated simultaneously with chemicals to modify its metabolism provided interesting results. Administration of phenobarbital or ethanol alone to mice increased the transformation rate of their splenic lymphocytes. To handle this problem, respective controls that

TABLE 4

LYMPHOCYTE TRANSFORMATION IN MOUSE SPLENIC CULTURES FROM ANIMALS EXPOSED TO VINYL CHLORIDE AND CULTURES INCUBATED IN THE PRESENCE OF VINYL CHLORIDE (VC) ATMOSPHERE*

Animal Group	Incubation Atmosphere	DPM/10^6 Cells (without mitogen)	Stimulation Index with PHA	Stimulation Index with PWM
Control	Normal	6699 ± 1473	8.8 ± 1.5	7.6 ± 1.1
	VC	5080 ± 1804	11.3 ± 2.2	8.2 ± 1.6
VC-exposed	Normal	10262 ± 1107	8.5 ± 3.2	10.8 ± 2.0
	VC	6385 ± 822	9.1 ± 3.3	9.3 ± 0.6

*Splenic cells from either control or vinyl chloride-exposed (1000 ppm, 6 hrs/day, 5 days/wk, for 8 weeks) mice were cultured in appropriate chambers containing either 5% CO_2 in air or 1000 ppm vinyl chloride in CO_2:air atmosphere. The values are mean ± S.E. for 3 animals, each culture run in quadruplicate.

were given these chemicals were exposed to room air simultaneously in control chambers. Saline treated controls were also used in both VC-exposed and unexposed groups. Since VC exposure increases the lymphocyte transformation in culture, the differences of ^{3}H-thymidine uptake in VC-exposed animals and nonexposed animals were calculated for each drug treatment. The results are shown in TABLE 5. As established previously, both spontaneous and mitogen-induced thymidine uptake were considerably increased by exposing animals to vinyl chloride. Administration of 80 mg/kg/day phenobarbital prior to VC exposure reduced this increment in lymphocyte transformation. Administration of 3.2 g/kg of ethanol prior to VC exposure decreased markedly the stimulating effect of VC exposure to an extent that when the cultures were incubated in the presence of PHA and PWM, their transformation rate, as indicated by tritiated thymidine uptake, was lower than that for animals treated with ethanol and not exposed to VC. This lower transformation rate is the reason for the last two negative values presented in TABLE 5.

DISCUSSION

The results reported here suggest that vinyl chloride stimulates the immune responsiveness of mice. The enhanced responsiveness develops rapidly upon exposure

to high levels, whereas 4 weeks of repeated exposure to low levels (10 ppm) are needed to elicit a discernible response. Blast formation of lymphocytes in splenic cell cultures, as evidenced by an increased incorporation of tritiated thymidine into cellular materials was the most sensitive indication of increased immune responsiveness. Vinyl chloride exposure at all levels increased the spontaneous lymphocyte transformation in splenic cell cultures, the effect being similar to that produced by injection of antigenic substances. The stimulating effect of two plant lectins, phytohemagglutinin and pokeweed mitogen, was also increased, further indicating an enhanced immune reactivity in these VC-exposed animals.

The stimulating effect of vinyl chloride exposure on immune responsiveness does not appear to be a nonspecific phenomenon. It is unlikely that this phenomenon is stress induced since stress is generally known to cause immunosuppression. The decrease in lymphocyte transformation in splenic cultures observed in the control group of mice with increasing time interval appears to be consistent with stress-induced immune suppression, since the exposure environment may provide some stress.

The immunostimulatory effect of VC was not always dose-related. After a 2-week exposure period only mice exposed to 1000 ppm VC showed such an effect. After 4 and 8 weeks of exposure the effect was seen at all levels of VC exposure. However, the effect in mice exposed to 1000 ppm VC for 8 weeks was less pronounced than observed in mice exposed for shorter duration. This suggests that with continued exposure to high levels of VC, the initial increase in immune responsiveness may reverse either as a result of adaption or a direct depressive effect of high levels of VC exposure.

TABLE 5

CHANGE IN LYMPHOCYTE TRANSFORMATION IN MICE SPLENIC CULTURES PRODUCED BY THE INHALATION OF 1000 ppm VINYL CHLORIDE FOR ONE WEEK AND IN MICE TREATED WITH PHENOBARBITAL AND ETHANOL TO ALTER THE METABOLISM OF VINYL CHLORIDE

Animals Treated with	Δ DPM/10^4 Cells* in the presence of		
	No mitogen	PHA	PWM
Saline	43,113	121,976	167,844
Phenobarbital	27,770	26,147	55,478
Ethanol	4,929	−147,786	−78,668

*(DPM in animals exposed to vinyl chloride)−(DPM in animals held in control chamber). Each value is obtained from an average of 4 animals and cultures of each animal run in triplicate. Negative values shown in last two columns were obtained because ethanol-treated mice had lower counts in those treated with ethanol and also exposed to vinyl chloride.

The metabolism of vinyl chloride has been shown to be a saturable process in the body.[16] A dose-related saturable binding of vinyl chloride has also been shown in rat liver. Also, protein alkylating metabolites of vinyl chloride in an *in vitro* liver microsomal system have been demonstrated.[15] In view of these results, it is not surprising that the immunostimulating effect of vinyl chloride is saturable since it too may possibly be mediated subsequent to biotransformation of VC in the body. It has been speculated that chloroethylene oxide is the reactive intermediate in the biotransformation of vinyl chloride. Formation of this alkylating intermediate, although not conclusively shown, has been hypothesized as a possible proximate carcinogen. Alkylation of a normal body protein by such a chemical may produce an antigenic determinant and the resultant immunostimulation. Whether such possibility exists needs to be investigated further.

The immunostimulatory effects of vinyl chloride agreed with the recent reports[5] that the vinyl chloride syndrome may involve an autoimmune phenomenon. The studies reported by these workers included patients with clinical effects of VC exposure. In our animal studies, the effects are shown after a relatively short duration of exposure. In these animal studies, an increased reactivity of lymphocytes, including those of "T" cells occurred since PHA is somewhat selective to mouse T-cells. A stimulation of T-cells has been reported to be an indication of an autoimmune phenomenon,[8] a finding also consistent with the hypothesis proposed by Ward *et al.*[5]

The immune stimulation caused by vinyl chloride exposure may be considered similar to the immune modulation caused by levamisole.[17] This chemical, which was first introduced as an animal anthelmintic, has been tried as a therapeutic adjunct in cancer patients and has been shown to prolong the disease-free interval in patients with lung and breast cancer and malignant melanoma.[18,19] At the same time, this chemical enhances survival of allogeneic tumors in animal models and also in some patients. In the case of vinyl chloride, its carcinogenic property may be distinct from its immune effects, but the latter may enhance the carcinogenic process once initiated even if not actually caused by it.

Other reports involving the immunostimulating properties of different chemicals have recently appeared. Renoux *et al.*[20] reported immunostimulating property of propylene glycol, a common solvent and vehicle for medicinal preparations. Goodman and Weigle[21] have reported a dose-related lymphocyte stimulation by 2-mercaptoethanol and α-thioglycerol. These chemicals have a thiol moiety in common in their chemical structure, a property also shared by the molecule of levamisole.[22] Two major metabolites of vinyl chloride, i.e., thiodiglycolic acid and *N*-acetyl-*S*-(hydroxyethyl)-cysteine, have been described[16] and are a result of glutathione conjugation by this chemical. It is possible that these metabolites may somehow be involved in the immunostimulatory property of vinyl chloride.

Direct evidence that metabolism of vinyl chloride is a determining factor for its lymphocyte-stimulating property is derived when its metabolism is altered. Ethanol is known to decrease the metabolism of VC.[23] and simultaneous administration of ethanol interfered with the immunostimulating property of VC in splenic cultures of exposed mice. Use of phenobarbital also exhibited a similar phenomenon. In studies reported previously, vinyl chloride metabolism was not enhanced by microsomes obtained from phenobarbital-treated animals.[15] Similarly, although the rate of VC metabolism was not altered, its binding in hepatic tissue was increased.[24] This suggests that phenobarbital may induce metabolism through another pathway leading to the formation of a product which more readily binds to macromolecules in liver and thus have little effect on lymphocytes. A slight reduction of lymphocyte-stimulating activity in VC-exposed animals by simultaneous administration of phenobarbital may be explained on this basis.

The suggestion that the epoxide metabolite of VC, chloroethylene oxide, may be responsible for stimulated lymphocyte transformation is also supported by our other studies (unreported previously) with chemicals possessing a similar chemical structure to that of vinyl chloride. A derivative of vinyl chloride, vinyl benzene (styrene monomer), has no effect on lymphocyte transformation when added directly to lymphocyte cultures *in vitro*. Addition of styrene oxide, on the other hand, in concentrations as low as 2.5×10^{-7}M caused a remarkable increase in such mitotic activity of lymphocytes in splenic cultures.

These studies, together with our other reports that vinyl chloride substantially increases lymphocyte mitosis in splenic cultures provide credence to previous reports by Ward *et al.*[5] that vinyl chloride syndrome may involve an immunologic phenomenon. A recent report[25] involving peripheral blood lymphocyte cultures from normal

and VC-exposed workers has, although, failed to support this hypothesis. The response of blood lymphocytes in persons exposed occupationally to VC was essentially unaltered.

SUMMARY

Male CD-1 mice were exposed to 10, 100 or 1000 ppm vinyl chloride (VC) for 2–8 weeks @ 6 hr/day, 5 days/week. A slight increase in the spleen weight of mice was noted at the highest exposure level. Spleens were obtained from these animals (4 mice/group after 2, 4, and 8 weeks of exposure) and their lymphocytes cultured *in vitro* with or without the presence of phytomitogens, phytohemagglutinin (PHA) and pokeweed mitogen (PWM). Relative blast formation and the DNA synthesis was measured by the incorporation of ^{3}H-thymidine in the cultured cells. The response of splenic lymphocytes to the phytomitogens was increased several-fold by VC exposure. The effects were apparent at 1000 ppm VC after 2 weeks of exposure and at all levels of VC exposure after 4–8 weeks. The effects were generally more pronounced at 100 ppm VC exposure than those at 1000 ppm. *In vitro* culture of splenic lymphocytes from control or VC-exposed mice in the VC atmosphere did not show an enhancement of blast formation. Alteration of VC metabolism during the VC exposure *in vivo* yielded results that indicated that metabolites of VC may be responsible for the stimulation of lymphocyte transformation observed in splenic cultures.

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