Vol. 39, pp. 79-91, 1981

Environmental Chemical-Induced Macrophage Dysfunction

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Immunomodulation by environmental chemical contaminants and the role immune parameters play in toxicity and risk assessment studies is of increasing concern. Although considerable evidence has indicated that various xenobiotics may be immunosuppressive, little attention has been directed toward ascertaining a specific cellular locus which could be responsible for the impaired immune responsiveness. Since previous studies had suggested a macrophage defect in xenobiotic-induced immunosuppression and since macrophages are integral components of an immune response, an in-depth evaluation of macrophage function was conducted in xenobiotic-exposed mice. Macrophages isolated from mice receiving PCB, HCB, and dieldrin had no alteration in their *in vitro* O_2 consumption while at rest or during phagocytosis. In addition, no alteration in *in vitro* phagocytic activity, phagocytic capacity or microbicidal activity was demonstrated. However, a significant impairment in the *in vivo* phagocytic clearance of a labelled antigen and an altered tissue distribution of the antigen was observed and was, perhaps, related, in part, to a significant decrease in serum fibronectin, an opsonic α_2 surface-binding glycoprotein. Furthermore, animals exposed to HCB and dieldrin, but not to PCB, had a profound decrease in their resistance to a challenge tumor cell implant which was related to a select alteration in tumor cell killing. The adherent spleen cells from HCB-treated mice had a profound suppression in their tumoricidal activity which was in contrast to dieldrin-treated mice, where the target cell type appeared to be the nonadherent cells. However, although dieldrin-exposed adherent cells (macrophages ?) did not appear to have an altered tumoricidal capacity, all four macrophage types isolated from dieldrin-treated mice had a significantly impaired ability to process a cellular antigen. Splenic and alveolar macrophages appeared to be the most sensitive cell types to dieldrin. The present studies suggest that macrophage dysfunction may be an integral part of xenobiotic-induced immunosuppression and that the effector but not affector component of macrophage function may be the site of alteration.

Introduction

Inadvertent modification of the immune response by environmental chemicals and food additives has been of increasing concern. Numerous studies which have utilized organohalides (*1-3*), organophosphates (*4*), carbamates (*5*), heavy metals (*6-8*), and food additives (*9*) have demonstrated that these chemicals have the potential to be immunosuppressive. One extrapolation of the immunosuppression induced experimentally by these compounds is an increased susceptibility to a challenge with infectious pathogens and tumor cell implants. Indeed, following exposure to various xenobiotics, an enhanced sensitivity to bacteria (*10*), viruses (*11*), protozoan parasites (*12*), gram-negative endotoxin (*13*), and tumor cell challenges (*14*) has been demonstrated.

Potential tissue destruction by the environmental chemicals resulting in either the release of "hidden" self-antigens or the formation of altered tissue macromolecules has raised the question of

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In this regard, dieldrin (*15*) and vinyl chloride (*16*) exposure have been reported to elicit autoimmune effects. Without the consideration that the toxicity and pathologic sequelae observed following chemical exposure may possibly be autoimmune phenomena, the toxic effects seen after chemical exposure would be incorrectly attributed to the direct action of the compound and/or its metabolites.

An additional consideration of the role of the immune system in toxic manifestations of chemicals of environmental importance is the possible influence of immunocompetent cells on xenobiotic metabolism. Since most environmental chemicals fulfill the Landsteiner criteria of haptens, a host response to these compounds would alter both their vascular clearance and tissue distribution and hence their catabolism. In support of this hypothesis have been the studies of Authrup et al. (*17*) and Wasserman et al. (*18*) which have demonstrated that immunocompetent cells and/or the immune response *per se* can alter the *in vitro* metabolism of xenobiotics and their *in vivo* pharmacokinetics.

The altered host defense status in environmental chemical treated animals may be suggested to be due to a compromised macrophage function since an essential role of fixed tissue macrophages in both host resistance to infectious agents and in immune responsiveness has been amply demonstrated. Biozzi et al. (*19*) reported that an enhanced phagocytic function of the reticuloendothelial (RE) cells is the primary mechanism of host defense against gram-negative and gram-positive bacteria. In reviewing the literature concerning virus-macrophage interactions, Mims (*20*) reported that macrophages phagocytize virus particles and either support their growth, allow their survival until they are passed to other cells, or destroy them. The principal functions of macrophages in relation to hemoflagellates and hemosporidea were initially recognized by Linton (*21*) and Taliaferro (*22*), who observed that certain parasitoses were primarily infections of the RE cells and that in malaria the chief defense was phagocytosis by RE cells (*23*).

In addition to the integral function of macrophages in defense against certain bacteria, viruses and protozoa, they are also essential in various immunological responses. Activation of the RE cells by a variety of techniques enhances the immune response to particular antigens, while a depression of RE function results in a decreased immune response (*24*, *25*). Also, macrophages have been suggested to act as initiators of the immune response (*26-28*), and to be essential in thymocyte mitogenesis (*29*, *30*). Furthermore, the role of macrophages in the selective killing of neoplastic demonstrated (*31*). Their ability to perform these various roles is related to their functional state.

The intent of the present manuscript is to describe a series of experiments examining the role of the macrophage in environmental chemical-induced immune alterations. While it is recognized that macrophages cooperate in an integrated manner with lymphocytes in the elaboration of an immune response, the present study is restricted to macrophages, whereas lymphocyte function will be reviewed elsewhere by Silkworth (*32*).

Materials and Methods

Animals

Male Balb/c mice (18-20 g) were used throughout all studies except certain tumor studies where appropriate syngeneic strains were used. All mice were fed powdered Wayne lab diet.

Chemicals

Polychlorinated biphenyl [Aroclor 1242 (Monsanto)], hexachlorobenzene (Eastman), and dieldrin (Shell) were administered in the diet. Test parameters were evaluated following 3, 6, and 18 weeks dietary exposure.

Cell Isolation

Elicited peritoneal macrophages (PM) were obtained by saline lavage of the peritoneal cavity four days after an intraperitoneal (IP) injection of 1 ml of 6% Na caseinate. Cells were washed two times in sterile saline and then resuspended to a concentration of 2.0×10^6 cells/ml in Hanks Balanced Salt Solution (HBSS, Gibco) with 2% fetal calf serum (FCS, Gibco). The total cell suspension contained 80-85% macrophages (as determined by eosin Y ingestion), and about 5% PMN's and lymphocytes as determined by using Turk's stain. The average total cell yield was approximately 1×10^7 cells/mouse.

Elicited polymorphonuclear leukocytes (PMN's) were obtained in the same manner as the peritoneal macrophages except they were harvested 24 hr after IP injection of the Na caseinate. The average cell yield was approximately 1.5×10^7 cells per mouse, and approximately 85% were PMN's (as determined by Turk's stain).

Alveolar macrophages were obtained by saline lavage of the lungs in ether anesthetized mice via a cannula inserted into the trachea. Cells from two

10^6 cells/ml and served as one data point in each assay. All other cell suspensions required only one animal per data point.

Splenic marcophages were isolated from a suspension of spleen cells that had been obtained by finely mincing spleens in HBSS. The cells were then suspended in HBSS with 2% FCS and then incubated in tissue culture dishes (10 × 15 mm) at 37°C in a 5% CO_2 humidified atmosphere for 3 hr. Adherent cells were collected, resuspended in HBSS with 2% FCS, and adjusted to a concentration of 2.0×10^6 cells/ml.

All cell counts were made by using a hemocytometer, and viability was assessed by trypan blue dye exclusion.

Oxygen Consumption

Oxygen consumption was measured by using a Gilson recording oxygraph equipped with a Clarke O_2 electrode as previously described (*33*). A volume of 1.6 ml containing 2.0×10^6 cells/ml in HBSS with 10% FCS was added to the chamber at 37°C and the resting O_2 consumption was measured for 8 min. After this time, 100 μl of an opsonized Fleishmann's household yeast (*Saccharomyces cerevisiae*) cake suspension was added to yield a yeast:cell ratio of 1: 25. The yeast suspension was prepared by adding approximately 1 g of Fleishmann's cake yeast to 100 ml of sterile 0.9% saline. This preparation was boiled for 15 min, cooled, and boiled again for 15 min to kill the yeast. Yeast viability was assessed by methylene blue exclusion. The yeast then were opsonized in FCS for 30 min at 37°C and added to the cell suspension to yield a final serum concentration of 20%. Oxygen utilization during the phagocytic event was measured for an additional 8 min.

Using the standardizing buffer (O_2 Probe Solution, Yellow Springs Instrument Company), the recording oxygraph was calibrated for 16 cm. The constant used for 16 cm is 50.4 and the formula is y/x (constant) = μM O_2/hr/10^6 cells.

Phagocytosis and Killing Assay

The method of Simpson et al. (*34*) was used and is described briefly. Equal volumes of test cells, opsonized live yeast in a ratio of 1: 10 with test cells and methylene blue (8×10^{-4} *M*) were placed into sterile polypropylene 12 mm × 75 mm test tubes with caps and were incubated at 37°C in a 5% CO_2 humidified atmosphere on an aliquot shaker for 30 min. The cell suspensions were then centrifuged (478g at 4°C for 6 min), and the supernatant was μl of sterile saline, and wet mounts were made and examined under oil immersion. A minimum of 100 cells distributed over five fields were examined. The number of phagocytically active cells, the total number of ingested yeast and the number of killed ingested yeast were enumerated. Thus, three parameters were measured: (a) the phagocytic capacity, (b) the phagocytic activity, and (c) the microbicidal activity:

$$\text{Phagocytic capacity} = \frac{\text{number of live and dead yeast ingested}}{\text{100 cells}}$$

$$\text{Phagocytic activity} = \frac{\text{number of cells containing} > \text{2 yeast}}{\text{100 cells}}$$

$$\text{Microbicidal activity} = \frac{\text{number of dead ingested yeast}}{\text{phagocytic capacity}}$$

Fibronectin Assay

Electroimmunoassay or "rocket" immunoelectrophoresis was used to quantitate plasma fibronectin or opsonic protein levels as previously described (*35, 36*). Experimental serum was assayed for immunoreactive fibronectin. Blood samples obtained from the abdominal vena cava were allowed to clot for 60 min at 25°C prior to centrifugation to obtain serum. It is recognized that the serum concentration of circulating fibronectin (opsonic glycoprotein) is consistently less than that of plasma due to the incorporation or covalent binding of plasma fibronectin to fibrin in the presence of Factor XIII (*37*). This difference is minimal if the blood is allowed to clot at room temperature prior to collection of serum (*38*). All samples for analysis were carefully handled under such constant conditions so that serum could be the test media used for the immunoassay as previously standardized (*35*). The serum was diluted to 10%, and 10 μl was added to each well cut into the solidifed agarose–antiserum solution layer on the glass plate (5 × 10 in.) used in the electroimmunoassay. The samples were then moved electrophoretically toward the anode at a voltage of 7.5 V/cm at 4°C for 22 hr by using an LKB multiphore system. The plates were washed overnight, pressed and dried, and subsequently stained (*35*). Rocket heights were used as a quantitative index of immunoreactive opsonic α_2SB glycoprotein

millimeters and a double reciprocal standard plot (1/mm vs. 1/μg opsonic or 1/mm vs. 1/% serum) was defined with a DEC-10 computer for protein standards at varying concentrations. This standard curve was used to determine serum immunoreactive opsonic α_2SB glycoprotein in μg/ml (*36*).

Tumor Susceptibility Study

Syngeneic mice were maintained on diets containing 5 or 100 ppm of PCB 1242 or HCB or 1 or 5 ppm of dieldrin and then challenged at 3, 6, or 18 weeks with appropriate syngeneic ascites-form tumor cells. DBA/2 male mice (18-20 g) were challenged with the methylcholanthrene-induced lymphomas, P388 or L1210. Male Balb/c mice (18-20 g) were challenged with the SV-40 virus-induced Balb/c kidney tumor, mKSA. Male C57B1/6 mice (18-20 g) were challenged with the benzo(a)pyrene-induced lymphoma EL-4. Tumor lines were maintained in ascites form by weekly intraperitoneal (IP) inoculation and serial passage. Tumor cells were obtained for passage by peritoneal lavage with a 22 gauge needle and 5 ml of sterile HBSS. Tumor cells were washed three times in cold, sterile HBSS, counted, and suspended to the appropriate concentration in sterile HBSS for IP inoculation of 0.5 ml of the suspension into isogeneic mice. The following doses of live tumor cells were used: P388, 0.5×10^6; EL-4, 2×10^5; mKSA, 1×10^7; and L1210, 0.5×10^5. The Litchfield test (*39*) was used for an analysis of time versus effect, and linear regression analysis was used to compute the mean survival time.

Cytotoxicity Assay

Tumor cell (mKSA) killing was assessed *in vitro* by ^{51}Cr-release from labelled tumor target cells as described by Canty and Wunderlich (*40*). The effector: target cell ratio was 40:1. The cells were suspended in RPMI 1640 (without phenol red) supplemented with 20% FCS and incubated at 37°C for 24 hr in a humidified atmosphere of 95% air:5% CO_2. Cytotoxicity was calculated by the method of Nathan et al. (*41*) as follows:

$$\frac{\text{Treated } ^{51}\text{Cr release} - \text{Control } ^{51}\text{Cr release}}{\text{Maximum } ^{51}\text{Cr release} - \text{Control } ^{51}\text{Cr release}} = \% \text{ Cytotoxicity}$$

Maximum release of the ^{51}Cr label was determined from the supernatant of thrice freeze-thawed tumor cells. Statistical differences were assessed by the Student *t*-test, and $p < 0.05$ was considered significant.

The mKSA tumor target cells were harvested 10 days following their IP inoculation. Spleen cells were obtained from nontumor-bearing mice which had been receiving a dietary administration of test chemicals for 3, 6, or 18 weeks. Spleens were removed asceptically and then teased through a 10XX silk cloth. Adherent cells were obtained by incubating approximately 1×10^7 spleen cells in 90 mm culture plates, previously coated with poly-L-lysine, with RPMI 1640 supplemented with 10% FCS at 37°C for 3 hr in a 95% air: 5% CO_2 humidified atmosphere. Nonadherent cells were decanted off, and adherent cells were removed by gentle rubbing with a rubber policeman. All cell preparations were washed three times in HBSS before use.

Intravascular Clearance and Antigen Processing

Phagocytic Determinations. The *in vivo* phagocytic response of dieldrin treated mice to a particulate antigen was measured by the vascular clearance of ^{51}Cr-labeled SRBC. Following administration of the chemical for two weeks, all groups of mice, treated and controls, received an IV administration of 0.1 cc of 20% SRBC labeled with 7.2 μCi of $Na_2{}^{51}CrO_4$. The ^{51}Cr-SRBC were prepared by the technique of Gray and Sterling (*42*). After IV injection of the ^{51}Cr-SRBC suspension, 0.01 cc aliquots of heparinized blood were collected from the tail veins at 1, 3, 5, 7, and 9 min. The blood radioactivity, as percentage of the injected dose, was plotted semilogarithmically against time in minutes and the intravascular half-time was calculated. Tissue distribution of the ^{51}Cr-SRBC suspension was measured at 11 min. after its injection, and the radioactivity of liver, lungs, and spleen expressed as a percentage of the injected dose on a weight and total organ basis.

Antigen Processing. Male Balb/c mice (18-20 g) received a dietary administration of 0.5, 5.0, and 50.0 ppm of dieldrin for two weeks. Following the two-week exposure period, macrophages were harvested and allowed to phagocytize, *in vitro*, opsonized sheep erythrocytes. Peritoneal macrophages were obtained by peritoneal lavage four days following an IP injection of 1 cc of 6% Na caseinate. Splenic macrophages were obtained by surface adsorption to poly-L-lysine-coated tissue culture plates. Alveolar macrophages were obtained by isotonic saline lavage of the lungs. Hepatic Kupffer cells were obtained by enzymatic digestion of the liver following an *in situ* perfusion (*43*).

3 hr at 37°C on an aliquot mixer. The opsonized ^{51}Cr-SRBC (*44*) were seeded onto macrophage monolayers at an SRBC: macrophage ratio of 100:1 and phagocytosis was allowed to occur for 4 hr. After this time, the plates were gently rinsed with HBSS and the macrophages with ingested ^{51}Cr-SRBC were removed by a rubber policeman. An aliquot of 1×10^7 macrophages, from dieldrin or control mice and containing, on the basis of radioactivity and light microscopic examination, essentially equal numbers of SRBC was injected into control Balb/c mice. Splenic plaque forming cells were enumerated by the Jerne and Nordin (*45*) technique on the peak response day. Preliminary studies revealed day 4 to be peak response day.

Statistical Analysis

All data, where appropriate, were analyzed by using the Student *t*-test. A probability level $p < 0.05$ was considered to be statistically significant. All data are presented as the mean ± SEM.

Results

Oxygen Consumption, Phagocytic and Microbiocidal Activity

The present studies indicate that PCB 1242 or HCB administered in the diet at 167 ppm for 3, 6, or 18 weeks did not significantly alter, in any consistent manner, oxygen utilization, phagocytic activity or microbicidal activity in phagocytic cells (Table 1). However, certain individual parameters resting O_2 consumption of PMNs from HCB treated mice was significantly lower than controls at 3 weeks but this change was not reflected in any functional alterations. In general, the resting rate of O_2 consumption as well as the respiratory burst increased with age in all groups, treated as well as controls, which possibly represents a maturational development.

The phagocytic activity, phagocytic capacity and microbicidal activity of alveolar, splenic and peritoneal macrophages and PMNs was not altered, in any consistent manner, by PCB 1242 or HCB following dietary administration lasting up to 18 weeks. Furthermore, the isolated incidences where alterations did occur were not related to any previously determined (*12*, *13*) *in vivo* host defense and/or immunological measurements (Table 2).

A modulator of cell surface activity, i.e, fibronectin, a surface binding glycoprotein, was measured and was found to be significantly decreased, primarily at the 18 week test period (Table 3). In general, an approximate 40% reduction, below control values, was observed in the fibronectin levels in the chemical treated mice following 18 weeks of dietary administration of the chemicals. Coincident with the decrease in serum fibronectin was a significant increase in the susceptibility of the chemical-treated mice to a challenge of ascites tumor cells (Tables 4-7). This increased susceptibility was most marked at the 18 week test period.

Tumor susceptibility of PCB 1242, HCB, and dieldrin treated syngeneic mice was assessed using ascites form of tumor cell implants from chemical and/or virus transformed cell lines bearing diverse H-2 and Mls antigens. The DBA/2 tumors (P388 and

Table 1. Influence of PCB 1242 and HCB on oxygen utilization by macrophages and neutrophils.[a]

		Oxygen utilization, μM O_2/hr/10^6 cells							
		Alveolar macrophages		Splenic macrophages		Peritoneal macrophages		PMN's	
	Time, weeks	Resting	% Burst	Resting	% Burst	Resting	% Burst	Resting	% Burst
Control	3	20.3 ± 2.7	15.1 ± 8.6	3.3 ± 0.5	16.3 ± 7.6	5.9 ± 0.4	48.1 ± 10.9	5.0 ± 0.4	160.4 ± 28.6
	6	23.4 ± 3.4	13.9 ± 14.5	3.4 ± 0.7	10.9 ± 4.2	7.2 ± 1.0	53.4 ± 11.8	8.2 ± 0.4	144.2 ± 29.4
	18	44.9 ± 5.5	27.3 ± 6.6	9.5 ± 2.8	24.4 ± 4.1	10.7 ± 1.0	42.7 ± 8.4	10.9 ± 0.7	133.8 ± 24.2
PCB 1242	3	18.1 ± 2.6	17.8 ± 9.3	2.9 ± 0.5	28.3 ± 6.1	6.3 ± 0.5	36.1 ± 4.8	6.6 ± 1.1	142.0 ± 30.0
	6	18.4 ± 2.1	37.7 ± 11.4	2.9 ± 0.3	30.6 ± 14.6	8.5 ± 0.7	36.2 ± 8.0	8.3 ± 0.5	110.9 ± 81.1
	18	48.1 ± 6.5	16.8 ± 5.0	9.2 ± 1.9	25.7 ± 8.4	10.5 ± 0.6	50.2 ± 14.3	10.7 ± 0.7	162.2 ± 33.6
HCB	3	23.2 ± 4.2	15.8 ± 3.1	3.2 ± 0.5	14.9 ± 5.8	6.3 ± 0.4	25.8 ± 8.6	6.3 ± 0.3*	96.6 ± 25.9
	6	18.1 ± 2.4	39.4 ± 15.9	2.9 ± 0.4	20.0 ± 4.0	7.7 ± 1.2	30.1 ± 9.3	6.9 ± 0.6	97.8 ± 18.2
	18	43.1 ± 6.6	28.2 ± 6.5	12.3 ± 2.2	24.8 ± 3.6	11.1 ± 0.7	31.2 ± 5.5	9.6 ± 0.5	106.6 ± 14.2

[a]PCB 1242 and HCB were administered in the diet for 3, 6, or 18 weeks at 167 ppm. Controls received Wayne lab diet without chemicals. The cells were prepared as described in Materials and Methods. All data are presented as the mean ± SEM and significance at $p < 0.05$ indicated by an asterisk. All data points consist of ≥ 9 individual studies.

Table 2. Influence of PCB 1242 and HCB on the phagocytic capacity, phagocytic activity, and microbicidal activity of macrophages and PMN's.[a]

	Times, weeks	Phagocytic capacity			Phagocytic activity			Microbicidal activity		
		C	PCB	HCB	C	PCB	HCB	C	PCB	HCB
Alveolar macrophages	3	119.4 ± 8.4	114.7 ± 13.3	73.1 ± 5.6	62.6 ± 6.6	64.0 ± 5.7	11.5 ± 2.4	14.4 ± 2.4	14.4 ± 3.3	12.2 ± 2.2
	6	120.7 ± 2.6	82.0 ± 9.5	101.0 ± 10.8	67.3 ± 5.8	53.3 ± 4.7	61.7 ± 6.6	13.5 ± 2.5	19.8 ± 5.8	17.5 ± 7.9
	18	172.2 ± 17.3	178.0 ± 17.0	206.4 ± 14.5	88.0 ± 4.4	91.0 ± 3.1	90.7 ± 3.4	37.2 ± 3.4	42.8 ± 4.8	35.0 ± 4.7
Splenic macrophages	3	99.7 ± 7.7	96.2 ± 16.1	110.5 ± 9.0	63.3 ± 4.6	57.6 ± 5.9	74.7 ± 5.2	8.9 ± 1.4	6.8 ± 2.1	7.7 ± 2.5
	6	95.0 ± 6.8	85.0 ± 6.8	106.7 ± 6.3	63.0 ± 5.3	53.3 ± 5.9	64.7 ± 3.6	9.8 ± 1.5	15.5 ± 1.6	14.3 ± 2.3
	18	140.3 ± 7.3	141.0 ± 7.8	132.0 ± 11.9	89.0 ± 12.3	89.0 ± 12.3	87.0 ± 3.4	71.6 ± 5.2	69.5 ± 4.0	72.7 ± 4.6
Peritoneal macrophages	3	255.0 ± 27.0	236.0 ± 35.3	164.0* ± 19.8	67.0 ± 7.0	71.3 ± 8.3	59.0 ± 7.7	8.7 ± 1.5	9.7 ± 2.5	13.0 ± 2.0
	6	323.3 ± 33.7	387.0 ± 30.4	323.3 ± 30.1	86.0 ± 5.1	93.0 ± 4.1	74.7* ± 3.8	13.4 ± 1.9	13.6 ± 3.9	11.3 ± 1.3
	18	216.0 ± 19.6	200.0 ± 15.0	215.0 ± 27.2	81.0 ± 2.3	81.0 ± 3.8	81.0 ± 3.8	33.5 ± 4.0	36.2 ± 2.6	34.5 ± 4.5
PMN's	3	272.9 ± 20.1	267.5 ± 18.1	248.5 ± 11.2	94.5 ± 2.4	92.5 ± 2.5	86.0 ± 3.7	19.5 ± 1.7	18.9 ± 1.3	19.9 ± 2.0
	6	281.5 ± 22.4	264.0 ± 16.6	328.0 ± 11.2	92.7 ± 2.4	88.7 ± 2.8	91.2 ± 0.8	15.0 ± 1.1	15.3 ± 2.4	14.0 ± 1.1
	18	278.8 ± 11.3	259.6 ± 29.9	181.6* ± 37.7	99.2 ± 0.53	98.8 ± 0.6	30.7 ± 3.3	36.6 ± 1.8	33.6 ± 2.1	

[a] The phagocytic and microbicidal function of macrophages and PMNs was ascertained as described in Materials and Methods. Dietary administration of PCB 1242 and HCB at 167 ppm was for 3, 6, or 18 weeks. Data are presented as the mean ± SEM with an asterisk (*) denoting significance at $p < 0.05$; $n = \geq 9$; C = Control.

1242, HCB, or dieldrin.[a]

Treatment	Fibronectin concentration, % of control		
	3 weeks	6 weeks	18 weeks
PCB			
5 ppm	139	83	40*
100 ppm	99	78*	45*
HCB			
5 ppm	115	72	74
100 ppm	92	70*	52*
Dieldrin			
1 ppm[b]	42*	131	86
5 ppm	107	100	51*

[a]Presented as % of control mean with an asterisk (*) denoting significance at $p < 0.05$; n = 7-10 for each group.
[b]Dieldrin (1 ppm) study was conducted independently and significance is based on its own control group.

Table 4. Mean survival time in mice fed PCB 1242, HCB, or dieldrin and challenged with mKSA tumor cells.[a]

Treatment	Mean survival time, days		
	3 weeks	6 weeks	18 weeks
Control	19.8	20.0	21.3
PCB			
5 ppm	19.2	19.2	20.0
100 ppm	17.6	18.1	18.0
HCB			
5 ppm	19.8	18.8	18.5
100 ppm	20.6	14.0*	11.4*
Dieldrin			
1 ppm[b]	24.0	19.4	14.3*
5 ppm	17.4	18.2	14.2*

[a]Ascites tumor cells mKSA-Tu5 (1.0×10^7/ml) were injected intraperitoneally (IP) in 0.5 ml sterile isotonic saline following a 3, 6, or 16 week dietary administration of PCB 1242, HCB or dieldrin. The percent cumulative mortality was recorded and the mean survival time was determined. The asterisk (*) denotes significance at $p < 0.05$; n = 7-10 for each group.
[b]Dieldrin (1 ppm) study was conducted independently and significance is based on its own control group.

L 1210) both have H-2^d and Mls^1 antigens whereas the Balb/c tumor mKSA has an H-2^d and Mls^2 antigen and the C57B1/6 tumor line EL-4 has an H-2^b and Mls^2 antigen. The Mls locus resides outside the H-2 complex and is an important stimulus in the mixed lymphocyte response (MLR).

As can be seen in Tables 4-7, PCB 1242 at dose levels of 5 or 100 ppm did not alter tumor susceptibility for periods up to 18 weeks dietary exposure. This lack of susceptibility to tumor challenge was observed in all four tumor models. However, a marked increase in susceptibility to tumor challenge was observed in mice administered HCB or

dieldrin and challenged with EL-4 tumor cells.[a]

Treatment	Mean survival time, days		
	3 weeks	6 weeks	18 weeks
Control	28.6	30.1	31.1
PCB			
5 ppm	26.7	27.9	31.0
100 ppm	18.8*	29.8	32.3
HCB			
5 ppm	27.0	27.3	28.1
100 ppm	22.4*	19.2*	13.9*
Dieldrin			
1 ppm[b]	21.6*	23.4*	22.1*
5 ppm	20.7*	17.7*	9.0*

[a]Ascites tumor cells EL-4 (2×10^5) were injected IP in 0.5 ml sterile isotonic saline following a 3, 6, or 18 week dietary administration of the chemicals. The percent cumulative mortality was recorded and the mean survival time was determined. The asterisk (*) denotes significance at $p < 0.05$; n = 7-10 for each group.
[b]Dieldrin (1 ppm) study was conducted independently and had its own control group.

Table 6. Mean survival time in mice fed PCB 1242, HCB, or dieldrin and challenged with P388 tumor cells.[a]

Treatment	Mean survival time, days		
	3 weeks	6 weeks	18 weeks
Control	24.3	25.9	25.5
PCB			
5 ppm	23.9	24.8	27.0
100 ppm	22.7	25.3	27.1
HCB			
5 ppm	21.0	20.1*	19.3*
100 ppm	20.2*	16.5*	11.7*
Dieldrin			
1 ppm[b]	24.0	22.2*	19.1*
5 ppm	19.0	15.0*	9.1*

[a]Ascites tumor cells P388 (0.5×10^6) were injected IP in 0.5 ml sterile isotonic saline following a 3, 6, or 18 week dietary administration of the test chemicals. The percent cumulative mortality was recorded and the mean survival time was determined. The asterisk (*) denotes significance at $p < 0.05$; n = 7-10 for each group.
[b]Dieldrin (1 ppm) study was conducted independently and had its own control group.

dieldrin in their diets and challenged with either of the four tumor cell lines. This increased susceptibility was demonstrated to be related to the length of dietary exposure to HCB and dieldrin and to the dosage of the chemical. The H-2 complex or Mls locus did not appear to be an important factor in that differential susceptibility to either of the tumor cell lines was not evident.

To ascertain a possible cellular lesion responsible

Table 7. Mean survival time in mice [illegible], or dieldrin and challenged with L 1210 tumor cells.[a]

Treatment	Mean survival time, days		
	3 weeks	6 weeks	18 weeks
Control	18.1	17.5	18.4
PCB			
5 ppm	18.0	18.4	19.2
100 ppm	17.5	18.1	19.7
HCB			
5 ppm	17.0	14.1*	11.2*
100 ppm	10.5*	7.2*	6.4*
Dieldrin			
1 ppm[b]	17.7	16.0	14.0*
5 ppm	12.1*	10.2*	5.1*

[a]Ascites tumor cells L 1210 (0.5×10^5) were injected IP in 0.5 ml sterile isotonic saline following a 3, 6, or 18 week dietary administration of the test chemicals. The percent cumulative mortality was recorded and the mean survival time was determined. The asterisk (*) denotes significance at $p < 0.05$; n = 7-10 for each group.

[b]Dieldrin (1 ppm) study was conducted independently and had its own control group.

Table 8. Influence of PCB 1242, HCB, and dieldrin on splenic cytocidal activity against mKSA tumor cells.[a]

Time, weeks	Cytotoxicity, % of control					
	PCB		HCB		Dieldrin	
	5 ppm	100 ppm	5 ppm	100 ppm	1 ppm	5 ppm
3	98	100	95	82*	92	77*
6	100	95	87*	71*	91	48*
18	92	98	80*	52*	84*	34*

[a]The cytotoxic activity of a total spleen cell preparation against ^{51}Cr-mKSA tumor cells was assessed using isolated spleen cells from nontumor-bearing PCB; HCB; or dieldrin-treated mice. Data are presented as percent of control with an asterisk (*) denoting significance at $p < 0.05$; n = 7-10 per group. PCB and HCB were administered in the diet at 5 and 100 ppm and dieldrin was administered at 1 and 5 ppm.

for the enhanced susceptibility of HCB and dieldrin-treated mice to the tumor cell challenges, splenic macrophage and lymphocyte tumoricidal activity was evaluated. In Table 8 the total splenic tumoricidal activity of a heterogenous population of spleen was assessed against mKSA tumor cells. PCB 1242 at 5 or 100 ppm did not alter splenic tumoricidal activity. However, HCB and dieldrin at their respective maximal doses, 100 and 5 ppm, respectively, significantly impaired tumor cell killing as early as three weeks after dietary exposure. At 5 ppm, HCB impaired tumor cell killing by 6 weeks whereas at 1 ppm dieldrin did not alter tumor cell killing

[illegible]uence o[illegible], and dieldrin on splenic nonadherent cell killing of mKSA tumor cells.[a]

Time, weeks	Cytotoxicity, % of control					
	PCB		HCB		Dieldrin	
	5 ppm	100 ppm	5 ppm	100 ppm	1 ppm	5 ppm
3	120*	105	87	83*	77*	54*
6	118	100	89	81*	70*	30*
18	100	101	90	84*	73*	23*

[a]The cytotoxic activity of nonadherent spleen cell against ^{51}Cr-mKSA tumor cells was assessed using spleen cells from non-tumor bearing mice administered PCB; HCB; or dieldrin for 3, 6, or 18 weeks. All data are presented as percent of control with an asterisk (*) denoting significance at $p < 0.05$; n = 7-10 per group. PCB and HCB were administered in the diet at 5 and 100 ppm and dieldrin was administered at 1 and 5 ppm.

activity of spleen cells until 18 weeks after dietary exposure.

Further studies to delineate the cell type involved were conducted using nonadherent and adherent spleen cells. PCB 1242 (5 ppm) had a mild stimulatory effect on nonadherent (lymphocyte) spleen cell tumor cell killing (Table 9). The high dose of PCB 1242 (100 ppm) was ineffective in altering splenic nonadherent cell tumor cell killing. HCB at a concentration of 100 ppm induced an approximate 20% reduction in tumor cell killing. This reduction was constant from 3 to 18 weeks of the dietary administration of HCB. At 1 ppm dieldrin induced an approximate 30% reduction in splenic nonadherent cell tumoricidal activity which was held constant for up to 18 weeks. However, at 5 ppm dietary administration of dieldrin, an approximate 50% reduction in splenic nonadherent cell tumor cell killing ability was manifested. This reduction was exacerbated with increased time of dietary exposure in that by 18 weeks, the 5 ppm dieldrin-treated animals had only 23% of control value tumoricidal activity (Table 9).

Adherent cell (macrophage ?) tumoricidal activity was not altered by either the low dose of PCB 1242 (5 ppm) or dieldrin (1 ppm) administered for 3, 6, or 18 weeks (Table 10). However, at the high dose of PCB and dieldrin, 100 ppm and 5 ppm, respectively, a significant decrease in tumor cell killing was observed at each integral tested. Adherent cells from the high dose PCB and dieldrin manifested a tumoricidal activity which was approximately 80% of control values (Table 10). This reduction was not exacerbated with time on diet. In contrast, HCB at a dietary administration of 5 and 100 ppm produced a time- and dose-related decrease in adherent cell tumoricidal activity. By

Table 10. Influence of PCB 1242, HCB, and dieldrin on splenic adherent cell killing of mKSA tumor cells.[a]

Time, weeks	Cytotoxicity, % of control					
	PCB		HCB		Dieldrin	
	5 ppm	100 ppm	5 ppm	100 ppm	1 ppm	5 ppm
3	97	82*	75*	50*	94	83*
6	97	80*	60*	27*	96	80*
18	94	76*	52*	11*	93	80*

[a]The cytotoxic activity of adherent spleen cell against ^{51}Cr-mKSA tumor cells was assessed using spleen cells from non-tumor bearing mice administered PCB; HCB; or dieldrin for 3, 6, or 18 weeks. All data are presented as percent of control with an asterisk (*) denoting significance at $p < 0.05$; $n = 7$-10 per group. PCB and HCB were administered in the diet at 5 and 100 ppm and dieldrin was administered at 1 and 5 ppm.

18 weeks after dietary administration of 5 ppm of HCB, adherent cells had only 52% of control cell tumor cell killing activity and 100 ppm only 11% (Table 10).

An evaluation of the *in vivo* phagocytic activity of fixed tissue macrophages of the RES revealed that a dose-related decrease in the vascular clearance of ^{51}Cr-SRBC was associated with an impaired hepatic and splenic, but not alveloar, clearance of the ^{51}Cr-SRBC in dieldrin-treated mice (Table 11). Although on a per total organ basis the hepatic localization of the ^{51}Cr-SRBC was greater in the dieldrin-treated mice, the content of the ^{51}Cr-SRBC expressed on a per gram basis was decreased. This may be a reflection of dieldrin-induced hepatic parenchymal cell hypertrophy and/or hyperplasia and a decrease in number and/or function of hepatic Kupffer cells.

To further ascertain the functionality of macrophages in dieldrin-treated mice, peritoneal, splenic, hepatic, and alveolar macrophages were isolated from mice which received a dietary administration of 0.5, 5.0, or 50.0 ppm of dieldrin for 2 weeks and evaluated for their ability to present an adequate immunogen to control mice (Table 12). Immunogen transfer was assessed by splenic plaque forming cell development in recipient mice. As can be seen in Table 12, all four populations of macrophages obtained from high dose (50 ppm) treated mice had an impaired ability to present an adequate immunogen to control mice. A dose-related decrease was most evident in splenic and peritoneal macrophages. However, splenic and alveolar macrophages appeared to be the most sensitive, with regard to dosage, in that both populations manifested a significant reduction in antigen processing when exposed to only 0.5 ppm of dieldrin.

Discussion

Macrophages have been recognized for many years to play a crucial role in resistance to intracellular infection, to act as accessory cells in the immune response, and to function as cytotoxic cells acting against tumor cells or virally infected cells. Their ability to perform these various roles is related to their functional state (*46*).

Although previous studies which have demonstrated an impaired antibody formation and heightened sensitivity to endotoxin and malaria in xenobiotic-treated mice suggested a macrophage defect, specific functional assessments were not conducted. As our knowledge of cellular cooperation in the generation of an immune response expands, the designation, at the present time, of a

Table 11. Vascular clearance and tissue distribution of ^{51}Cr-SRBC in dieldrin-treated mice.[a]

Dieldrin, ppm	$t_{1/2}$, min[c]	Tissue distribution[b]					
		Liver		Lung		Spleen	
		% ID/g	% ID/TO	% ID/g	% ID/TO	% ID/g	% ID/TO
0	5.7 ± 0.2	23.9 ± 1.2	67.3 ± 3.3	5.4 ± 0.8	0.93 ± 0.1	16.6 ± 1.0	2.2 ± 0.2
0.5	7.1 ± 0.5	20.0 ± 1.8	69.9 ± 5.4	4.0 ± 0.5	0.88 ± 0.1	15.2 ± 1.1	2.0 ± 0.1
5.0	11.9 ± 1.1*	12.2 ± 1.1*	77.6 ± 4.8	5.3 ± 0.8	0.92 ± 0.6	9.1 ± 0.9*	1.6 ± 0.8
50.0	20.2 ± 2.3*	5.4 ± 2.9*	84.4 ± 7.1*	5.9 ± 0.6	0.80 ± 0.9	6.0 ± 1.8*	1.0 ± 0.1*

[a]Mice were maintained on diet for 2 weeks after which all mice received 0.1 cc of 20% SRBC labeled with $Na^{51}Cro_4$ containing 7.05 μCi.

[b]Tissue distribution of the ^{51}Cr-SRBC was measured 11 min after injection, and tissue radioactivity is expressed as percentage of the injected dose (%ID) on a weight and total organ basis. Data expressed as mean ± SEM, where an asterisk (*) denotes significance at $p < 0.05$; $n = 10$ throughout.

[c]Vascular clearance was determined by taking 0.1 cc aliquots of blood from the tail vein at 1, 3, 5, 7, and 9 min. The blood radioactivity, as percentage of the injected dose, was plotted semilogarithmically against time in minutes, and the intravascular half-time $t_{1/2}$ calculated.

Table 12. Comparative influence of transferred antigen-exposed macrophages from normal and dieldrin-treated Mice on splenic plaque formation in control naive mice.[a]

Dieldrin, ppm	PFC/spleen[b]			
	Peritoneal macrophages	Splenic macrophages	Alveolar macrophages	Kupffer cells
0	34,651 ± 1,477	43,869 ± 3,871	22,207 ± 1,970	17,041 ± 976
0.5	29,020 ± 1,641	33,298 ± 2,710*	15,001 ± 1,432*	14,277 ± 1,122
5.0	15,775 ± 2,013*	12,656 ± 1,174*	13,795 ± 987*	13,998 ± 1,002
50.0	7,897 ± 696*	9,023 ± 862*	10,625 ± 1,125*	9,844 ± 1,217*

[a]Balb/c male mice were maintained on dieldrin-containing diets for 2 weeks.
[b]Peritoneal and alveolar macrophages were harvested by saline lavage. Hepatic Kupffer cells were isolated by an enzymatic procedure (*42*) and splenic macrophages were isolated by surface adsorption. SRBC were opsonized in 40% FCS for 30 min and labeled with $Na^{51}CrO_4$. Macrophage cultures were seeded with the opsonized ^{51}Cr-SRBC at a SRBC: macrophage ratio of 100:1 and co-incubated for 3 hr. An inoculum of macrophages (1×10^7) containing a uniform radioactivity (control = experimental) was injected into control Balb/c mice. Peak splenic PFC formation (day 4) was determined. Data are presented as the mean ± SEM, where an asterisk (*) denotes significance at $p < 0.05$; $n = 10$ throughout.

specific cellular lesion being responsible for xenobiotic-induced immune dysfunction may be naive. Nonetheless, for experimental purposes the immune system does lend itself to dissection of its cellular components and in this way discrete cellular functions may be ascertained.

An attempt has been made to conceptualize the possible modes of action of environmental chemical contaminants relative to their influence on immune parameters. As can be seen in Figure 1, we feel the chemicals may act either directly or indirectly on immunocompetent cells inducing a stimulation or suppression, which may be time- and dose-related. The direct influence may be via plasma membrane alterations, molecular interference, or by cytotoxic actions. Indirect actions via metabolic perturbations mediated by altered nutritional and/or endocrine parameters must also be considered.

In the present study, an assessment of *in vivo* and *in vitro* macrophage function was conducted. The overall design was directed toward evaluating what we felt would be a measure of affector and effector macrophage function using *in vitro* and *in vivo* studies.

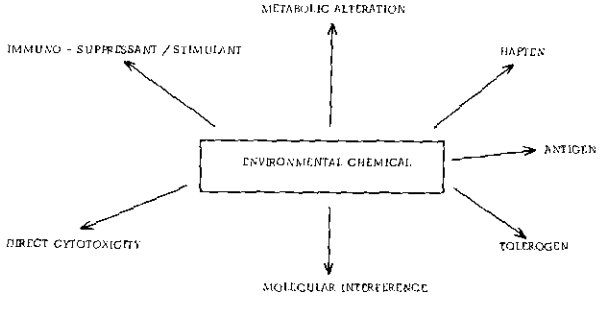

FIGURE 1. Possible roles of environmental chemicals in induction of immune alterations.

Oxygen consumption as a concomitant of the phagocytic event has been utilized as an index of the metabolic status of the macrophage. The respiratory burst which occurs during phagocytosis is energy dependent (*47*). The demonstration that isolated macrophages from chemical-treated mice have no alteration in either their resting O_2 consumption or in the O_2 consumption during phagocytosis suggests that the phagocytic event is not impaired (Table 1). In support of this observation is the demonstration that these cells have no alteration in their phagocytic activity or phagocytic capacity (Table 2). However, the *in vitro* phagocytic and O_2 utilization studies used particles opsonized with fetal calf serum which would not allow a detection of a serum defect. In this regard, the serum concentration of fibronectin, an α_2 opsonic surface-binding glycoprotein, was shown to be decreased (Table 3). Therefore, the decreased vascular clearance of the ^{51}Cr-labeled SRBC (Table 9) and its altered tissue distribution may be due to a serum opsonin (fibronectin) deficit rather than a phagocytic defect in the macrophage. The utilization of control serum and treated cells and vice versa (treated serum and treated cells) allows a discrimination between a cellular defect versus a humoral defect in an evaluation of macrophage phagocytosis.

The phagocytic clearance capability of macrophages that comprise the majority of the reticuloendothelial system (i.e., hepatic Kupffer cells and splenic macrophages) has classically been measured by the vascular clearance of gelatinized colloid. The clearance capacity of this system of sessile phagocytes toward gelatin-stabilized colloid has been shown to not only depend upon delivery of colloid to the RES or the innate phagocytic capability of the cells but also upon a specific plasma protein for-

nr :or or opsonic α_2 surf glycoprotein (*35, 36*). Recently this protein has been shown to be identical to a plasma protein originally called cold-insoluble globulin, but now more commonly called plasma fibronectin (*36*).

Studies investigating the participation of the RES in host defense have demonstrated that RES depression following traumatic injury, sepsis, or during tumor growth (*48*) is associated with decreased amounts of circulating opsonic fibronectin or a depression in the ability of fibronectin in the plasma to mediate *in vitro* macrophage uptake of test colloids. Thus, the presence of adequate levels of circulating opsonic fibronectin is important in the maintenance of host defense mechanisms that are dependent upon RES macrophage function. The site of synthesis of this important plasma protein has yet to be determined. However, tissue culture studies have revealed that a variety of cell types of mesenchymal origin synthesize a glycoprotein associated with the cell surface which mediates cell attachment to substratum. This glycoprotein is antigenically related to plasma fibronectin and is significantly decreased on the fibroblast surface following oncogenic transformation of the cells (*49*). Thus, loss of growth control observed in transformed cell lines may in part be dependent upon an inability to synthesize adhesive fibronectin. Although the cell associated form of fibronectin and that found in the circulation are antigenically related, the two forms of the molecule are not identical. The plasma form and cellular form differ with respect to certain structural and functional properties (*49*). The majority of the cell surface form exists in a polymerized form while that in the plasma is mainly dimeric in nature consisting of two disulfide crosslinked monomers, each with a molecular weight of 220,000. The protein found in fibroblast-conditioned medium, although consisting of a high proportion of the dimeric form, is much more active than the plasma form in the agglutination of fixed sheep red blood cells. Other studies have demonstrated slight differences in molecular weight between cell and plasma form which may suggest that the cell surface form is a precursor to the plasma form (*50*).

Studies from this laboratory have demonstrated that the toxicological agents polychlorinated biphenyl (PCB) and hexachlorobenzene (HCB), which are common environmental chemical contaminants, result in the depression of plasma fibronectin levels in mice that was temporally associated with an increase in the sensitivity of the mice to the mKSA ascites tumor (*51*). This was not due to a general decrease in plasma protein synthesis or to hemodilution, since there were no alterations in nese *in vivo* alterations in host defense there was an inverse relationship between plasma fibronectin levels and spontaneous macrophage spreading as measured *in vitro*. It can therefore be hypothesized that certain environmental toxicological agents may depress synthesis or enhance degradation of plasma opsonic fibronectin thus compromising RES host defense mechanisms by severely limiting macrophage phagocytic clearance function.

In the present study, *in vivo* host defense was evaluated by assessing the response of the treated mice to an injection of a tumor implant. The hypothesis that macrophages play a vital function in tumor rejection is now supported by many investigators. This was initially based upon the evidence of tissue histiocytes in animal tumors and the tumoricidal activity of activated macrophages. Furthermore, stimulation of the RES with immunological adjuvants or with a variety of chronic infections has been shown to enhance the resistance of animals to unrelated infections as well as to tumors (*52*). This resistance has been shown to be mediated by activated macrophages. Macrophage tumoricidal activity does not require phagocytosis of the tumor cell but does parallel the level of protein synthesis (*53, 54*) and has been correlated with enhanced microbicidal activity in activated macrophages (*55, 56*). The significantly impaired resistance to challenge with various types of tumor cell lines (Tables 4-7) which was demonstrated in HCB and dieldrin exposed mice may be a reflection of an altered tumoricidal activity of macrophages and/or lymphocytes from the xenobiotic-treated mice.

To assess this possibility, spleen cell tumoricidal activity was evaluated using a total spleen cell preparation from PCB, HCB and dieldrin treated mice as well as a spleen cell preparation that contained mainly either adherent cells (macrophages ?) or nonadherent cells (lymphocytes ?). The unfractionated spleen cell population as used in the present study has similarly been used to detect natural killer (NK) cells which have been suggested to play a role in resistance to some tumors *in vivo* (*57*). NK cells have been demonstrated to be distinct from activated macrophages in physical characteristics, target selectivity, genotype distribution and the mechanism of cytolysis (*57*). However, since *in vivo* treatment with antimacrophage agents, such as silica and carrageenan, depress NK activity *in vitro* and since macrophages and NK cells are relatively radioresistant, an interaction between NK and macrophages in their tumoricidal activity may be occurring *in vivo* (*57*). The absence of any alteration in the unfractionated spleen cell

cate the absence of any alteration in NK cell activity. However, such was not the case in HCB and dieldrin-exposed mice in that both groups, dieldrin more so than HCB, had a significant depression in tumor cell killing ability. However, when the spleen cells were fractionated into adherent and nonadherent populations it appeared that the adherent cell population was the cell type influenced most by HCB, whereas the target cell for dieldrin appeared to be the nonadherent spleen cell. Of interest was the observation of a slight stimulation of nonadherent cell tumoricidal capability in PCB-exposed mice and a depression in adherent cell cytocidal capacity. Since NK cells as well as activated macrophages may kill target cells by an antibody-dependent as well as an antibody-independent means (*58*, *59*), the present results may suggest that NK cells are the target cells which are impaired in dieldrin-treated mice and that macrophages, perhaps activated *in vitro* by surface adsorption, are the target cells which are impaired in HCB-treated mice and PCB-treated mice. Since it is possible that NK activity is modulated by macrophages, the use of the beige mouse mutant which has impaired NK activity (*60*) but not macrophage activity may be useful in dissecting the influence of the xenobiotics on NK versus macrophage tumor cell killing.

Since it has been demonstrated that macrophage processing of antigen may be an important determinant for tumor immunity (*61*), the present study examined antigen processing ability of diverse populations of macrophages from dieldrin-exposed mice. In this study, it was discovered that macrophages from dieldrin-treated mice which were allowed to ingest a standard antigen load manifested a significant impairment in their ability to transfer an adequate immunogen to naive control mice. Although peritoneal and splenic macrophages demonstrated the greatest degree of suppression (approximately 5-fold) at the highest dose of dieldrin (50 ppm), splenic and alveolar macrophages were the most sensitive macrophage types in that they manifested a decreased response at the lowest dieldrin dose.

Perhaps diversion of lysosomal enzymes away from antigen digestion and/or the induction of a population of macrophages (suppressor ?, scavenger ?) unable to induce an antibody-forming response in recipient mice may account for the depressed response in the animals which received the transferred macro phages. These results, in conjunction with the previously described studies, may be interpreted to indicate either that the effector but not affector function of the macrophage the result of a selective induction of a population of macrophages, normally heterogeneous in function, which are now homogenous in function.

REFERENCES

1. Koller, L. D., and Thigpen, J. R. Biphenyl-exposed rabbits. Am. J. Vet. Res. 34: 1605 (1973).
2. Vos, J. G. Immune suppression as related to toxicology. CRC Crit. Rev. Toxicol. 5: 67 (1977).
3. Loose, L. D., Pittman, K. A., Benitz, K.-F., Silkworth, J. B., Mueller, W., and Coulston, F. Environmental chemical-induced immune dysfunction. Ecotox. Environ. Safety 2: 173 (1978).
4. Shtenberg, A. I., and Dzhunusova, R. M. Depression of immunobiological reaction of an animal under the influence of certain organic phosphorous pesticides. Bull. Exptl. Biol. Med. U.S.S.R. 33: 86 (1968).
5. Shtenberg, A. I., Ashmenaskas, Y. I. and Kusavasky, I. A. Immunobiological reactivity changes under the influence of some pesticides belonging to the group of carbamate and dithiocarbamate compounds. Vop. Pitan. (31) 1: 58 (1972).
6. Koller, L. D. Immunosuppression produced by lead, cadmium and mercury. Am. J. Vet. Res. 34: 1457 (1973).
7. Treagen, L. Metals and the immune response; a review. Chem. Pathol. Pharmacol. 12: 189 (1975).
8. Koller, L. D., Exon, J. H., and Roan, J. G. Humoral antibody response in mice after single dose exposure to lead or cadmium. Proc. Soc. Exptl. Biol. Med. 151: 339 (1976).
9. Archer, D. L. Immunotoxicology of foodborne substances: an overview. J. Food Prot. 41: 983 (1978).
10. Thigpen, J. E., Faith, R. E., McConnell, E. E., and Moore, J. A. Increased susceptibility to bacterial infections as a sequela of exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Infect. Immun. 12: 1319 (1975).
11. Gainer, J. J., and Pry, T. W. Effect of arsinicals on viral infections in mice. Am. J. Vet. Res. 33: 2299 (1972).
12. Loose, L. D., Silkworth, J. B., Pittman, K. A. Benitz, K.-F., and Mueller, W. Impaired host resistance to endotoxin and malaria in polychlorinated biphenyl- and hexachlorobenzene-treated mice. Infect. Immun. 20: 30 (1978).
13. Loose, L. D., Silkworth, J. B., Mudzinski, S. P., Pittman, K. A., Benitz, K.-F., and Mueller, W. Modification of the immune response by organochlorine xenobiotics. Drug Chem. Toxicol. 2: 111 (1979).
14. Dean, J. H., Padarathsingh, M. L., Jerrells, T. R., Keys, L., and Northing, J. W. Assessment of immunobiological effects induced by chemicals, drugs, or food additives. II. Studies with cyclophosphamide. Drug Chem. Toxicol. 2: 133 (1979).
15. Hamilton, H. E., Morgan, D. P., and Simmons, A. A pesticide (dieldrin)-induced immunohemolytic anemia. Environ. Res. 17: 155 (1978).
16. Ward, M. Immunological mechanisms in the pathogenesis of vinyl chloride disease. Brit. Med. J. 1: 936 (1976).
17. Authrup, H., Harriss, C. C., Stoner, G. D., Selkirk, J. K., Schafer, P. W., and Trump, B. F. Metabolism of (^{3}H) benzo(a)pyrene by cultured human bronchus and cultured human pulmonary alveolar macrophages. Lab. Invest. 38: 217 (1978).
18. Wasserman, M., Wasserman, D., Kedar, E., and Djavaherian, M. Immunological and detoxification interactions in *p,p'*-DDT-fed rabbits. Bull. Environ. Contam. Toxicol. 6: 426 (1971).
19. Biozzi, G., Halpern, B. N., Benacerraf, B., and Stiffel, C.

Symposium , p. 204.
20. Mims, C. A. Aspects of the pathogenesis of virus diseases. Bacteriol. Rev. 28: 30 (1964).
21. Linton, R. W. Reticulo-endothelial system in protozoan infections. Arch. Pathol. 8: 488 (1929).
22. Talioferro, W. H. The Immunology of Parasitic Infections. Century Publishing Co., New York, 1929.
23. Goble, F. C., and Singer, I. The reticuloendothelial system in experimental malaria and trypanosomiasis. Ann. N.Y. Acad. Sci. 88: 149 (1960).
24. Thorbecke, G. J., and Benacerraf, B. The reticuloendothelial system and immunological phenomena. Progr. Allergy 6: 559 (1962).
25. Schwartz, R. S., Ryder, R. J. W., and Gottlieb, A. A. Macrophages and antibody synthesis. Progr. Allergy 14: 81 (1970).
26. Fishman, M., Hamerstrom, R. A., and Bond, V. P. *In vitro* transfer of macrophage RNA to lymph node cells. Nature 198: 549 (1963).
27. Franzl, R. E., and McMaster, P. D. The primary immune response in mice. I. The enhancement and suppression of hemolysin production by a bacterial endotoxin. J. Exptl. Med. 127: 1087 (1968).
28. Frei, P. C., Benacerraf, B., and Thorbecke, G. J. Phagocytosis of the antigen, a crucial step in the induction of the primary response. Proc. Natl. Acad. Sci. (U.S.) 53: 20 (1965).
29. Mills, G., Monticone, V., and Paetkau, V. The role of macrophages in thymocyte mitogenesis. J. Immunol. 117: 1325 (1976).
30. Rosenstreich, D. L., Farrar, J. J., and Dougherty, S. Absolute macrophage dependency of T-lymphocyte activation by mitogens. J. Immunol. 116: 131 (1976).
31. Tagliabue, A., Mantovani, A., Kilgallen, M., Herberman, R. B., and McCoy, J. L. Natural cytotoxicity of mouse monocytes and macrophages. J. Immunol. 122: 2363 (1979).
32. Silkworth, J. B., and Loose, L. D. Assessment of environmental contaminant-induced lymphocyte dysfunction. Environ. Health Perspect. 39: 105 (1981).
33. Loose, L. D., Silkworth, J. B., and Warrington, D. Cadmium-induced depression of the respiratory burst in mouse pulmonary alveolar macrophages, peritoneal macrophages and polymorphonuclear neutrophils. Biochem. Biophys. Res. Comm. 79: 326 (1977).
34. Simpson, D. W., Roth, R., and Loose, L. D. A rapid, inexpensive and easily quantified assay for phagocytosis and microbicidal activity of macrophages and neutrophils. J. Immunol. Methods 29: 221 (1979).
35. Blumenstock, F. A., Saba, T. M., Weber, P., and Laffin, R. Biochemical and immunological characterization of human opsonic α_2 SB glycoprotein identity with cold insoluble globulin. J. Biol. Chem. 253: 4287 (1978).
36. Saba, T. M. Aspecific opsonins. In: The Immune System and Infectious Disease (Proc. 4th Intern. Convocation on Immunology), S. Karger Basel, (1975), pp. 489-504.
37. Mosher, D. F. Action of fibrin-stabilizing factor in cold-insoluble and a_2 macroglobulin in clotting plasma. J. Biol. Chem. 251: 1639 (1976).
38. Mosesson, M. W. Cold-insoluble globulin (Clg): a circulating cell surface protein. Thrombosis Hemostatis 38: 742 (1977).
39. Litchfield, J. T., Jr. A method for graphic solution of time-percent effect curves. J. Pharmacol. Exptl. Therap. 49: 399 (1949).
40. Canty, T. G., and Wunderlich, J. R. Quantitative *in vitro* assay of cytotoxic cellular immunity. J. Natl. Cancer Inst. 45: 761 (1970).
ar cytolysis macrophages and granulocytes. II. Hydrogen peroxide as a mediator of cytotoxicity. J. Exptl. Med. 149: 100 (1979).
42. Gray, S. J., and Sterling, K. The tagging of red cells and plasma proteins with radioactive chromium. J. Clin. Invest. 29: 1604 (1950).
43. Stege, T. E., Loose, L. D., and DiLuzio, N. R. Comparative uptake of sulfobromophthalein by isolated Kupffer and parenchymal cells. Proc. Soc. Exptl. Biol. Med. 149: 455 (1975).
44. Loose, L. D., Cook, J. A., and DiLuzio, N. R. Malaria immunosuppression - a macrophage mediated defect. Proc. Helm. Soc. Wash. 39 (special issue): 484 (1972).
45. Jerne, N. K., and Nordin, A. A. Plaque formation in agar by single antibody-producing cells. Science 140: 405 (1963).
46. Ogmundsdottir, H. M., and Weir, D. M. Mechanisms of macrophage activation. Clin. Exp. Immunol. 40: 223 (1980).
47. Sbarra, A. J., and Karnovsky, M. L. The biochemical basis of phagocytosis. I. Metabolic changes during the ingestion of particles by polymorphonuclear leukocytes. J. Biol. Chem. 234: 1355 (1959).
48. Saba, T. M. Physiology and physiopathology of the reticuloendothelial system. Arch. Int. Med. 126: 1031-1052 (1970).
49. Vaheri, A., Ruoslahti, E., Westermark, B., and Ponten, J. A common cell-type specific surface antigen (SF) in cultured human glial cells and fibroblasts: loss in malignant cells. J. Exptl. Med. 143: 64 (1976).
50. Yamada, K. M., and Kennedy, D. W. Fibroblast cellular and plasma fibronectins are similar but not identical. J. Cell Biol. 80: 492 (1979).
51. Loose, L. D., Mueller, W., Charbonneau, T., and Blumenstock, F. Influence of xenobiotics on host defense. In: The Biological Relevance of Immune Suppression as Induced by Genetic, Therapeutic and Environmental Factors. J. Dean and M. Padarathsignth, Eds., Van Nostrand-Reinhold, in press.
52. Hibbs, J. B., Lambert, L. H., and Remington, J. S. Macrophage mediated nonspecific cytotoxicity--possible role in tumour resistance. Nature 235: 48 (1972).
53. Keller, R., Keist, R., and Ivath, R. J. Functional and biochemical parameters of activation related to macrophage cytostatic effects on tumor cells. Intern. J. Cancer 14: 675 (1974).
54. Sharma, S. D., and Piessens, W. F. Tumor cell killing by macrophages activated *in vitro* with lymphocyte mediators. II. Inhibition by inhibitors of protein synthesis. Cell. Immunol. 38: 264 (1978).
55. Chapman, H. A., Jr., and Hibbs, J. B., Jr. Modulation of macrophage tumoricidal capability by components of normal serum: A central role for lipid. Science 197: 282 (1977).
56. Loose, L. D., Astry, C. L., and Megirian, R. Dose-dependent macrophage activation by *Corynebacterium parvum*. J. Immunopharmacol., in press.
57. Roder, J. C., Lohmann-Matthes, M.-L., Domzig, W., Kiessling, R., and Holler, O. A functional comparison of tumor cell killing by activated macrophages and natural killer cells. Eur. J. Immunol. 9: 283 (1979).
58. Ojo, E., Haller, O., and Wigzell, H. *Corynebacterium parvum*-induced peritoneal exudate cells with rapid cytolytic activity against tumor cells are non-phagocytic cells with characteristics of natural killer cells. Scand. J. Immunol. 8: 215 (1978).
59. Macfarlan, R. I., Burns, W. H., and White, D. O. Two cytotoxic cells in peritoneal cavity of virus-infected mice: Antibody-dependent macrophages and nonspecific killer cells. J. Immunol. 119: 1569 (1977).

selectively impairs natural kill cell function. Nature 278: 451 (1979).

antigen for induction of tumor immunity. Cancer Res. 37: 1838 (1977).