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 xenobiots 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 \textit{in vitro} O_{2} consumption while at rest or during phagocytosis. In addition, no alteration in \textit{in vitro} phagocytic activity, phagocytic capacity or microbicidal activity was demonstrated. However, a significant impairment in the \textit{in vitro} 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 \( \alpha_{2} \) 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 xenobiots, 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
the possible induction of auto-immune diseases. 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 hapten, 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 parasites 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 cells both specifically and nonspecifically has been 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 × 10⁶ 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 × 10⁷ cells/mouse.

Elicited polymophonuclear 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 × 10⁷ 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
Animals were pooled and adjusted to 0.5 x 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 macrophages 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 x 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 x 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 (32). A volume of 1.6 ml containing 2.0 x 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 x 10^4 M) were placed into sterile polypropylene 12 mm x 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 discarded. The cell pellet was resuspended in 20-50 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:

Phagocytic capacity =
\[
\text{number of live and dead yeast ingested} / 100 \text{ cells}
\]

Phagocytic activity =
\[
\text{number of cells containing > 2 yeast} / 100 \text{ cells}
\]

Microbicidal activity =
\[
\text{number of dead ingested yeast} / \text{phagocytic capacity}
\]

**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 solidified agarose–antiserum solution layer on the glass plate (5 x 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.
concentration. Rocket heights were recorded in 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 α2SB glycoprotein in μg/ml (38).

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-forming 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 C57Bl/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 isogenic mice. The following doses of live tumor cells were used: P388, 0.5 × 10⁶; EL-4, 2 × 10⁶; mKSA, 1 × 10⁷; and L1210, 0.5 × 10⁶. 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 assayed in vitro by ⁵¹Cr-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% CO₂. Cytotoxicity was calculated by the method of Nathan et al. (41) as follows:

\[
\text{Treated } ⁵¹\text{Cr release} - \text{Control } ⁵¹\text{Cr release} \\
\text{Maximum } ⁵¹\text{Cr release} - \text{Control } ⁵¹\text{Cr release} = \% \text{ Cytotoxicity}
\]

Maximum release of the ⁵¹Cr 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 aseptically and then teased through a 10XX silk cloth. Adherent cells were obtained by incubating approximately 1 × 10⁹ 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% CO₂ 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 ⁵¹Cr-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 Na₂⁵¹CrO₄. The ⁵¹Cr-SRBC were prepared by the technique of Gray and Sterling (42). After IV injection of the ⁵¹Cr-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 ⁵¹Cr-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).

Environmental Health Perspectives
Sheep erythrocytes were opsonized in 40% FCS for 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 $1 \times 10^7$ 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 $\pm$ SEM.

**Results**

**Oxygen Consumption, Phagocytic and Microbicidal 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 were altered but not in any temporal pattern. The 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 chemotherapy 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

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**Table 1. Influence of PCB 1242 and HCB on oxygen utilization by macrophages and neutrophils.**

| Time, weeks | Control | PCB 1242 | HCB |
|-------------|---------|----------|-----|
|              | Alveolar macrophages | Splenic macrophages | Peritoneal macrophages | PMN's |
|              | Resting | % Burst | Resting | % Burst | Resting | % Burst | Resting | % Burst |
| 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 |
|              | 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 |
| 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 | 20.4 ± 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 |

*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 $\pm$ SEM and significance at $p < 0.05$ indicated by an asterisk. All data points consist of $\geq$ 9 individual studies.

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| Times, weeks | Phagocytic capacity | Phagocytic activity | Microbicidal activity |
|-------------|---------------------|---------------------|----------------------|
|             | C       | PCB  | HCB  | C       | PCB  | HCB  | C       | PCB  | HCB  |
| Alveolar    |         |      |      |         |      |      |         |      |      |
| 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     |         |      |      |         |      |      |         |      |      |
| macro-      |         |      |      |         |      |      |         |      |      |
| 3           | 99.7 ± 7.7 | 96.2 ± 16.1 | 110.5 ± 9.0 | 63.3 ± 4.6 | 57.6 ± 5.9 | 74.7 ± 6.2 | 8.9 ± 1.4 | 6.8 ± 2.1 | 7.7 ± 2.5 |
| 6           | 95.0 ± 6.8 | 95.0 ± 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  |         |      |      |         |      |      |         |      |      |
| macro-      |         |      |      |         |      |      |         |      |      |
| 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 | 327.0 ± 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 | 287.5 ± 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           | 201.5 ± 22.4 | 204.0 ± 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 | 33.6 ± 2.1 |

* 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 = 9; C = Control.
Table 3. Serum fibronectin concentrations in mice fed PCB 1242, HCB, or dieldrin.*

| 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     |          |
| 5 ppm     | 107     | 100     | 51*      |

*aPresented as % of control mean with an asterisk (*) denoting significance at p < 0.05; n = 7-10 for each group.

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

| 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*    |

*aAscites 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 18 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.

Table 5. Mean survival time in mice fed PCB 1242, HCB, or dieldrin and challenged with EL-4 tumor cells.*

| 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*     |

*aAscites 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.

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

| 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*     |

*aAscites tumor cells P388 (0.5 × 10^8) 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.

L 1210) both have H-2d and Mls1 antigens whereas the Balb/c tumor mKSA has an H-2d and Mls2 antigen and the C57B1/6 tumor line EL-4 has an H-2b and Mls2 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 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 fed PCB 1242, HCB, or dieldrin and challenged with L 1210 tumor cells.*

| Treatment | Mean survival time, days |
|-----------|-------------------------|
|           | 3 weeks | 6 weeks | 18 weeks |
| Control 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     | 17.7    | 16.0    | 14.6*   |
| 5 ppm     | 12.1*   | 10.2*   | 5.1*    |

*Ascites tumor cells L 1210 (0.5 × 10⁸) 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.

Table 8. Influence of PCB 1242, HCB, and dieldrin on splenic cytotoxic activity against mKSA tumor cells.*

| Cytotoxicity, % of control |
|----------------------------|
| Time, weeks | 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* |

*The cytotoxic activity of a total spleen cell preparation against ⁵⁷Cr-mKSA tumor cells was assessed using isolated spleen cells from non-tumor-bearing mice; 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 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).

Table 9. Influence of PCB 1242, HCB, and dieldrin on splenic nonadherent cell killing of mKSA tumor cells.*

| Cytotoxicity, % of control |
|----------------------------|
| Time, weeks | 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* |

*The cytotoxic activity of nonadherent spleen cell against ⁵⁷Cr-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.
Table 10. Influence of PCB 1242, HCB, and dieldrin on splenic adherent cell killing of mKSA tumor cells.a

| Time, weeks | PCB 5 ppm | 100 ppm | HCB 5 ppm | 100 ppm | Dieldrin 1 ppm | 5 ppm |
|-------------|-----------|---------|-----------|----------|----------------|------|
| 3           | 97        | 82*     | 75*       | 50*      | 94             | 83*  |
| 6           | 97        | 80*     | 60*       | 27*      | 96             | 80*  |
| 18          | 94        | 76*     | 62*       | 11*      | 93             | 80*  |

aThe cytotoxic activity of adherent spleen cell against 51Cr-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.

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 spleenic 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 51Cr-SRBC in dieldrin-treated mice.a

| Dieldrin, ppm | t1/2, min | Liver | Lung | Spleen |
|---------------|-----------|-------|------|-------|
|               |           | % ID/g | % ID/TO | % ID/g | % ID/TO | % ID/g | % ID/TO |
| 0             | 5.7 ± 0.2 | 22.9 ± 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 | 68.9 ± 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* |

aMice were maintained on diet for 2 weeks after which all mice received 0.1 cc of 20% SRBC labeled with Na51Cro4 containing 7.05 µCi.

bTissue distribution of the 51Cr-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.

cVascular 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 t1/2 calculated.

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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 | Peritoneal macrophages | Splenic macrophages | Alveolar macrophages | Kupffer cells |
|--------------|------------------------|---------------------|----------------------|--------------|
| 0            | 34,651 ± 1,477         | 43,889 ± 3,871      | 22,207 ± 1,970       | 17,041 ± 976 |
| 0.5          | 29,020 ± 1,641         | 33,298 ± 2,710*     | 15,001 ± 1,422*      | 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* |

aBalb/c male mice were maintained on dieldrin-containing diets for 2 weeks.
bPeritoneal 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 with *asterisk* co-incubated for naive.

The 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 ascertainment.

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](image.png)

**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 suggest 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 51Cr-labeled SRBC (Table 9) and its altered tissue distribution may be due to a serum opsonin (fibronectin) defect 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-
merly designated as specific opsonin, humoral recognition factor or opsonic \( \alpha_2 \) surface binding (SB) 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 \textit{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 of 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 other plasma protein levels. In association with these \textit{in vivo} alterations in host defense there was an inverse relationship between plasma fibronectin levels and spontaneous macrophage spreading as measured \textit{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, \textit{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 \textit{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 \textit{in vivo} treatment with antimacrophage agents, such as silica and carrageenan, depress NK activity \textit{in vitro} and since macrophages and NK cells are relatively radioreistant, an interaction between NK and macrophages in their tumoricidal activity may be occurring \textit{in vivo} (57). The absence of any alteration in the unfractionated spleen cell
tumoricidal activity in PCB-treated mice may indicate 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 cytoidal capacity. Since NK cells as well as activated macrophages may kill target cells by an antibody-dependent means 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 macrophages. These results, in conjunction with the previously described studies, may be interpreted to indicate either that the effector but not afferent function of the macrophage is impaired by xenobiotics and/or that this may be the result of a selective induction of a population of macrophages, normally heterogeneous in function, which are now homogenous in function.

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