

Macrophage Induction of T-Suppressor Cells in Pesticide-Exposed and Protozoan-Infected Mice

by Leland D. Loose*

The use of infectious pathogens has allowed the detection of the development of synergism between pathogens and ubiquitous environmental chemical contaminants. This synergism has been demonstrated to result in a state of immunosuppression which either did not occur in the independent and singular presence of the chemical or pathogen and/or was greater than additive when both were combined. The immunosuppression was distinct with regard to the organochloride used and, therefore, is not a ubiquitous characteristic of all organohalides. The production of a macrophage soluble factor which appeared to induce T-suppressor cells was demonstrated in hepatic Kupffer cells from mice administered 5 ppm of dieldrin for 10 weeks and then infected with *Leishmania tropica* promastigotes. The factor was not generated in mice administered dieldrin and infected with malaria nor in mice administered only dieldrin nor in mice only infected with *Leishmania*. Additional studies revealed a profound impairment in macrophage antigen processing with macrophages obtained from mice administered dieldrin. The use of pathogen models may allow the immunosuppressive potential of environmental chemical contaminants to be expressed in a more sensitive manner.

Introduction

Previous studies have demonstrated that environmental chemical contaminants impair host defense responses to a malaria challenge and to gram-negative endotoxin (1). In conjunction with these alterations, a significant reduction in antibody formation to sheep erythrocytes, (SRBC), a T-dependent and macrophage-processed antigen, was observed (2). These functional alterations were demonstrated in the absence of any lymphoid histopathologic changes. Further studies to examine cell-mediated immunity (CMI) in the xenobiotic treated mice revealed an essentially normal CMI responsiveness as measured by the graft-versus-host assay, mixed lymphocyte culture reaction,

polyclonal mitogen activity and lymphocytotoxicity response (3). Since it was felt that the CMI parameters which were measured and which were unaltered were, in general, macrophage-independent whereas the response to malaria, endotoxin and SRBC were macrophage-dependent, the cellular lesion responsible for the impaired malaria, endotoxin and SRBC responses could reside at the level of the macrophage.

Measurements of macrophage effector activity, i.e., cellular respiration, phagocytic activity, phagocytic capacity and chemotaxis revealed no alteration in these parameters (4). However, macrophage effector activity, i.e., antigen processing and tumor cell killing were significantly impaired (5). Therefore, it was speculated that if the cellular lesion responsible for the xenobiotic-induced immunosuppression was at the level of the macrophage, that the lesion could be due to: a functional impairment in the macrophage, induction of a population of suppressor macrophages, production of suppressor

*Albany Medical College, Albany, New York 12208. Present address: Department of Pharmacology, Pfizer Central Research, Groton, Connecticut 06340.

factors from the macrophage or a combination of one of the above.

Therefore, further studies were conducted to determine the contribution of the macrophage to xenobiotics-induced immunosuppression. The possible development of a synergistic reaction between the influence of the xenobiotics on the immune response and a concomitant protozoan infection was ascertained concurrently.

Materials and Methods

Animals

Male BALB/c mice (18-20 g) were used throughout all studies.

Chemicals

Dieldrin (Shell) and hexachlorobenzene (HCB, Eastman) were administered in the diet at 1 and 5 ppm and 5 and 100 ppm, respectively. Control mice received untreated powdered Wayne Lab Blox. Food and water were provided *ad libitum*, and a 12:12 day:night photoperiod was maintained. Dietary administration of the test chemicals was for 3, 5 or 10 week periods.

Parasites

Plasmodium berghei (NYU-2) was passaged weekly by intraperitoneal (IP) injection of 1×10^6 infected erythrocytes. The survival time of infected mice injected with this strain of murine malaria at the stated inoculum was approximately 10-12 days. *Leishmania tropica* was initially derived from a biphasic blood agar medium and then adapted to a defined culture medium consisting of MEM 199 with 10% heat inactivated fetal calf serum. Subcultures were made weekly. The parasite stage used was a promastigote in a mid- to late-log phase. Survival time of BALB/c mice injected intradermally (ID) with 1×10^6 promastigotes was approximately 15-20 weeks.

Antibody Formation

Antibody production was assessed using the direct Jerne and Nordin (6) plaque assay as previously described (2). Plaque formation to polyvinylpyrrolidone (PVP, Sigma), a T-independent antigen, utilized passively sensitized SRBC coated with PVP by the method of Boyden (7). Preliminary studies revealed that the kinetics of splenic plaque formation in experimental and control mice were compa-

able and, therefore, only day 4 was used to subsequently enumerate plaques.

Mononuclear Spleen Cell Populations

Splenic T- and B-lymphocytes were enumerated as previously described (8). T-cells were detected by the presence of theta antigen (Thy-1) and subsequent cytotoxicity induced by anti-theta serum. B-cells were detected by the presence of surface immunoglobulin (Ig) using fluorescein-tagged, goat anti-mouse Ig. Splenic macrophages were stained by the method of Yam, Li and Crosby (9), which detects the presence of surface neutral esterase, a cytochemical marker for macrophages.

Lymphocyte Blastogenesis

Spleens were removed aseptically from mice, controls and experimentals, at the stated intervals (see Tables 5-8), and teased apart in cold RPMI 1640 and strained through a 10xx silk cloth. This single cell suspension was then passaged through nylon wool (Leuko-Pak leukocyte filter, Fenwal Labs, Deerfield, Ill.) which was soaked in saline for 2 hr at 37°C then rinsed three times in doubly distilled water for 3 days, autoclaved and then dried. A 60-ml disposable syringe barrel was then packed with 6 g of nylon wool and then washed with 20 ml of RPMI 1640 containing 5% fetal calf serum. The columns were then drained and placed in a 37°C incubator for 1 hr prior to being loaded with 10^8 spleen cells in 20 ml of RPMI 1640. The column, with the cells, was incubated at 37°C for 45 min prior to eluting the cells at a 1 ml/min rate. The T-cell enriched suspension was then concentrated by centrifugation at 250 g for 15 min. Cell viability, as determined by trypan blue dye exclusion, was > 85%, and > 95% of the cells were theta positive. The enriched T-cell population was then dispensed in 200 μ l volumes containing 4×10^5 cells into Linbro flat-bottomed microtest plates (Flow Lab). The RPMI 1640 medium was supplemented with 2mM L-glutamine, 10% fetal calf serum, 50-100 units of penicillin and 50-100 μ g of streptomycin/ml. In addition, phytohemagglutinin (PHA-M, B grade, Difco) was added at a concentration of 0.1 μ g/well. The plates were incubated at 37°C in humidified 5% CO₂ for 48 hr, after which 0.1 μ Ci of ³H (specific activity 5 Ci/mmol) was added and the plates incubated for an additional 16 hr prior to harvest. Cell harvesting was conducted as previously described (3), and data are presented as cpm $\times 10^3$. Anti-theta serum treatment of the nylon wool enriched T-cell preparation utilized a 1:80 dilution of anti-Thy-1 antiserum (Cedarlane Labs) and a 1:10 dilution of

Low-Tox rabbit complement incubated together for 30 min at 37°C. Mitomycin C treatment consisted of incubating the nylon wool passaged cells (10^7 /ml) with 20 µg of mitomycin C (Sigma) for 25 min at 37°C. The cells were washed three times in RPMI 1640 prior to use in blastogenesis studies (3).

Macrophage Assay

Alveolar, splenic and peritoneal macrophages were isolated as previously described (4). Briefly, alveolar macrophages were isolated by lavage of the lung with saline, peritoneal macrophages were isolated by saline lavage of the peritoneal cavity, and splenic macrophages were isolated by surface adsorption. Hepatic Kupffer cells were isolated by partial enzymatic digestion of the liver as described by Stege et al. (10). The macrophage preparations were incubated overnight in RPMI 1640 plus 10% FCS in a 5% CO₂ humidified atmosphere at a cell concentration of 1×10^7 cells/culture plate. The following day the cell supernatant was decanted off and centrifuged at 250 g for 20 min to remove cell debris. A 50 µl aliquot of this supernatant was then added to the T-cell blastogenesis cultures, replacing 50 µl of the RPMI 1640 medium. PHA-induced blastogenesis studies were then conducted as described above.

Macrophage antigen processing of SRBC, a T-dependent antigen which requires macrophage cooperation for immunogenicity, was conducted as previously described (5).

Statistics

All data are reported as the mean with significance denoted by an asterisk where $p < 0.05$. Significance was determined by using the Student's *t* test. Significance for mortality data was calculated using the time-effect evaluation of Litchfield (11).

Results

As can be seen in Table 1, the addition of either 1 or 5 ppm of dieldrin to the diet of mice exacerbated the lethality induced by a challenge infection with either the malaria parasite *Plasmodium berghei* (NYU-2) or *Leishmania tropica*. The lethality synergized by dieldrin and the parasites appeared to be both time and dose related. However, mice administered 5 or 100 ppm of HCB and then challenged with malaria or Leishmania expressed a synergy only with Leishmania and not malaria. The mortality induced by Leishmania was more marked in dieldrin-exposed mice than in HCB-exposed mice.

When the dieldrin and HCB-treated mice which were infected with malaria or Leishmania and then evaluated for their ability to produce antibody to SRBC, a suppressive synergism was noted primarily in HCB-treated mice (Table 2). In these mice, the splenic plaque forming response was more suppressed in the combination of HCB + infected mice than in those only HCB-treated or only infected. In dieldrin-treated mice, a synergism was observed only in mice which received 5 ppm of dieldrin plus infection with Leishmania.

Although a suppressed SRBC response was noted in HCB-treated mice infected with either malaria or Leishmania, a decreased antibody formation to PVP, a T-independent antigen, was observed only in Leishmania-infected mice exposed to dieldrin or HCB. Malaria did not act synergistically with dieldrin or HCB to elicit an impaired response to PVP (Table 3). The decreased antibody formation to PVP in dieldrin- and HCB-treated mice infected with Leishmania was time- and dose-related in dieldrin-treated mice but was only time-related in HCB-treated mice.

An examination of the number of splenic T-cells, B-cells and macrophages revealed no consistent significant alterations in the cell types in either

Table 1. Mortality in *Plasmodium berghei* and *Leishmania tropica* infected mice administered dieldrin or HCB.*

Weeks	Mean survival time, days ^b							
	Dieldrin, 1 ppm		Dieldrin, 5 ppm		HCB, 5 ppm		HCB, 100 ppm	
	Malaria	Leishmania	Malaria	Leishmania	Malaria	Leishmania	Malaria	Leishmania
3	10.4	123.5	10.8	80.8*	12.0	97.6*	11.1	73.7*
6	10.6	81.2*	7.2*	57.7*	11.9	83.1*	9.8	60.0*
10	7.1*	53.9*	4.7*	23.0*	10.8	75.6*	7.0*	41.1*
Control:	12.3	119.0						

*Male BALB/c mice (18-20 g) were administered dieldrin or HCB at 1 and 5 or 5 and 100 ppm, respectively, for 3, 6 or 10 weeks and then challenged with either 1×10^5 *Plasmodium berghei* (NYU-2) infected erythrocytes (IP) or 1×10^6 *Leishmania tropica* promastigotes injected ID.

^bMean survival time (days) is denoted for experimental and control mice and significance is indicated by an asterisk where $p < 0.05$; $n = 12$ per group. Following injection of the parasite(s) all dietary administration of HCB or dieldrin ceased.

Table 2. Direct splenic plaque formation to SRBC in mice infected with malaria or Leishmania with administration of dieldrin or HCB.^a

Time, weeks	Chemical treatment		Splenic PFC/10 ⁶ cells		
	Agent	Concn, ppm	No infecting organism	Malaria	Leishmania
—	None	—	255 ^b	107 ^c	186 ^c
3	Dieldrin	1	240 ^d	128	190
		5	137 ^d	91	76
6	Dieldrin	1	200 ^d	117	133
		5	91 ^d	72	43
10	Dieldrin	1	107 ^d	99	101
		5	50 ^d	38	21
3	HCB	5	280 ^d	148	107
		100	172 ^d	72	58
6	HCB	5	248 ^d	109	82
		100	103 ^d	40	37
10	HCB	5	176 ^d	77	49
		100	69 ^d	19	15

^aSplenic PFC/10⁶ cells determined on day 4 following 10 infection of 0.1 ml of 10% SRBC. Malaria-infected mice (1 × 10⁶ parasites, IP) were immunized on day 3 post infection, and Leishmania infected mice (1 × 10⁶ promastigotes, ID) were immunized 20 days post infection.

^bControl consisting of untreated but immunized mice.

^cControl consisting of PFC responses in infected mice receiving no chemical treatment.

^dControl consisting of chemically treated, on infected mice.

chemical treated, infected or chemical-treated plus infected mice as compared to control values (Table 4). The only statistically significant alteration occurred in the 5 ppm dieldrin-treated plus Leishmania-infected mice at the 10 week exposure period in which a significant decrease in macrophages, T-cells and B-cells was observed as compared to normal controls, infected animals, or 5 ppm dieldrin at the 10 week exposure period.

In contrast to the apparent absence of any consistent alteration in T-cell numbers in chemical-treated

plus infected mice, a significant depression in HCB and dieldrin-treated plus Leishmania-infected mice to the T-cell mitogen, PHA, was demonstrated (Table 5). Isolated splenic T-cells from Leishmania-infected mice which had been administered dieldrin or HCB had a significantly depressed response to the polyclonal T-cell mitogen PHA. Neither dieldrin or HCB alone or malaria or Leishmania infection alone caused any change in the splenic T-cell enriched population response to PHA. Furthermore, malaria infection in dieldrin or HCB-treated mice

Table 3. Direct splenic plaque formation to PVP in mice infected with malaria or Leishmania with administered dieldrin or HCB.^a

Time, weeks	Chemical treatment		Splenic PFC/10 ⁶ cells		
	Agent	Concn, ppm	No infecting organism	Malaria	Leishmania
—	None	—	287 ^b	180 ^c	267 ^c
3	Dieldrin	1	290	205	199
		5	250	222	133
6	Dieldrin	1	277	227	150
		5	233	197	99
10	Dieldrin	1	249	173	93
		5	200	172	42
3	HCB	5	269	293	174
		100	241	214	142
6	HCB	5	248	254	129
		100	223	188	101
10	HCB	5	257	231	87
		100	189	153	76

^aPVP (Sigma) was administered IP at a dose of 2.5 µg/mouse to mice infected with *P. berghei* (1 × 10⁵ parasites, IP) on day 4 post-immunization and to mice infected with *L. tropica* (1 × 10⁶ promastigotes, ID) on day 20 post infection.

^bUntreated, uninfected mice.

^cDirect plaques were enumerated on day 4 post immunization, n = 12.

Table 4. Mononuclear (T, B, Mφ) cells in mice infected with malaria or Leishmania and receiving dieldrin or HCB.^a

Time, weeks	Chemical treatment		Infecting organism	Mononuclear cells		
	Agent	Concn, ppm		T, %	B, %	Mφ, %
-	None	-	None	30	51	12
-	None	-	Malaria	34	60	15
-	None	-	Leishmania	31	58	13
3	Dieldrin	1	None	33	54	15
			Malaria	33	50	12
			Leishmania	30	50	15
3	Dieldrin	5	None	20	49	18
			Malaria	24	56	10
			Leishmania	32	47	10
6	Dieldrin	1	None	29	48	12
			Malaria	30	52	15
			Leishmania	24	41	12
6	Dieldrin	5	None	30	42	10
			Malaria	27	57	12
			Leishmania	24	40	8
10	Dieldrin	1	None	37	50	12
			Malaria	27	46	9
			Leishmania	23	37	10
10	Dieldrin	5	None	27	40	14
			Malaria	20	60	12
			Leishmania	19	41	5
3	HCB	5	None	30	49	12
			Malaria	32	54	12
			Leishmania	33	50	10
3	HCB	100	None	27	57	14
			Malaria	31	50	15
			Leishmania	30	46	9
6	HCB	5	None	28	51	15
			Malaria	32	51	14
			Leishmania	37	49	11
6	HCB	100	None	25	54	11
			Malaria	31	46	12
			Leishmania	29	40	11
10	HCB	5	None	34	47	10
			Malaria	30	47	16
			Leishmania	28	47	9
10	HCB	100	None	27	42	13
			Malaria	26	40	11
			Leishmania	27	48	9

^aMononuclear splenic cells (T-cells, B-cells, and macrophages) as mean percentages; $n = 12$. Cellular populations were enumerated on day 4 in malaria-infected mice (*P. berghei*, 1×10^5 , 1P) and day 20 in Leishmania-infected (1×10^6 promastigotes, 1D) mice.

did not alter PHA responsiveness. The dieldrin + Leishmania-induced impairment in PHA activity was time- and dose-related, whereas the change seen in HCB + Leishmania-infected animals was only time-related, i.e., exacerbation with extended dietary administration of HCB.

To further evaluate the impaired PHA responsiveness in dieldrin-treated mice infected with Leishmania, the splenic T-cell enriched population was subjected to mitomycin C and anti-theta (anti-Thy-1) serum. Mitomycin C and anti-theta serum abolished the mitogenic response to PHA, as measured by ³H-thymidine incorporation. When splenic T-cells taken from dieldrin + Leishmania infected mice were added at one-half the normal cell concen-

tration (4×10^5) to normal (control) splenic T-cells a significant inhibition was demonstrated (Tables 6 and 7). This inhibition could be blocked by pretreating the dieldrin + Leishmania-infected mouse derived T-cells with mitomycin C which suggested an active cell-mediated suppressor. Similarly, when the splenic T-cells from the dieldrin + Leishmania-infected mice were pre-treated with anti-Thy-1 antiserum their inhibitory or suppressive effect was also abolished. These data suggested the presence of a Thy-1 positive suppressor cell in the spleen of the dieldrin-treated/Leishmania-infected mice.

Since the Leishmania promastigote has been described as a parasite infecting and proliferating in macrophages it was subsequently decided to de-

Table 5. PHA response of splenic T-cell nylon wool-enriched populations from mice infected with malaria or Leishmania and receiving dieldrin or HCB.*

Time, weeks	Chemical treatment		PHA response, cpm × 10 ³		
	Agent	Concn, ppm	No infecting organism	Malaria	Leishmania
—	—	—	39	41	44
3	Dieldrin	1	48	43	40
		5	53	39	22
6	Dieldrin	1	49	37	36
		5	45	41	16
10	Dieldrin	1	41	40	29
		5	44	36	11
3	HCB	5	54	36	33
		100	50	37	30
6	HCB	5	42	39	29
		100	41	41	22
10	HCB	5	37	35	22
		100	39	33	19

*Splenic cells were isolated from malaria- and Leishmania-infected mice on days 4 and 20, respectively, post infection and eluted from a washed nylon wool (Fenwal) column (60 ml syringe barrel) with RPMI 1646 with 5% ICS at a rate of 1 ml/min. PHA (Difco) was added at a concentration of 0.1 µg/well containing 200 µl of a 2 × 10⁶ cell/ml suspension. All plates (Flow, Linbro microtest) were inoculated with PHA for 48 hr with 0.1 µCi of ³H (5 Ci/mole) added 16 hr before harvest.

Table 6. PHA response of mitomycin C-treated splenic T-cell enriched population from Leishmania-infected mice administered dieldrin.*

Cell origin	PHA response, cpm × 10 ³
Normal	43
Normal + mitomycin C (20 µg/ml)	2
Leishmania (d. 20)	44
Leishmania + mitomycin C	6
Dieldrin (5 ppm, 10 wk)	40
Dieldrin + mitomycin C	3
Dieldrin/Leishmania	10
Dieldrin/Leishmania + Normal	15
Dieldrin/Leishmania (mitomycin C) + Normal	26
Half number of normal	24
Normal + normal mitomycin C-treated cells	22

*Cell preparations were used as previously described in Table 5. The enriched T-cell population at a concentration of 1 × 10⁷ cells/ml was incubated with 20 µg/ml of mitomycin C (Sigma) for 25 min at 37°C. The cells were washed three times with fresh medium and numbers adjusted for subsequent use. Each study was conducted in triplicate.

Table 7. PHA response of anti-theta treated splenic T-cell enriched population from Leishmania-infected mice administered dieldrin.*

Cell origin	PHA response, cpm × 10 ³
Normal	49
Normal + anti-theta	4
Leishmania (d. 20)	43
Leishmania + anti-theta	5
Dieldrin (5 ppm, 10 wk)	48
Dieldrin + anti-theta	2
Dieldrin/Leishmania	12
Dieldrin/Leishmania + Normal	14
Dieldrin/Leishmania (anti-theta) + Normal	30
Half number of normal	21
Normal + normal anti-theta treated cells	20

*All cells were prepared as described. Nylon-wool passaged spleen cells were incubated with a 1:80 dilution of anti-thy-1 antiserum (Cederlane Labs) and a 1:10 dilution of rabbit complement.

termine if macrophage dysfunction contributed to the induction of the Thy-1 positive suppressor cells. Hepatic Kupffer cells, alveolar, splenic and peritoneal macrophages isolated from dieldrin treated (5 ppm, 10 wk) Leishmania-infected (day 20) mice were cultured for 24 hr, after which their supernatant, following centrifugation at 250g at 4°C for 15 min, was added to cell cultures simultaneously with PHA. Supernatant from hepatic Kupffer cells, and to a lesser extent from splenic macrophages, but not alveolar or peritoneal macrophages, when added

to either control T-cells or T-cells from Leishmania-infected mice did not alter their response to PHA, however, when added to T-cells from dieldrin- or HCB-treated mice or from dieldrin/Leishmania-infected mice, the supernatant suppressed the T-cell response to PHA (Table 8). Supernatant from macrophage populations from dieldrin-treated mice or Leishmania-infected mice did not alter the response to PHA. The suppressive activity was removed from the supernatant when dialyzed against phosphate-buffered saline overnight at 4°C.

The production by hepatic Kupffer cells, and to a lesser extent splenic macrophages, of a suppressive

Table 8. Macrophage induced alteration of PHA response of splenic T-cell enriched population from Leishmania-infected mice administered dieldrin.^a

Cell origin	PHA response, cpm × 10 ³
Normal	48
Normal + Mφ	46
Leishmania (d. 20)	43
Leishmania + Mφ	49
Dieldrin (5 ppm, 10 wk)	43
Dieldrin + Mφ	18
Dieldrin/Leishmania	11
Dieldrin/Leishmania + Mφ	6
HCB (100 ppm, 10 wk) + Mφ	15
Half number of normal	25

^aCells were prepared as described. Hepatic Kupffer, alveolar, splenic and peritoneal macrophage were isolated from dieldrin-treated Leishmania infected (d. 20) mice and cultured in RPMI 1640 + 10% FCS for 24 hr. Their supernatant was added to lymphocyte cultures simultaneously with the PHA; 50 μl of the supernatant was contained in each well.

factor which impaired T-cell responses to PHA was correlated with the ability of hepatic macrophage from dieldrin/Leishmania treated mice to process

an antigen and to transfer an adequate immunogen to a recipient control animal. Similar alterations were observed with splenic macrophages but to a lesser degree (Table 9). The impaired antigen processing capability was correlated with dietary exposure time.

Discussion

Extensive interest in immunotoxicology has been generated within the past 5-6 years by the demonstration that various environmental chemical contaminants may, in laboratory situations, be immunosuppressive. However, apart from contact dermatitis, clinical evidence indicating environmental chemical-induced immune alteration has not been available; although several examples of autoimmune diseases generated by chemical exposure have been reported (12, 13).

Experimental immunotoxicologic data have primarily focused on evaluating the immunosuppressive effect of various environmental chemical

Table 9. Macrophage presentation of antigen in mice infected with malaria and Leishmania and receiving dieldrin or HCB.^a

Time, weeks	Chemical	Macrophage donor type	PFC/10 ⁶ spleen cells		
			No infecting organism	Malaria	Leishmania
3	Dieldrin (5 ppm)	Peritoneal	110	90	79
		Splenic	150	105	83
		Alveolar	91	79	64
		Hepatic	77	56	43
		Peritoneal	91	77	69
		Splenic	109	120	71
	HCB (100 ppm)	Alveolar	82	84	74
		Hepatic	86	61	30
		Peritoneal	99	93	86
		Splenic	130	101	80
		Alveolar	115	89	69
		Hepatic	79	77	32
6	Dieldrin (5 ppm)	Peritoneal	76	61	43
		Splenic	85	103	32
		Alveolar	70	79	58
		Hepatic	71	44	77
		Peritoneal	68	89	61
		Splenic	117	90	63
	HCB (100 ppm)	Alveolar	103	76	53
		Hepatic	58	84	26
		Peritoneal	54	48	30
		Splenic	71	74	20
		Alveolar	54	87	37
		Hepatic	68	37	16
10	Dieldrin (5 ppm)	Peritoneal	61	77	32
		Splenic	102	79	56
		Alveolar	89	83	49
		Hepatic	41	50	20

^aMacrophages were isolated from dieldrin (5 ppm)- and HCB (100 ppm)-treated mice infected with malaria or *Leishmania* on days 4 and 20, respectively. SRBC were opsonized in 50% FCS for 30 min and labeled with Na⁵¹CrO₄. A standardized inoculum of ⁵¹Cr-SRBC was injected IV into control BALB/c mice and PFC/10⁶ cells were determined on day 4; n = 10.

contaminants, food additives, drugs and physical agents while, in general, disregarding the possible immunostimulation that may occur. In this regard, time and dose may be the critical determinants deciding whether a chemical contaminant is considered to be an immunostimulant or immunosuppressant. Furthermore, the catabolic capability of the lymphoid-macrophage elements at both a basal level and at an immune and/or stimulated level have been neglected.

Since the immune response of an organism is a complex multicellular and humoral event an evaluation of a chemical as an immunosuppressant should initially encompass an immune parameter which is a maximal determination of the host immune capacity. Such a measurement is best reflected in the assessment of the host defense using a challenge with a pathogen. The pathogen used may be a bacterium, virus or complex parasite. Indeed, a systematic evaluation of cell-mediated immunity and humoral immunity may be conducted using a series of various pathogens.

In addition to microbial pathogens, suitable parasitic models are also available. Commonly used parasites for immunological studies are: *Schistosoma mansoni*, *Trichinella spiralis*, *Plasmodium berghei*, *Toxoplasma gondii*, *Trypanosoma cruzi*, and *Leishmania tropica*. The present study utilized two of these parasites, *Plasmodium berghei* (NYU-2) and *Leishmania tropica*, to evaluate the influence of two common environmental chemical contaminants dieldrin and hexachlorobenzene (HCB) on host defense capacity and immune competence. *Plasmodium berghei* (NYU-2) is a lethal murine malaria which evokes a T-cell, B-cell and macrophage response. Although it is lethal, survival time is a reflection of immune competence. The *L. tropica* promastigote is a macrophage parasite which may or may not survive and proliferate in tissue macrophages depending on their functional status (14).

In the present study host defense mechanisms were more significantly impaired in dieldrin-treated mice than in mice receiving HCB. Indeed, the *Leishmania* model did appear to be a more sensitive indicator of immunotoxicity since mice bearing this parasite had a marked lethality whether treated with dieldrin or HCB (Table 1). However, immunosuppressive synergism to a T-dependent antigen, SRBC, in the parasite and chemical-treated mice was more evident in mice exposed to HCB than to dieldrin (Table 2). It appeared that the chemical and not the parasite was the determining factor in the antibody response to SRBC. However, when a T-independent antigen was used, i.e., PVP, the only statistically significant immunosuppressive synergism occurred in dieldrin and HCB-treated mice

bearing the *Leishmania* parasite. Since the antibody response to PVP has been reported to be macrophage-independent (15) it would suggest that B-cells/plasma cells are the target cell type involved. Lack of an altered response to T-independent antigens in malaria infected mice has been reported. Since a significant decrease in splenic T-cells, B-cells and macrophages was only seen in the dieldrin/*Leishmania* treated mice in the 5 ppm-10 week group, a change in gross cellularity would seem not to be a causal factor. However, a functional alteration can obviously not be excluded.

In this regard, the marked synergism between *Leishmania* in both dieldrin- and HCB-treated mice in regard to their response to PHA denotes a very selective cellular impairment in T-cell functionality. Since the 5 ppm-10 wk dieldrin/*Leishmania*-treated group demonstrated the most consistent alteration in immune parameters, i.e., depressed SRBC response, depressed PVP responses, and decreased T, B and M ϕ numbers and depressed PHA response, this group of animals was examined in greater detail for cellular hypofunctionality.

Treatment of splenic T-cell enriched populations from the dieldrin/*Leishmania* treated mice with mitomycin C to inhibit DNA synthesis or with antiserum to the Thy-1 antigen blocked their ability to inhibit the PHA response of normal T-cells. The inhibitory or suppressing activity suggested the presence of Thy-1 positive DNA synthesizing T-cells. Since induction of T-suppressor cells by macrophages has been demonstrated (16) the contribution of macrophages from dieldrin/*Leishmania* to the induction of T-suppressor cells (T_s) was investigated.

Isolated hepatic, and to a lesser extent splenic, macrophages produced a dialyzable factor that when added with PHA to splenic T-cell cultures, suppressed PHA stimulation of the T-cells. This macrophage T-cell suppressor factor did not influence normal control cells nor T-cells from *Leishmania* infected mice. However, when added to T-cells from dieldrin/*Leishmania* mice or dieldrin or HCB-treated mice it inhibited their proliferative response. The inhibition seen in HCB derived T-cells suggests that the factor may act on organohalide-primed cells. Although activated macrophages have been demonstrated to secrete inhibitory factors (17) previous studies have shown that macrophages from organohalide xenobiotics are not activated (4). Indeed, as observed in Table 9, the inability of the macrophages to present an adequate immunogen to naive hosts indicates a suppressed state.

The demonstration that maximal suppressor factor was found associated with hepatic Kupffer cells may be, in part, related to hepatotoxicity of the two xenobiotics. If this is the case, it may provide a

valuable tool in the study of the relationship between hepatotoxicity and immunosuppression.

Further studies to elucidate the factor produced by the suppressor macrophages will evaluate interferon production as well as prostaglandins which have immunomodulatory roles (18). In addition, macrophage secreted factors may also compete with thymidine for its uptake into DNA by T-cells.

The incorporation of infectious disease models into immunotoxicologic studies may be a more relevant as well as more sensitive indicator of immunotoxicity. Although most studies have utilized mature animals, the use of neonates and aged animals in conjunction with stress may be a more accurate measurement of immune potential.

REFERENCES

1. 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. Immunol.* 20: 30-35 (1978).
2. 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: 173-198 (1978).
3. Silkworth, J. B., and Loose, L. D. The assessment of environmental contaminant-induced lymphocyte dysfunction. *Environ. Health Perspect.* 39: 105-128 (1981).
4. 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. Padarathsingh, Eds., Van Nostrand Reinhold, 1981, pp. 259-274.
5. Loose, L. D., Silkworth, J. B., Charbonneau, F., and Blumenstock, F. Environmental chemical-induced macrophage dysfunction. *Environ. Health Perspect.* 39: 79-91 (1981).
6. Jerne, N. K., and Nordin, A. A. Plaque formation in agar by single antibody-producing cells. *Science* 140: 405-406 (1963).
7. Boyden, S. V. The absorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by anti-protein sera. *J. Exptl. Med.* 93: 107-112 (1951).
8. LaVia, M. F., Loose, L. D., LaVia, D. S., and Silberman, M. S. The immunodepressive effect of phenol derivatives. *Adv. Exptl. Biol. Med., Macrophages and Lymphocytes, Part A*, M. Escobar and H. Friedman, Eds., Plenum Press, New York, 1980, pp. 523-538.
9. Yam, L. F., Li, C. Y., and Crosby, W. H. Cytochemical identification of monocytes and granulocytes. *Am. J. Clin. Pathol.* 55: 283-290 (1971).
10. 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: 455-461 (1975).
11. Litchfield, J. T., Jr. A method for graphic solution of time-percent effect curves. *J. Pharmacol. Exptl. Therap.* 49: 399-408 (1949).
12. Hamilton, H. E., Morgan, D. P. and Simmons, A. A pesticide (dieldrin)-induced immunohemolytic anemia. *Environ. Res.* 17: 155-164 (1978).
13. Ward, M. Immunological mechanisms in the pathogenesis of vinyl chloride disease. *Brit. Med. J.* 1: 936-940 (1976).
14. Chang, K.-P. *Leishmania* infection of human skin fibroblasts *in vitro*: absence of phagolysosomal fusion after induced phagocytosis of promastigotes, and their intracellular transformation. *Amer. J. Trop. Med. Hyg.* 27: 1084-1096 (1978).
15. Wong, D. M., and Herscovitz, H. B. Immune activation by T-independent antigens: lack of effect of macrophage depletion on the immune response to TNP-LPS, PVP and dextran. *Immunol.* 37: 765-775 (1979).
16. Bash, J. A., and Cochran, F. R. Carrageenan-induced suppression of T lymphocyte proliferation in the rat: *in vitro* production of a suppressor factor by peritoneal macrophages. *J. Reticuloendothel. Soc.* 28: 203-211 (1980).
17. Nelson, D. S. Production by stimulated macrophages of factors depressing lymphocyte transformation. *Nature* 246: 306-308 (1973).
18. Gordon, D., Bray, M. A., and Morley, J. Control of lymphokine secretion by prostaglandins. *Nature* 262: 401-405 (1976).