Polychlorinated Biphenyl-Induced Modification of Lymphocyte Response to Plant Mitogens in Rats[1]

M. BONNYNS[2] and C. H. BASTOMSKY

McGill University Clinic, Royal Victoria Hospital, 687 Pine Avenue West, Montreal (Quebec H3A 1A1, Canada), 13 October 1975.

Summary. In PCB-treated rats total white cell and differential counts and lymphocyte proliferative response to PWM were unaltered. The lymphocyte response to PHA was increased in treated animals.

Polychlorinated biphenyls (PCBs) are widespread environmental contaminants[3,4] and accidental human exposure to large amounts has occurred[5]. Several studies indicate that PCBs may be immunosuppressive[4]. Thus in rabbits[6] and guinea-pigs[7] PCBs induced thymic atrophy and lymphopenia. Guinea-pigs, treated with PCBs showed suppression of both humoral and cell-mediated immunity[7].

It is generally accepted that thymus-dependent (T) lymphocytes are responsible for cell-mediated immunity and that bursa-dependent (B) lymphocytes are antibody-producing cells[8]. In the mouse and man plant mitogens in the soluble form stimulate lymphocyte transformation; phytohemagglutinin (PHA) stimulates T cells and pokeweed mitogen (PWM) stimulates both T and B cells[9]. We therefore examined the effects of PCBs in rats upon lymphocyte responses (^{3}H-thymidine incorporation into DNA) to PHA and PWM.

Materials and methods. Aroclor 1254, a PCB mixture containing 54% by weight of chlorine, was added to powdered Purina Rat Chow to give a concentration of 250 mg/kg. Male Sprague-Dawley rats (260–340 g) were fed Purina Chow, with or without PCB, for 7 days. 2 ml blood was drawn under light ether anesthesia and sterile conditions from an external jugual vein and mixed with 20 U heparin before and after the 7 day period. The animals were then killed and the livers and thymuses were weighed.

Lymphocyte responses to PHA and PWM were determined as described in detail elsewhere (BONNYNS and MCKENZIE, in preparation). In brief, 25 µl samples of heparinized blood were added to sterile polystyrene tubes containing 550 µl of tissue culture medium (RPM 11640, Gibco, Grand Island, N.Y.) and penicillin (100 U/ml) and Streptomycin (50 µg/ml). PHA (Difco Laboratories, Detroit, Mich.) 1 or 4 mg/ml, or PWM (Gibco) 50 or 250 µg/ml (final concentrations) were added just before the start of incubation. The tubes were incubated at 33–34 °C for 24 h (PHA) or 5 days (PWM); then 0.5 µCi ^{3}H-thymidine (New England Nuclear Corp., Boston, Mass., 2 Ci/mmole) was added and incubation was continued for another 24 h. Erythrocytes were lysed with distilled water and leukocytes were collected on Millipore filters (Millipore Corp., Boston, Mass.). The DNA in the trapped cells was precipitated with trichloracetic acid, the filters were dissolved in 1 ml of ethylene glycol monomethyl ether and 12 ml of Bray's solution was added. Radioactivity (dpm) was determined in a liquid scintillation counter with automatic quench correction. All values were the means of triplicates.

Total serum protein concentration and paper electrophoresis of serum proteins were determined by conventional techniques in 5 normal serum pools from 4 rats each and in 1 serum pool from the PCB-treated animals.

Results. Table I shows that body weight was similar in both groups of rats. Liver weight was increased by about 60% in the PCB group. Thymus weight was similar in both groups, but when related to body weight was reduced in PCB-treated rats.

Total white cell counts were similar in control (6670 ± 143/mm^3; mean ± SD) and PCB-treated (6170 ± 139) rats. The differential counts (90% lymphocytes and 10% neutrophils) were identical in both groups.

Table II shows lymphocyte responses to PHA and PWM. In the control group there was no difference in responses between the first and second bleeding (paired *t*-test). But in the PCB group the response to PHA 1 mg/ml was greater after treatment (paired *t*-test). This was also apparent when the responses to PHA 1 mg/ml in the second bleeding of the PCB group was compared to the control group; in this instance there was also a significantly greater response to PHA 4 mg/ml in the PCB group. On the other hand, PCB did not influence the response to PWM.

Total serum protein and α_1,- α_2- and γ-globulin fractions were reduced in PCB-treated rats. The values in the pool of serum (g/100 ml) from treated rats with normal ranges in parentheses are: total protein 6.4 (6.7–7.8), albumin 2.98 (2.90–3.45), α_1 globulin 1.25 (1.30–1.67), α_2 globulin 0.44 (0.47–1.20), β-globulin 1.32 (1.13–1.39), γ-globulin 0.28 (0.38–0.58).

Discussion. The increase in liver weight in PCB-treated rats confirms previous observations[3,4] and indicates that the PCB was exerting bilogical effects despite the relatively short period of administration. Body weights were

Table I. Effect of PCB on body, liver and thymus weights

Weight	Group		
	Control	PCB	p[a]
Body (g)	291 ± 22	299 ± 22	NS
Gain in weight (g)	22 ± 9	17 ± 10	NS
Liver (g)	11.8 ± 1.6	18.9 ± 1.8	< 0.001
g/100 g	4.0 ± 0.3	6.3 ± 0.8	< 0.001
Thymus (mg)	651 ± 80	517 ± 137	NS
mg/100 g	224 ± 26	173 ± 44	< 0.05

Each value is the mean ± SD of 5 animals. [a] Student's *t*-test.

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[2] Nato Research Fellow. Present address: Laboratory of Experimental Medicine, University of Brussels, Saint-Pierre Hospital, B-1000 Bruxelles, Belgium.

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Table II. Effect of PCB on ^{3}H-thymidine incorporation (dpm $\times 10^{-3}$) into DNA by mitogen-stimulated lymphocytes

Mitogen		Control		p[a]	PCB		p[a]
		1st bleed	2nd bleed		1st bleed	2nd bleed	
PHA	(1 mg/ml)	25.31 ± 16.58	47.64 ± 12.06	NS	21.11 ± 15.36	70.93 ± 12.90[b]	< 0.005
	(4 mg/ml)	54.73 ± 18.84	60.56 ± 8.17	NS	64.81 ± 32.25	87.26 ± 23.73[b]	NS
PWM	(50 µg/ml)	13.84 ± 6.61	20.73 ± 6.52	NS	14.44 ± 7.66	18.88 ± 7.01	NS
	(250 µg/ml)	14.21 ± 2.23	15.22 ± 4.44	NS	10.29 ± 5.67	12.81 ± 2.82	NS

Each value is the mean ± SD of 5 animals. [a] Student's t-test for paired differences; [b] $p < 0.05$ vs second bleed controls (Student's t-test).

similar in control and treated rats, which tends to rule out non-specific effects related to cachexia or stress. Thymus weight was reduced in the PCB group, in keeping with previous observations[6,7] and suggesting possible immunosuppression.

Lymphocyte responses to PHA and PWM are often used in human and animal studies as indices of cell-mediated immunity and antibody production[10]. In our experiments the response to PHA was unexpectedly enhanced in lymphocytes from PCB-treated rats. At first glance this seems to be at variance with the report by Vos and VAN DRIEL-GROOTENHUIS[7], who showed suppression of cell-mediated immunity in PCB-treated guinea-pigs by delayed hypersensitivity skin reactions to tuberculin. However, our data need not necessarily be contradictory. Allergic skin reactions and lymphocyte transformation in response to PHA need not necessarily correlate[11]. Moreover, in guinea-pigs treated with cyclophosphamide, a well recognized suppressor of cell-mediated immunity, the lymphocyte response to PHA was not suppressed[12] and in some patients treated with cyclophosphamide the proliferative response of lymphocytes to PHA was increased[13]. Two possible mechanisms for this paradoxical effect were suggested[13], first a selective depletion of B lymphocytes not activated by PHA and an increase in the T:B ratio and secondly a non-lethal injury of PHA responsive lymphocytes, with the enhanced proliferative response reflecting chromosomal repair. Similar mechanism(s) may be responsible for the enhanced lymphocyte response to PHA in PCB-treated rats. Thymic atrophy and hypogammaglobulinemia in PCB-treated animals certainly do suggest immunosuppression.

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Familial Association in Serum IgE Levels

KATRIN WILSON and K. J. TURNER[1]

Clinical Epidemiology Unit, Department of Medicine, University of Western Australia; and Clinical Immunology Unit, Department of Microbiology, University of Western Australia, Princess Margaret Hospital, Subiaco (Western Australia 6008), 10 November 1975.

Summary. The serum IgE levels measured by RIST correlated closely with those obtained by the single radioimmunodiffusion method. Values for husband and wife were closely related as were those between father and daughter but in contrast to previous observations no significant relationship existed between IgE levels for mother and son.

The population of Busselton, a small rural town in Western Australia, has been subject since 1960 to mass health examination surveys at three yearly intervals. Since the population is relatively static for most of the time and only resident families are included in the survey this would therefore appear to be an ideal situation in which to examine matters of epidemiological interest.

TURNER, ROSMAN and O'MAHONY[2] reported the prevalence of asthma, hay fever and eczema in the Busselton school children aged 6 to 17 years who were interviewed during the Spring of 1970. They also assayed serum IgE levels in 1,069 of the children and related the serum IgE levels to the prevalence of allergic disease. As anticipated from the results of other studies[3–5] elevated serum IgE levels correlated well ($p < 0.001$) with the incidence of allergic disease. Evidence was presented for an inheritance pattern of both asthma and hay fever and genetic factors were also suggested by correlation of serum IgE levels. It appeared that these were sex-linked in that mothers' IgE values correlated more closely with that of their sons

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