Immunology 1983 **50** 207

A chemical carcinogen, 3-methylcholanthrene, alters T-cell function and induces T-suppressor cells in a mouse model system

L. J. ALFRED, A. WOJDANI, MARIA NIETO, R. PEREZ & G. YOSHIDA *Pathology Department, Charles R. Drew Postgraduate Medical School, and UCLA Cancer Center (L.J.A.), Los Angeles, California, U.S.A.*

Accepted for publication 4 May 1983

Summary. The *in-vivo* effects of a polycyclic aromatic hydrocarbon (PAH), 3-methylcholanthrene (MCA), on *in-vitro* mitogen activation, cell-mediated lympholysis (CML) and T-cell subset distribution in mouse splenic lymphocyte populations were measured. Three inbred mouse strains were treated with a single intraperitoneal injection of corn oil alone or with different doses of MCA in oil (0·5–50 mg kg^{-1}). One to ninety days after injection, splenic lymphocytes were isolated, and assayed for blastogenesis, CML and the percent T-helper and T-suppressor cells using monoclonal antibodies. High doses of MCA suppressed mitogen activation (15·2–53·6%) and CML (69–90%) within 24 hr in lymphocytes from PAH-responsive mice (C57 and C3H). Blastogenesis was stimulated and CML was suppressed to a lesser degree (5–45%) in lymphocytes from non-responsive mice (DBA). MCA induced an increase in T-suppressor cells in responsive mice, but there was no change in DBA mice. These studies suggest a correlation between immunocytotoxicity of PAH compounds on T-cell subsets and the responsiveness of mouse strains to these carcinogens.

INTRODUCTION

Initial events associated with the effects of carcinogenic chemicals on cell-mediated immune functions relative to tumourigenesis have not been described. Polycyclic aromatic hydrocarbon (PAH) compounds represent a class of ubiquitous environmental chemicals which are mutagenic and carcinogenic (Sims & Grover, 1974; Heidelberger, 1975; Gelboin & Tso, 1978; DePierre & Ernster, 1978; Gehly *et al.*, 1982). These substances depress the homograft reaction and graft *vs* host reactivity (St. Jernswald, 1965; Outzen, 1980), and the cytotoxicity of natural killer cells (Kalland & Forsberg, 1982).

The animal's immune response to PAH-induced tumours might stimulate or inhibit tumour growth and progression (Prehn, 1977; Outzen, 1980). Little attention, on the other hand, has been given to early PAH-induced alterations in host effector cells which are ultimately required for epitope recognition and destruction of allogeneic preneoplastic and tumour cells.

Recent studies in this laboratory showed a PAH dose- and interstrain-related suppression of blastogenesis of mouse splenic T-cells from PAH-responsive and non-responsive mice (Alfred & Wojdani, 1983; Wojdani & Alfred, 1982). In the present study, we compared *in-vitro* mitogen activation, cell-mediated lympholysis (CML) and the percent distribution of T-cell subsets among splenic lymphocytes from control and 3-methylcholanthrene (MCA)-injected responsive and non-responsive mice. We observed a correlation between the PAH-induced depression of

Correspondence: Dr Lawrence J. Alfred, Charles R. Drew Postgraduate Medical School, 1621 East 120th Street, Los Angeles, CA 90059, U.S.A.

cell-mediated immune function, the induction of T-suppressor cells and tumour susceptibility to these substances.

MATERIALS AND METHODS

Animals

Eight-week-old inbred strains of mice used in this investigation were C3H/fCUM, C57BL/6 CUM and DBA/2 CUM, purchased from the University of California at Berkeley. Based on PAH-induced levels of aryl hydrocarbon hydroxylase (AHH) activity and tumour susceptibility, C3H/fCUM and C57BL CUM are considered as PAH-responsive strains and DBA/2 CUM as a non-responsive or low-responsive strain.

Chemicals and biologicals

MCA was purchased from Sigma Chemical Company, St. Louis, MO; [^{3}H]-methyl-thymidine, $Na_2[^{51}Cr]O_4$, Lyt-1 and Lyt-2 monoclonal antibodies and fluorescinated anti-mouse IgM were obtained from New England Nuclear Corp., Boston, MA; phytohaemagglutinin (PHA-M) was purchased from Burroughs Wellcome Laboratory, Raleigh, NC; RPMI 1640, calf serum and penicillin-streptomycin were purchased from Grand Island Biologicals, Santa Clara, CA; lymphocyte separation medium (LSM), a brand of Ficoll–Hypaque, was purchased from Litton Bionetics Inc., Rockville, MD.

Treatment of mice and lymphocyte preparation

Mice used for the blastogenic assay were injected once with varying doses of MCA (0·5, 5·0 or 50·0 mg kg^{-1}). Splenic lymphocytes were isolated after 24 hr and tested for mitogen activation as previously described (Alfred & Wojdani, 1983). Another group of mice was similarly injected with oil alone or with MCA (50 mg kg^{-1}). After 1, 7, 30 and 90 days, splenic lymphocytes from six control or MCA-treated animals of each strain were used for blastogenesis, CML and T-helper and T-suppressor cell assays.

Mitogenic assay

Five hundred thousand lymphocytes per 0·1 ml culture medium (CM) were cultured in quadruplicate in flat-bottom microtitre plate wells, without and with PHA (25 μg/ml). After 48 hr of incubation at 37° in a water saturated atmosphere of 95% air and 5% CO_2, cells were pulsed with tritiated thymidine 2 μCi/well for 16–18 hr.

Incorporation of [^{3}H]-thymidine into cellular DNA was determined by harvesting the cultures in a Mash II unit. Radioactivity was measured by liquid scintillation counting. The percentage of induction or suppression of ^{3}H-uptake was determined by the following formula:

$$\% \text{ of induction or suppression} = \left(1 - \frac{\text{c.p.m. in presence of carcinogen}}{\text{c.p.m. in absence of carcinogen}}\right) \times 100$$

The mean c.p.m. ($\pm$SD) was determined from quadruplicate wells. The variability in this assay was less than 20%.

Murine effector cells

Primary alloimmune effector cells were obtained from spleens of C57BL/6, C3H mice or DBA which had been previously immunized for 10 days (i.p.) with 2×10^7 EL4 lymphoma or P815 tumour cells. Splenic lymphocytes were separated as described above, and placed onto a pre-soaked nylon-wool column for 1 hr at 37°C as described by Julius, Simpson & Herzenberg (1973). Non-adherent T cell-enriched fractions were resuspended in CM and the viability (routinely was 95–98%) was determined by trypan-blue exclusion.

Murine target cells

P815 mastocytoma or EL4 lymphoma cells were maintained in ascites form in DBA/2 (H-2^d) or C57BL/6 (H-2^b) mice, respectively. Five to seven days after injection (i.p.), cell populations were taken for experimental use. Tumour cells were harvested aseptically and washed once in RPMI 1640 and then with 0·17 M ammonium chloride in 0·1 M tris buffer (pH 7·2) for lysis of contaminating red blood cells. Finally, after two additional washes with RPMI, tumour cells were placed onto pre-soaked nylon-wool columns as described above. Non-adherent cells were resuspended in CM and the percent viability was determined.

Cell mediated lympholysis

A modified ^{51}Cr-release assay as described by Brunner *et al.*, 1968 was employed. ^{51}Cr-release (experimental release—*ER*) was determined by centrifuging the plates at 1000 *g* for 5 min and harvesting 0·1 ml of the supernatant for counting in a gamma counter. Total release (*TR*) was determined by addition of 1·0 ml 1% Triton X-100, and spontaneous release (*SR*) was determined by the addition of target cells in medium.

The percent ^{51}Cr-release was determined by the following formula:

$$\% \ ^{51}\text{Cr-release} = \frac{ER-SR}{TR-SR} \times 100$$

The percent suppression of CML by MCA was determined as:

$$\left(1 - \frac{\% \ ^{51}\text{Cr-release in MCA-injected mice}}{\% \ ^{51}\text{Cr-release in oil-injected mice}}\right) \times 100.$$

Determination of percent helper and suppressor cells using Lyt-1 and Lyt-2 monoclonal antibodies

T-cell enriched populations were prepared as described by Julius *et al.* (1973). Five million T cells were mixed with 1:40 dilutions of Lyt-1 or Lyt-2 monoclonal antibody preparations, incubated for 1 hr at 4° with occassional mixing, pelleted by centrifugation at 600 *g* for 5 min. Cells were resuspended in RPMI, washed three times and incubated with fluoresceinated anti-mouse IgM for 30 min at 4°. Cells were then washed twice with RPMI and scored for the percent of Lyt-1 (helper)- or Lyt-2 (suppressor)-positive cells. In each determination 500 cells were scored using a Leitz fluorescence microscope.

RESULTS

In the present study, inbred strains of PAH-responsive and non-responsive mice were injected with various concentrations of MCA. The short- (24 hr) and long-term (3 months) *in-vivo* effects of this carcinogen on *in-vitro* mitogen activation and the ability of antigen-specific splenic effector T cells to kill alloreactive target tumour cells using two different targets were determined. We also measured the percent distribution of Lyt-1 and Lyt-2 cells in splenic tissue at different intervals after treatment.

Effect of MCA on mitogen activation

Mice were injected with oil alone or with MCA in oil at 0·5, 5 or 50 mg kg^{-1}, and 24 hr later lymphocytes were isolated. A dose of 0·5 mg kg^{-1} of MCA had little or no effect on blastogenesis, while doses of 5 and 50 mg kg^{-1} significantly suppressed mitogen activation of lymphocytes from C3H and C57 mice. At 50 mg MCA, blastogenesis was suppressed 51–53% in lymphocytes from the above strains, compared to a 77% stimulation in DBA lymphocytes (Table 1). At intervals of 1 and 7 days after injection with 50 mg kg^{-1} of MCA, blastogenesis of lymphocytes from C3H and C57 mice was significantly suppressed (34–50%, respectively). After 30–90 days, the levels of blastogenesis in lymphocytes from these responsive mice approximated those of oil-injected controls. In comparison, MCA-stimulated mitogen activation of lymphocytes from DBA mice at 1, 7 and 30 days after injection (73, 50 and 24%, respectively), but after 90 days there was no significant difference in values from control and treated mice (Table 2).

Table 1. *In-vitro* mitogen activation of splenic lymphocytes from mice injected with different concentrations of MCA*

Mouse strain	[^{3}H]TdR c.p.m./10^6 cells × 10^{-3}] mg MCA/kg body weight 0	0·5	5	50
C57	33±3·6	35±4·5	28±3·8	16±1·4 (51±8)†
C3H	28±4·2	26±3·7	18±2·6	13±1·8 (54±4)
DBA	22±2·1	21±2·6	25±3·3	39±2·6 (77±8)‡

* Values are expressed as means±SD for three separate determinations. Mitogen activation of lymphocytes from C57 and C3H mice, compared to DBA mice, was significantly depressed with increasing doses of MCA ($P<0·005$).

† Percent suppression.

‡ Percent stimulation.

Effects of MCA on antigen-specific CML

The effects of MCA dose and mitogen activation on CML were measured. Data presented in Table 3 show that MCA suppressed CML of allosensitized lymphocytes in a strain-related fashion. At a dose of 5 mg kg^{-1}, MCA suppressed CML 69–82% in C57 and C3H mice, and only 5% in non-responsive (DBA) mice. At a dose of 50 mg kg^{-1}, the suppression of CML activity by lymphocytes from C57 mice increased to 75±3%, those from C3H mice increased to 90±2% and the activity of DBA lymphocytes increased to 45±5% (*see* Table 3).

Mitogen activation of allosensitized lymphocytes from control or MCA-injected mice increased the percent of target-cell killing by these effector cells. The MCA-induced suppression of CML was retained in 48-hr mitogen-treated lymphocyte cultures derived from all three strains (Table 4). In concurrent experi-

Table 2. Mitogen activation *in vitro* of splenic lymphocytes derived from three strains of mice injected with MCA for varying periods of time

Mouse strain	[^{3}H]TdR c.p.m./10 cells $\times 10^{-3}$ different days after injection							
	1 day		7 days		30 days		90 days	
	Oil	MCA	Oil	MCA	Oil	MCA	Oil	MCA
C57	28±5·6	14±4·2	33±4·0	19±1·0	36±2·3	31±1·2	49±2·8	53±4·1
C3H	35±4·2	19±6·0	41±4·9	27±1·4	50±6·3	42±4·2	63±6·4	59±8·4
DBA	26±3·0	45±2·0	24±3·5	36±3·5	25±3·2	31±2·6	26±3·5	30±2·6

* Means±SD.

The proliferation of lymphocytes from C3H and C57 mice was suppressed during 1–30 days; DBA mice were stimulated during the same period of time ($P < 0{\cdot}001$).

Table 3. *In-vivo* effects of MCA on antigen-specific CML of splenic lymphocytes *in vitro*

Mouse strain	^{51}Cr-release from alloreactive target tumour cells			
	MCA dose (mg kg^{-1})			
	0	0·5	5	50
C57	32±4·0	29±3·2	10±0·4 (69±2)*	8·0±0·6 (75±3)
C3H	38±3·2	42±6·0	7±1·0 (82±3)	4·0±0·2 (90±2)
DBA	20±1·2	18±0·8	19±0·6 (5±1)	11·0±1·1 (45±5)

* Percent suppression of CML. MCA suppressed CML activity of C57 and C3H allosensitized lymphocytes 69±2% to 90±2%, compared to 5±1% to 45±5% suppression of DBA T cells ($P < 0{\cdot}001$).

Table 4. Effects of mitogen activation on CML of effector T cells from antigen-specific control and MCA-treated mice

Mouse strain	% ^{51}Cr-release from alloreactive target tumour cells			
	Control mice		MCA-treated mice	
	−PHA	+PHA	−PHA	+PHA
C57	46±4	62±5	18±3	21±3
C3H	41±4	62±5	18±3	21±3
DBA	24±2	45±5	16±1	32±4

Effector cells from control mice showed the expected increase in target-cell killing following mitogen activation. Effector cells from MCA treated mice showed a depression in killing activity after mitogen activation.

ments we measured the long-term effects of MCA treatment of antigen-specific CML against respective haplotype non-identical target tumour cells. Data shown in Table 5 demonstrate that 15 days of MCA treatment produced a 60±8% suppression in the ability of C57 lymphocytes to kill target cells, 76±4% suppression of C3H effector cells and 29±2% depression in DBA effector cells. This MCA-induced suppression in CML activity is significantly reduced after 30 days, and approximated control levels after 90 days, (Table 5).

Effect of MCA on the percent T-helper and T-suppressor cells

With the use of monoclonal antibodies (anti-Lyt-1 and anti-Lyt-2), the percent distribution of T-helper and T-suppressor among splenic lymphocyte populations was measured in control and MCA-treated mice. As shown in Table 6, all three mouse strains had approximately the same percentage of Lyt-1- and Lyt-2-positive cells in spleens of injected or control mice after 1–90 days. MCA treatment produced a slight decrease in the percent of Lyt-1 cells and an increase in Lyt-2 cells in C57 and C3H mouse lymphocyte, but there was little or no change in the percent of these subsets in DBA splenic cells. The Lyt-1:Lyt-2 ratios in MCA-treated C57 and C3H were 3·4 and 3·7 24 hr after treatment, 1·4 and 1·6 30 days after treatment, and 1·8 and 2·0 90 days after treatment. The ratios in MCA-treated DBA mice were unchanged during these periods.

Table 5. Long-term effects of MCA treatment on antigen-specific CML of splenic T cells against alloreactive tumour target cells *in vitro*

		% ^{51}Cr-release from target cells					
		Days after MCA injection					
		15		30		90	
Mouse strain	Target cells	Oil	MCA	Oil	MCA	Oil	MCA
C57	P815	38±2	15±2 (60±8)*	40±3	22±2 (45±4)	38±4	34±3 (10±7)
C3H	P815	41±2	10±2 (76±4)	38±3	18±2 (52±6)	43±4	37±2 (14±6)
DBA	EL4	27±3	19±2 (29±2)	31±2	26±3 (16±9)	33±4	29±2 (12±5)

* Percent suppression of ^{51}Cr-release from alloreactive target cells. Values for MCA suppression at 90 days after injection were not significant.

Table 6. *In vivo* effect of MCA (50 mg kg^{-1}) on the percent distribution of T-helper (Lyt-1) and T-suppressor (Lyt-2) cells in splenic tissue

		% Lyt-1 or Lyt-2 positive cells, and Lyt-1:Lyt-2 ratio									
		Days after MCA injection									
		1		7		15		30		90	
Mouse Strain	Antibody	Oil	MCA	Oil	MCA	Oil	MCA	Oil	MCA	Oil	MCA
C57	anti-Lyt-1	70	69	67	62	66	59	71	54	68	56
	anti-Lyt-2	19	20	18	24	20	25	20	37	22	31
	Lyt-1:Lyt-2	3·6	3·4	3·7	2·6	3·3	2·3	3·5	1·4	3·0	1·8
C3H	anti-Lyt-1	69	70	70	62	73	64	71	56	74	60
	anti-Lyt-2	18	19	20	26	19	17	10	35	18	30
	Lyt-1:Lyt-2	3·8	3·7	3·5	2·4	3·8	2·4	3·5	1·6	4·1	2·0
DBA	anti-Lyt-1	70	68	73	71	75	72	74	72	73	75
	anti-Lyt-2	21	22	20	20	22	24	21	26	22	23
	Lyt-1:Lyt-2	3·3	3·1	3·6	3·5	3·4	3·0	3·5	2·8	3·3	3·2

Values shown above represent the means of two separate experiments. Enriched T-cell fractions of splenic lymphocyte populations were tested with Lyt-1 and Lyt-2 monoclonal antibody preparations as described in 'Materials and Methods'.

DISCUSSION

The metabolism, mutagenic and carcinogenic activity of PAH compounds have been studied previously (Heidelberger, 1975; Miller, 1978; Jerina *et al.*, 1980). Pertinent to the present study is the genetic regulation of the metabolism of PAH compounds, and the susceptibility of inbred strains of mice to PAH-induced tumours (Kouri & Nebert, 1977). We examined the *in-vivo* effects of different doses of MCA on the ability of splenic T cells for *in-vitro* blastogenesis and CML, and on the percent distribution of T-cell

subsets. MCA treatment results in an interstrain difference in the induced suppression of mitogen activation, in which lymphocytes from PAH-responsive strains, compared to those from non-responsive mice, are suppressed to a significantly greater degree (Tables 1, 2). This interstrain suppression in blastogenesis by MCA may be related to the genetically controlled differences in levels of metabolism of this compound via AHH or to percent differences in splenic T-cell subpopulations. Single injections of MCA produce a suppression of blastogenic activity in 1–7 days, but this function returns to control levels between 30–90 days. The restoration of normal blastogenesis suggests a repopulation of splenic tissue with mitogenic T cells or the effective excretion of toxic MCA products.

Splenic T-cells allosensitized with tumour target cells in MCA-treated mice and tested against haplotype non-identical cells *in vitro*, show a suppression in CML acitivity. C57 mouse T cells are suppressed $69 \pm 2\%$ to $75 \pm 3\%$, C3H T cells are suppressed 82 ± 3 to $80 \pm 2\%$ and DBA T cells are suppressed $5 \pm 1\%$ to $45 \pm 5\%$ (Tables 3, 5). The suppressive effect of MCA on antigen-specific CML was not observed 90 days after MCA treatment which may be correlated with the repopulation of splenic tissue with cytotoxic T lymphocytes. These results appear to be supported by the findings of Kallard & Forsberg (1982) on the transient inhibition of the lytic step of mouse NK cells by MCA.

In addition to mitogen activation and suppression of CML, we analysed the effects of MCA on the percent distribution of T-helper and T-suppressor cells in splenic tissue. In C57 mice a slight decrease in Lyt-1 cells was accompanied by a significant increase in Lyt-2-positive cells and a 40% decrease in the Lyt-1: Lyt-2 ratio 90 days after MCA injection, compared to controls. The Lyt-1:Lyt-2 ratio in C3H splenic lymphocyte populations was depressed 51% in 90 days, while that of DBA mice was unchanged during the 90-day period. The percent T-helper and T-suppressor cells in splenic tissue of the three strains is the same (see controls for all days, Table 6). MCA appears to exert a differential cytotoxic effect on T-helper and a stimulatory action on T-suppressor cells. We do not know if these effects are related to the carcinogenic index of PAH compounds. Other studies in this laboratory show that both 1, 2-benzanthracene (weak carcinogen) and 3, 4-benzo(a)pyrene (potent carcinogen) suppress mitogen activation of mouse splenic lymphocytes. This cytotoxic effect may be directed against macrophages or T-helper cells thus, an early carcinogen-induced alteration in the 'T-helper/T-suppressor switch', which persists for long periods of time, may be a predisposing factor for tumour formation. Consistent with this idea, are the findings of Haubeck & Kolsch (1982), who suggested that the induction of T-suppressor cells might be an early event in the formation of spontaneous tumours. We propose that the mouse model, using PAH-responsive and non-responsive strains, is an ideal system for ascertaining the role of T-suppressor cell induction in chemical carcinogenesis.

ACKNOWLEDGEMENTS

This research was supported by grants from NIH/MBRS (No. RR08140), NIH/MARC (No. 5 T34 GM07971) and EPA (R807046).

We are grateful to Dr B. Bonavida, UCLA School of Medicine for the generous gifts of P815 and EL4 target cells. The critical review of the manuscript by Dr S. Shacks, Assistant Professor, Department of Pediatrics, Charles R. Drew Postgraduate Medical School, is highly appreciated.

REFERENCES

ALFRED L. & WOJDANI A. (1983) Effects of methylcholanthrene and benzanthracene on blastogenesis and aryl hydrocarbon hydroxylase induction in splenic lymphocytes from three inbred strains of mice. *Internat. J. Immunopharm.* **5**, 123.

BRUNNER K.T., MAVEL J., CEROTINNI J.C. & CHAPUIS B. (1968) Quantitative assay of the lytic action of immune lymphoid cells on ^{51}Cr-labelled allogenic target cells *in vitro*. *Immunology*, **14**, 181.

DEPIERRE J.W. & ERNSTER L. (1978) The metabolism of polycyclic hydro-carbons and its relationship to cancer. *Biochim. biophys. Acta.* **473**, 149.

GEHLY E.B., LANDOLPH J.R., HEIDELBERGER C., NAGASAWA H. & LITTLE J.B. (1982) Induction of cytotoxicity, mutation, cytogenetic changes and neoplastic transformation by benzo(a)pyrene and derivatives in C3H/10T 1/2 clone 8 mouse fibroblasts. *Cancer Res.* **42**, 1866.

GELBOIN H.V. & TSO P.O.P. (1978) *Polycyclic Hydrocarbons and Cancer*, Vols 1 & 2. Academic Press, New York.

HAUBECK H.D. & KOLSCH E. (1982) Regulation of immune response against the syngeneic ADJ-PC-5 plasmacytoma in BALB/c mice. III. Induction of specific T-suppressor cells in the BALB/c plasmacytoma ADJ-PC-5 during early stages of tumorigenesis. *Immunology*, **47**, 503.

HEIDELBERGER C. (1975) Chemical carcinogenesis. *Ann. Rev. Biochem.* **44**, 79.

JERINA D.M., YAGI H., THAKKER D.R., LEHR R.E., WOOD

A.W., LEVIN W., & CONNEY A. (1980) Bay-region activation of polycyclic aromatic hydro-carbons to ultimate mutagens and carcinogens. In: *Microsomes, Drug Oxidations and Chemical Carcinogenesis* (eds M. J. Coon, A. H. Conney, R. W. Easterbrook, H. V. Gelboin, J. R. Gillette and P. J. O.Brien), Vol 2. Academic Press, New York.

JULIUS M., SIMPSON E. & HERZENBERG L.A. (1973) A rapid method for isolation of functional thymus-derived lymphocytes. *Eur. J. Immunol.*, **3**, 645.

KALLAND T. & FORSBERG J.G. (1982) 3-methylcholanthrene transiently inhibits lysis of mouse natural killer (NK) cells without affecting target cell binding. In: *15th International Leucocyte Culture Conference, Asilomar, CA*, p. 340.

KOURI R.E. & NEBERT D.W. (1977) Genetic regulation of susceptibility to polycyclic hydrocarbon-induced tumors in the mouse. In: *Origins of Human Cancer* (eds J. D. Watson and H. Hiatt) Cold Spring Harbor Laboratory Press, New York.

MILLER E.C. (1978) Some current perspectives on chemical carcinogenesis in humans and experimental animals. Presidential Address. *Cancer Res.* **38**, 1479.

OUTZEN H.C. (1980) Development of carcinogen-induced skin tumors in mice with varied states of immune capacity. *Int. J. Cancer*, **26**, 87.

PREHN R.T. (1977) Immunostimulation of chemical oncogenesis in the mouse. *Int. J. Cancer*, **20**, 918.

ST. JERNSWALD J. (1965) Immunosuppressive effect of 3-methylcholanthrene on antibody formation at the cellular level and reaction against weak antigenic homografts. *J. natn Cancer Inst.* **35**, 885.

SIMS P. & GROVER P. (1974) Epoxides in polycyclic aromatic hydrocarbon metabolism and carcinogenesis. *Adv. Cancer Res.* **20**, 165.

WOJDANI A. & ALFRED L. (1982) Effects of methylcholanthrene and benzanthracene on blastogenesis and aryl hydrocarbon hydroxylase induction in T-lymphocytes in inbred mice. *Proceedings of the 13th International Cancer Congress Seattle, Washington.*