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10. Using the thermodynamics from Day *et al.* (*8*), we obtain $k_{eq} = k_1/k_2 = 1.1 \times 10^{11}$ molecule cm^{-3}, with a probable uncertainty of about a factor of 2. Based on this, our value for the intercept k_1 (0.017 sec^{-1}) leads to a value for k_2 of 1.5×10^{-13} cm^3 $molecule^{-1}$ sec^{-1}, lower by a factor of about 7 than Callear's value (*9*). From the observed dependence on [CO] and $[O_2]^{-1}$, we obtain $k_2/k_3k_1 = 6.3 \pm 0.2 \times 10^6$ second, from which a value of 1.4×10^{-18} cm^3 $molecule^{-1}$ sec^{-1} can be derived for k_3. This is five orders of magnitude lower than Callear's value (*9*), which must be in error as a result of the complexity of the kinetic derivation in his flash photolysis system. The reproducibility and surface independence of our data imply that we are dealing with a purely homogeneous mechanism at these low concentrations.
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12. In our experience CO cyclinders contain variable amounts of metal carbonyls, usually iron, up to about 100 ppm. This is removed by passage over an iodinated charcoal trap at face velocities as fast as 20 sec^{-1}.
13. F. W. Sunderman, Jr., personal communication. In our calculations we assume instantaneous mixing and no effect on the lifetime arising from the presence of the rats. Both assumptions are subject to error.
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Aryl Hydrocarbon Hydroxylase Is Inhibited by Antibody to Rat Liver Cytochrome P-450

Abstract. *Antibody to the major purified cytochrome P-450 induced by 3-methylcholanthrene in rat liver strongly inhibits aryl hydrocarbon hydroxylase activity of uninduced and benz*[a]*anthracene-induced human monocytes and lymphocytes. Antibody to the cytochrome P-450 induced by phenobarbital has relatively little or no effect on the aryl hydrocarbon hydroxylase activity of the same human cells.*

Human monocytes and lymphocytes metabolize the carcinogenic polycyclic hydrocarbon benzo[*a*]pyrene (BP) (*1*). This activity is mediated by aryl hydrocarbon hydroxylase (AHH), a cytochrome P-450–dependent mixed function oxidase system in mammalian liver (*2, 3*), lung (*3, 4*), and other tissues (*3, 5*). The AHH activity is induced in mammalian liver by a wide variety of xenobiotics, including polycyclic hydrocarbons and phenobarbital. Induction in liver by polycyclic hydrocarbons results also in the appearance of a form of the cytochrome different from the major form present in the untreated animal. This new form of cytochrome P-450 exhibits different substrate specificity and has different susceptibility to the inhibitor 7,8-benzoflavone than the uninduced enzyme (*6*). Treatment with phenobarbital causes an increase in another form of cytochrome P-450 (*7*). Several of these cytochromes P-450 have been purified from rat (*8, 9*), rabbit (*10*), and mouse (*11*) liver. The AHH of human monocytes and lymphocytes is induced by polycyclic hydrocarbons in cell culture (*12*). However, since AHH in these cells is low and only very small amounts of cells from individual donors are available, direct studies of cytochrome P-450 multiplicity in monocytes and lymphocytes have not been reported. In this study rabbit antibodies (IgG, immunoglobulin G) to the major purified forms of cytochrome P-450 from rats treated with 3-methylcholanthrene (MC-IgG) and from rats treated with phenobarbital (PB-IgG) (*8, 13*) were used to investigate the nature and diversity of AHH activity of human monocytes and lymphocytes.

Monocytes and lymphocytes were isolated from leukocyte-rich plateletpheresis residues obtained from blood of normal volunteers, cultured, and harvested as described previously (*14, 15*). For inhibition experiments, monocytes (2.5×10^6 to 5×10^6 cells) and lymphocytes (5×10^6 to 10×10^6 cells) were first incubated at room temperature in 0.5 ml of 50 m*M* potassium phosphate buffer (*p*H 7.55) either alone or with IgG from rabbits prior to immunization, MC-IgG, or PB-IgG. Antibody was used at 1 mg per 0.5 ml, except where indicated otherwise. After the preliminary incubation, 0.5 ml of solution containing the remaining assay ingredients was added to give final concentrations of: 50 m*M* phosphate buffer, *p*H 7.55, 4 m*M* $MgCl_2$, 25 m*M* nicotinamide, 0.7 mg of bovine serum albumin per milliliter, 0.8 m*M* reduced nicotinamide adenine dinucleotide phosphate (NADPH), and 1.0 m*M* reduced nicotinamide adenine dinucleotide

Table 1. Effect of immunoglobulin G (IgG) from unimmunized animals (designated preimmune IgG), MC-IgG, and PB-IgG on AHH activity of human monocytes and lymphocytes.

Donor/additions	AHH activity* (percent of control)†			
	Monocytes		Lymphocytes	
	Uninduced	BA-induced	Uninduced	BA-induced
Donor 1				
None	0.51 ± 0.12 (100)	5.70 ± 0.72 (100)		
Preimmune IgG	0.55 ± 0.04 (108)	6.24 ± 0.33 (109)		
MC-IgG	0.24 ± 0.04 (47)	3.54 ± 0.43 (62)		
Donor 2				
None	1.29 ± 0.18 (100)	18.78 ± 5.21 (100)	0.38 ± 0.08 (100)	1.33 ± 0.17 (100)
Preimmune IgG			0.26 ± 0.06 (68)	0.83 ± 0.03 (62)
MC-IgG	0.43 ± 0.15 (33)	7.97 ± 2.54 (42)	0.06 ± 0.04 (16)	0.17 ± 0.04 (13)
PB-IgG	1.52 ± 0.04 (118)	13.50 ± 2.12 (72)	0.18 ± 0.01 (47)	0.90 ± 0.06 (68)
Donor 3				
None	0.35 ± 0.11 (100)	7.85 ± 2.21 (100)	0.47 ± 0.12 (100)	1.38 ± 0.06 (100)
MC-IgG	0.10 ± 0.05 (29)	2.72 ± 0.87 (35)	0.03 ± 0.03 (6)	0.15 ± 0.05 (11)
PB-IgG	0.42 ± 0.11 (120)	7.60 ± 1.24 (97)	0.63 ± 0.16 (134)	1.09 ± 0.00 (79)

*AHH activity is expressed as units per milligram of protein; values represent the means ± standard deviation (four to eight determinations) except that where PB-IgG was used only two to four determinations were made. †The activity of each cell sample in the absence of IgG (no additions) is designated as 100 percent. The other numbers in parentheses show AHH activity in comparison to the control.

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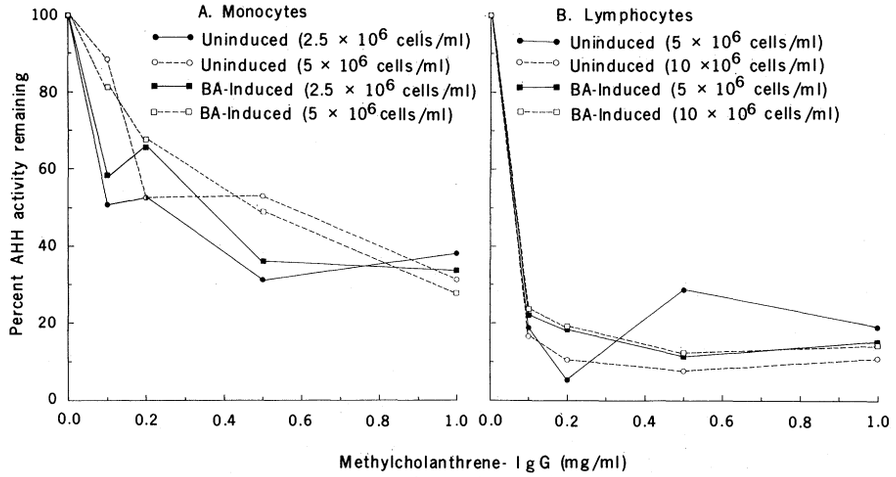

Fig. 1. Inhibition of AHH activity of human monocytes and lymphocytes by MC-IgG as a function of antibody concentration.

(NADH). The incubation for the AHH assay was started immediately after addition of 100 nmole of BP in 50 μl of absolute methanol and was continued in a water bath at 37°C with shaking under yellow light. After 30 minutes, the reaction was stopped by addition of 1 ml of cold acetone, and samples were processed (*15*). Both BP and BP metabolites were extracted with 3 ml of hexane, and the phenols were subsequently extracted with 1 ml of 1*N* NaOH. Fluorescence was then determined (Aminco SPF 500 spectrofluorometer; excitation wavelength, 392 nm with a 5-nm bandpass; emission wavelength, 522 nm with a 10-nm bandpass). The AHH activity was calculated from a standard curve of 3-hydroxybenzo[*a*]pyrene (3-OH-BP), with 1 unit being defined as that amount of enzyme producing fluorescence equivalent to that of 1 pmole of 3-OH-BP per minute.

The effect of antibody on AHH activity in untreated and benz[*a*]anthracene (BA)-treated monocytes and lymphocytes from three individuals is shown in Table 1. Uninduced and BA-induced monocyte AHH activity was not significantly affected by incubation with IgG from animals prior to immunization or by PB-IgG ($P > .05$). However, the AHH activity of these cells was very significantly inhibited by MC-IgG ($P < .005$ in all cases). The enzyme activity in the presence of MC-IgG was 29 to 62 percent of the activity in the absence of antibody. Lymphocyte AHH activity was sometimes inhibited by the PB-IgG (donor 2: uninduced, $P < .005$; BA-induced, $P < .01$; donor 3: uninduced, not significant; BA-induced, $P < .005$), but this inhibition was not significantly different from that obtained with IgG from animals prior to immunization. The MC-IgG reduced AHH activity of uninduced and BA-induced lymphocytes to 7 to 16 percent of the control and this inhibition was well above that observed in these cells with IgG from animals prior to immunization ($P < .0005$ in all cases). Sensitivity of the AHH activity of monocytes and lymphocytes to the antibody preparations was unaltered by BA treatment of the cells. In rat liver microsomes the sensitivity of AHH activity to the antibodies is enhanced by polycyclic hydrocarbon treatment (*13*). This suggests that the polycyclic hydrocarbon-induced cytochrome P-450 in liver is different from the major form present in uninduced liver. In contrast, the AHH of control and BA-induced monocytes and lymphocytes is similarly inhibited by the antibody preparations, although the AHH of the cells is elevated by BA treatment. This suggests that in human monocytes and lymphocytes the cytochrome P-450 forms induced by BA treatment are the same as the forms in the untreated cells. Our results also show that the cytochrome P-450 of both uninduced and BA-induced human monocytes and lymphocytes is antigenically similar to the major cytochrome P-450 form present in microsomes from MC-treated rat liver and relatively unrelated to the phenobarbital-induced liver cytochrome.

The AHH activity of lymphocytes was more strongly inhibited by MC-IgG than that of the monocytes (Fig. 1). With monocytes (Fig. 1A), inhibition of activity increased gradually with increasing antibody concentration and reached 50 percent at about 0.4 mg of MC-IgG per milliliter, whereas with lymphocytes (Fig. 1B) we observed an 80 percent inhibition at only 0.1 mg/ml. With both monocytes and lymphocytes, the extent of inhibition was relatively independent of the cell concentration used. Thus, our results suggest either different sets of cytochrome P-450's in monocytes and lymphocytes or possibly different accessibility of the enzyme to antibody in the different cells.

It has been proposed that the susceptibility of individuals to the development of cancer may be related to their ability to metabolize carcinogenic compounds (*16*). This capacity is under some degree of genetic control (*15, 17*), at least part of which may be reflected in the proportions of the various cytochromes P-450 in the target tissue. Our results open a new approach to the examination of human AHH, and may be helpful in determining the heterogeneity of cytochrome P-450 in different tissues of different individuals. This assessment may lead to an understanding of the relation of mixed function oxidase profile and susceptibility to chemical carcinogenesis.

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Insertion of a New Gene of Viral Origin into Bone Marrow Cells of Mice

Abstract. *DNA containing the herpes simplex virus thymidine kinase (HSVtk) gene was used to transform wild-type tk$^+$ mouse L cells to a tk^{++} status in vitro using methotrexate as a selective agent. HSVtk DNA was also used to transform mouse bone marrow cells in vitro. Transformed marrow cells injected into irradiated and methotrexate-treated recipient mice gave rise to proliferating cells which in some cases dominated the marrow population and which contained HSVtk gene sequences.*

Genetic manipulation of mammalian cells growing in tissue culture has been accomplished by various methods (*1*). Recently we described a technique for inserting genes for drug resistance into cells of living mice (*2*). In this procedure, calcium phosphate precipitates of mouse DNA containing multiple copies of the gene for dihydrofolate reductase (DHFR) were used to transform bone marrow cells to a state of increased resistance to the drug methotrexate. Transformed cells had a proliferative advantage over untransformed cells under the selective pressure of methotrexate administration. Ultimately, the transformed methotrexate-resistant cells dominated the proliferating hematopoietic cell population of the mice.

Two obvious potential uses of this gene-insertion technique are apparent for use in man: (i) induction of a higher degree of bone marrow resistance to the toxicity of anticancer drugs like methotrexate and (ii) the introduction of a new gene that, by itself, does not confer any proliferative advantage on recipient cells, by linking it to a second gene conferring drug resistance.

We set out to devise conditions in which we could use the herpes simplex virus (HSV) thymidine kinase (tk) gene as a selective marker even in tk$^+$ cells, which have the normal mammalian tk gene. We reasoned that use of appropriate levels of methotrexate and exogenous thymidine should cause cell growth to be limited by the thymidine kinase produced by the normal mammalian tk gene. Consequently, transformants carrying additional tk genes would have a selective advantage. Moreover, we noted that the HSVtk enzyme has a greater affinity for its substrate than does the normal mammalian enzyme and that this might confer an additional selective advantage.

We now describe our success in first transforming tk$^+$ mammalian tissue culture cells to drug resistance with herpesvirus tk gene in vitro and then selecting, in intact animals, bone marrow cells transformed with HSVtk gene.

We used the technique of Wigler *et al.* (*1*) to insert the herpesvirus tk gene (*3*) in tissue culture cells. Mouse L cells lacking thymidine kinase (tk$^-$) were incubated with a calcium phosphate precipitate of DNA containing the herpes tk gene in the plasmid vector pBR322 and then transferred to selective HAT medium (hypoxanthine, aminopterin, thymidine) (*1*). The anticipated results for transformation of Ltk$^-$ cells to tk$^+$ status were observed, and transformation efficiency was approximately the same as that reported by Wigler *et al.* (*1*) (Table 1). Thymidine kinase specific activity of different clones varied from 1.8 to 4.2 times the specific activity of wild-type (tk$^+$) cells.

Table 1. Thymidine kinase specific activity of mouse L cells transformed in vitro with HSVtk DNA. Clones were derived from the parent tk$^-$ L cell line or tk$^+$ wild-type L cells (NCTC) by transformation with calcium-precipitated HSVtk DNA (*1*), with 4 to 20 μg of tk DNA. The tk DNA was either in circular form in plasmid pBR322 or in plasmid that had been cut with sal and ligated (*9*). Clones of Ltk$^-$ lineage were selected in HAT medium. Cells of NCTC (wild-type) lineage were selected for in medium containing $10^{-4}M$ methotrexate and thymidine (0.5 μg/ml).

Clone	Cell lineage	Specific activity (cpm/μg)
	Untransformed	
	Ltk$^-$	0.08
	Ltk$^+$ (wild type)	4.9 to 5.5
	Transformed with HSVtk in plasmid	
207	Ltk$^-$	9.9
205	Ltk$^+$	10.3
214	Ltk$^+$	12.3
	Sal cut and ligated	
214-3	Ltk$^+$	22

In addition to transforming tk$^-$ cells to tk$^+$ status, we wished to transform wild-type (tk$^+$) cells so that they would contain additional copies of the tk gene of viral origin. Wild-type (tk$^+$) cells will grow in HAT medium by utilizing the available thymidine. Consequently, we explored a range of methotrexate and thymidine concentrations that would inhibit normal cells with a single copy of tk gene but allow cells with increased concentrations of tk to grow. These selective conditions required higher folate antagonist concentrations (methotrexate, $10^{-4}M$) and lower thymidine levels (0.5 μg/ml) than used in conventional HAT medium.

Wild-type (tk$^+$) L cells were exposed to herpesvirus tk DNA under transforming conditions and then cultured under selective conditions. A number of transformed clones were isolated, and some were grown to sufficient density to allow for measurement of thymidine kinase specific activity (Table 1) and for analysis of tk-specific gene sequences in DNA, which we identified by Southern (*4*) hybridization (data not shown). Transformation efficiency of tk$^+$→ tk^{++} varied between 0.2 and 5 colonies per 10^6 cells when 4 μg of HSVtk DNA was used. Spontaneous growth of tk$^+$ cells in the selective medium was < 1 per 10^8.

On the basis of the success of selecting for cells transformed to tk^{++} status in vitro, we undertook parallel experiments in mice. Our strategy was similar to that employed for selection of expression of the DHFR gene (*2*). Mouse bone marrow cells with a distinctive chromosomal marker (T6T6) were obtained from intact normal animals and treated in vitro with a calcium microprecipitate of herpesvirus tk gene (*3*, *5*). The treated marrow, presumed to contain a few stem cells transformed by viral gene, was mixed in a ratio of 1:1.5 with "mock" trans-

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