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CYTOCHROME P-450 IN A CULTURED HUMAN LYMPHOCYTE CELL LINE

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Currently, much attention is focused on the metabolism of numerous environmental carcinogens, especially benzo(a)pyrene (BP) and other polycyclic aromatic hydrocarbons which are metabolized by the aryl hydrocarbon hydroxylase (AHH) activity of the microsomal mixed function oxygenases. AHH activity has been reported in various human tissues, including monocytes [1], macrophages [2] and mitogen-activated lymphocytes [3,4].

AHH activity in mitogen-activated lymphocytes has received attention during the past five years: Kellermann et al. [5], Trell et al. [6], and more recently Rasco et al. [7] have reported a positive correlation between lymphocyte AHH activity and lung cancer risk in cigarette smokers. However, these reports have not been confirmed by Paigen et al. [8], who compared lymphocyte AHH inducibility of the progeny of lung cancer patients with a matched control population. Because of the significance of AHH, and also because of the limited availability of human tissues required for enzyme purification and detailed studies on the mechanism of human mixed function oxygenases, we investigated 75 stable lymphocyte cell lines in permanent culture. So far we have found three lymphocyte cell lines which, in the absence of mitogenic-prestimulation and under proper culture conditions, possess appreciable AHH activity. One of these cell lines (RPMI-1788), an Ig-synthesizing normal B-lymphocyte cell line, has AHH activity and inducibility comparable to fresh mitogen-activated lymphocytes [8,9]. This property of the cell line makes it a convenient source for large quantities of cells that are required for detailed studies on the metabolism of carcinogens, purification of the mixed function oxygenases and metabolically related enzymes, and for detailed studies on the mechanisms by which these enzymes activate and/or detoxify drugs, carcinogens and most other xenobiotics.

The RPMI-1788 cells were suspended at a density of 0.5×10^6 cells/ml in RPMI-1640 medium containing 10% heat inactivated fetal calf serum (FCS), 100 units/ml penicillin and 50 μg/ml streptomycin. The cultures were incubated at 37° in an atmosphere of 5% CO_2:95% air and 100% relative humidity.

Initially a lag period in cell growth of 12 hrs was observed; this was followed by a period of exponential growth until 50-60 hrs in five different experiments. Cells were harvested at 46-48 hrs after the initiation of the cultures, since this corresponds to maximal AHH activity which was determined by the fluorometric method of Nebert and Gelboin [10] as modified by Gurtoo et al. [9].

It is well established that AHH is inducible by many of its substrates [11]. For induction purposes the cells in culture were exposed to 0.3 μM dibenz(a,h)anthracene (DBA)

during the last 24 hrs. This concentration of DBA and exposure period were found to be optimal for this cell line [12]. In Table 1 are given the AHH activities (pmole equivalents of 3-hydroxy BP/10^6 cells/min) and inducibility ratios of RPMI-1788 cells for various inducers.

Table 1. AHH activity and inducibility in RPMI-1788 cells cultured during logarithmic growth phase.

The cells from stock cultures demonstrating 90% viability were seeded at 0.5×10^6 cells/ml in a total volume of 8 ml in Falcon T-30 flasks and incubated for 48 hrs at 37° in 5% CO_2:95% air. Some flasks received an inducer, shown in the table, 24 hrs prior to harvest. The harvested cells were assayed for AHH. Each value is the mean of two or three determinations. The concentrations of the inducers used are optimal for AHH induction in this cell line, as determined by dose response studies with various inducers, except for TCDD for which only 0.1 and 0.3 nM were tested.

Inducer[a]	AHH activity as % of control[b,c]
DBA (a,c) --- 0.3 μM	300
DBA (a,h) --- 0.3 μM	350
BA --- 10.0 μM	305
MC --- 1.0 μM	160
TCDD --- 0.3 nM	295

a) DBA, dibenzanthracene; BA, benzanthracene; MC, 3-methylcholanthrene; TCDD, 2,3,7,8-tetrachloro-dibenzo-p-dioxin.

b) Control activity is taken as 100% and this was equivalent to 0.12 + 0.01 (Mean + SE) pmoles of the phenolic metabolites formed/10^6 cells/min, using a 3-hydroxybenzo(a)-pyrene standard curve for quantitation.

c) The background fluorescence for the zero time controls was very little relative to sample incubations. The background activity ranged from 0.03 to 0.05 pmole phenolic metabolites formed/min. Since the reaction is linear up to 20×10^6 cells/incubation, background fluorescence does not limit the sensitivity of the assay. For example, with 10×10^6 cells/incubation the activity/min/incubation approximates 1.2 pmoles, i.e. 20-30 times the background value.

Fig. 1 shows the difference spectra of microsomes isolated from DBA-induced RPMI-1788 cells, and from the livers of rats pretreated with phenobarbital or 3-methylcholanthrene [11,13]. In accordance with several previous reports [13,14], difference spectral maxima of liver microsomes from phenobarbital and 3-methylcholanthrene treated rats are at 450 nm and 448 nm respectively. In contrast, the difference spectral maximum of the RPMI-1788 cell microsomes is at 451 nm. Our other studies [12] on the induction and inhibition specificities of AHH in this cell line indicate that the mixed function oxygenase-linked cytochrome is similar to rodent cytochrome P-448; however, the spectral examination reveals that, although in some enzymological properties it may be similar to rodent cytochrome P-448, spectrally it is a distinct cytochrome.

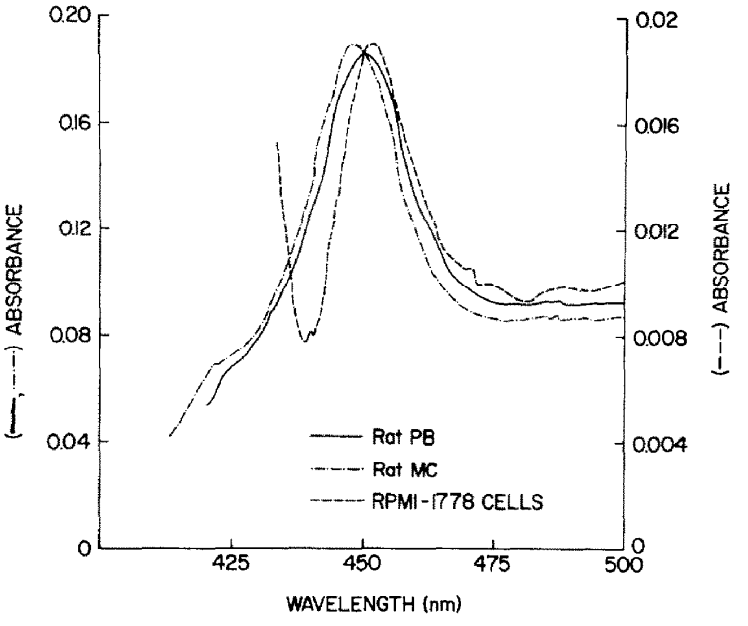

Fig. 1. Difference spectra of cytochrome P-450 in RPMI-1788 cells.

Cells (4×10^9) were suspended in 60 ml of 0.25 M sucrose-1 mM EDTA and homogenized, using a glass homogenizer, fitted with a Teflon pestle. The homogenate was centrifuged twice at 15,000 x g for 15 and 5 minutes, respectively. The 15,000 x g supernatant was centrifuged in a Beckman L5-50 Model ultracentrifuge at 105,000 x g for 60 minutes. The resulting microsomal pellet was suspended in 15 ml of 0.1 M potassium phosphate buffer (pH 7.4) and was centrifuged again at 105,000 x g for 60 minutes. The resulting pellet was suspended in 7 ml of the above buffer (8 mg protein/ml) containing 1 mM sodium cyanide. Three ml were transferred to two cuvettes. The baseline of equal light absorbance was recorded on an Aminco DW/2 spectrophotometer previously calibrated with homium oxide filter. A few mg of sodium hydrosulfite were added to both cuvettes; carbon monoxide was bubbled for 30 sec only through the contents of the sample cuvette and the spectrum was recorded. Using an extinction coefficient of 91 mM^{-1} cm^{-1} for the cytochrome P-450, the concentration was calculated to be 0.2 pmole cytochrome P-451/10^6 cells. For rat liver microsomes from phenobarbital or 3-methylcholanthrene-treated rats, spectrum was recorded under identical conditions except that cyanide was omitted. The concentration of rat liver microsomal protein used was as follows: phenobarbital (PB) 0.6 mg/ml; 3-methylcholanthrene (MC) 0.8 mg/ml.

The significant interpretation of the spectral properties of the cytochrome P-451 in RPMI-1788 cells leads us to suggest that mechanistic, physical and biochemical information obtained from studies with rodent liver cytochromes P-450 might not be directly applicable to humans; instead, information on human cytochrome P-450 should be obtained to explain

how humans metabolize drugs and carcinogens, what are the genetic bases of interindividual variations in the metabolism of xenobiotics and what specific mechanisms are involved to explain the effects of various environmental and physiological factors on the metabolism of drugs and carcinogens. To this end, the RPMI-1788 cell line is very useful, at least for some of the studies, as it can provide a convenient source of human cells in large quantities for detailed studies on metabolism, as well as may prove useful for the purification of mixed function oxygenases and metabolically related enzymes.

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REFERENCES

1. R.C. Bast, Jr., T. Okuda, E. Plotkin, R. Tarone, H.J. Rapp and H.V. Gelboin, Cancer Res. 36, 1967-1974 (1976).
2. E.T. Cantrell, G.A. Warr, D.L. Busbee and R.R. Martin, J. Clin. Invest. 52, 1881-1884 (1973).
3. D.L. Busbee, C.R. Shaw and E.T. Cantrell, Science 178, 315-316 (1972).
4. J.P. Whitlock, Jr., H.L. Cooper and H.V. Gelboin, Science 177, 618-619 (1972).
5. G. Kellermann, C.R. Shaw and M. Luyten-Kellermann, New England J. Med. 289, 934-937 (1973).
6. E. Trell, R. Korsgaard, B. Hood, P. Kitzing, G. Norden and B.G. Simonsson, Lancet 2, 140 (1976).
7. M.A. Rasco, T. Yamaguchi, D.A. Johnston and C.R. Shaw, Proc. Amer. Assoc. Cancer Res. 19, 189 (1978).
8. B. Paigen, H.L. Gurtoo, J. Minowada, L. Houten, R. Vincent, K. Paigen, N.B. Parker, B. Ward and N.T. Hayner, New England J. Med. 297, 346-350 (1977).
9. H.L. Gurtoo, N. Bejba and J. Minowada, Cancer Res. 35, 1235-1243 (1975); H.L. Gurtoo, J. Minowada, B. Paigen, N.B. Parker and N.T. Hayner, J. Natl. Cancer Inst. 59, 787-798 (1977).
10. D.W. Nebert and H.V. Gelboin, J. Biol. Chem. 243, 6242-6249 (1968).
11. A.H. Conney, Pharmacol. Rev. 19, 317-366 (1967).
12. H.J. Freedman, H.L. Gurtoo, J. Minowada, B. Paigen and J.B. Vaught, in preparation; H.J. Freedman, N.B. Parker, A.J. Marinello, H.L. Gurtoo and J. Minowada, in preparation.
13. A.P. Alvares, G. Schilling, W. Levin and R. Kuntzman, Biochem. Biophys. Res. Commun. 29, 521-537 (1967).
14. A.Y.H. Lu, S.B. West, D. Ryan and W. Levin, Drug Metab. and Dispos. 1, 29-39 (1973).