Biochemical Pharmacology Vol 35 No 13, pp 2069–2072 1986
Printed in Great Britain

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd

ARYL HYDROCARBON HYDROXYLASE ACTIVITY AND CYTOCHROME P_1-450 GENE EXPRESSION IN NEWLY ESTABLISHED HUMAN LYMPHOBLASTOID LINES

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(Received 4 March 1986; accepted 14 April 1986)

Human lymphoblasts having inducible aryl hydrocarbon hydroxylase (AHH) activity can be derived from circulating lymphocytes by activation with phytohaemagglutinin (PHA). Although mitogen-activated lymphocytes have been used in many studies in an attempt to correlate individual differences in AHH inducibility with susceptibility to environmental carcinogenesis, the results of these studies remain controversial (1). However, an association between high induced AHH levels and primary lung cancer has been shown (2), and inter-individual variability of lymphocyte inducibility is generally considered to be under genetic control (3,4). The enzyme proteins responsible for AHH activity are cytochrome P-450s. In rodent liver, cytochrome P_1-450 (P-450c) is the form most closely associated with inducible AHH activity (5). Jaiswal *et al.* (3) have shown an excellent correlation between inducible P_1-450 mRNA concentration and inducible AHH activity in mitogen-activated human lymphocytes.

Epstein Barr Virus transformed B lymphoblastoid cell lines, which remain diploid for up to 2 years in continuous culture, retain individual specific genetic markers (6), and can be readily established from most donors (7), are a potential source of sufficient material for the investigation of the molecular and cellular basis of individual differences in AHH inducibility. However, there have been few studies of AHH induction in B lines. Gurtoo and Marinello (8) examined 75 established lines and detected inducible AHH in only 3. This low frequency of AHH-inducible B lines probably discouraged further efforts to exploit them for studies of human variation of AHH expression. One line (RPMI 1788) has been characterized biochemically (9,10) and a cloned, AHH positive line (AHH-1) derived from it (11). Recently, Nagayama *et al.* (12) reported inducible AHH in B lines (of unspecified age) of several donors. They ascribed observed differences in AHH inducibility to genetic differences of the donors. The low frequency of AHH inducible lines reported by Gurtoo and Marinello (8) may be due to the age of the cultures they examined. To our knowledge, no information is available with respect to the stability of AHH expression in human B lines.

To evaluate the feasibility of using B cell lines for the study of AHH and cytochrome P-450 gene expression in humans, we examined AHH induction in newly-established lines from different donors during continuous culture. We show here that benzo(a)anthracene (BA)-induced AHH is initially high in most lines, but declines rapidly during culture. However, substantial activity, associated with detectable induced levels of a cytochrome P_1-450 mRNA, persists in some lines for more than 30 population doublings. Our results indicate that differences in AHH induction of B cell lines do not necessarily reflect genetic differences of the donors.

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MATERIALS AND METHODS

Lymphocytes were cultured in RPMI 1640 with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin and 100 µg/ml streptomycin. AHH and cytochrome c reductase were assayed (13) on 3-day PHA-activated cultures from 5 donors (including the donors of lines 4, 5, 8 and 9). The BA-induced levels of these cultures ranged from 0.16 to 0.21 units AHH/unit cytochrome c reductase. B lymphoblastoid lines were established by transformation with purified, mycoplasma-free Epstein Barr Virus (Showa Res. Inst., FL) and maintained according to Pauly et al. (7). Lines from 8 out of 10 donors were established (i.e. began logarithmic growth) within 27-41 days. Line CM (frozen immediately after establishment) was obtained from Dr R. Roy (Centre Hospitalier de l'Université Laval, Québec). BA-induced and control AHH and cytochrome c reductase activities were determined (9,10) on days 10, 26 and 68 after establishment of logarithmic growth (5, 14 and 30 population doublings respectively). Cell viability was routinely greater than 90%.

For the Northern analyses of P_1-450 mRNA induction, total cellular RNA, isolated by the guanidine thiocyanate/CsCl procedure (14), was denatured (5 min, 60°, 2.2 M formaldehyde, 50% formamide), electrophoresed through 1.2% agarose in the presence of 1.1 M formaldehyde, and transferred to nitrocellulose (15). The ^{32}P-labeled, nick-translated probe used (at 1.4×10^6 cpm/ml) was a 1200 base pair internal Pst I fragment (16) of a cloned rat P-450c cDNA (17) which shows 84% overall homology (including four 50-nucleotide segments of 94% homology) with the corresponding segment of human P_1-450 cDNA (18,19). The final wash was at 60° in 0.5X SSCE, 0.1% sodium dodecyl sulfate (1X SSCE is 0.15 M NaCl, 0.015 M sodium citrate, 0.002 M EDTA, pH 7.2).

RESULTS AND DISCUSSION

We measured BA-induced AHH of 9 newly established lines during 2.5 months (more than 30 cell generations) of continuous culture (Fig. 1). Except for line 8, the 6 lines tested on day 10 all had substantial BA-inducible AHH activity which was equal to, or greater than, that of line AHH-1 (20). Induced AHH declined rapidly thereafter but substantial activity was still present in lines 5, 6 and 9 on day 68. It is unlikely that the initial differences or the decline are due to differences in the degree of blast cell development of the cultures since the cytochrome c reductase levels (13) were similar for all lines and remained constant (legend, Fig. 1). Initial differences in inducible AHH could reflect viral transformation of different B cells of initially heterogenous populations. The decline in inducible AHH activity during culture could be due to a change in some component(s) of the enzyme induction mechanism or to a decrease in the number of inducible cells. In this context, it is of interest that Crespi and Thilly (11) determined by cloning that only a small portion of the cells of line RPMI 1788 had AHH activity. The decline in activity explains the paucity of AHH inducible lines among those maintained in continuous culture (8). A similar decline of AHH activity during early passage of hamster fetal cell cultures has been described (21). Line 8, which had the lowest activity, was derived from a donor whose PHA activated lymphocytes had a relatively high induced AHH level (0.21 units AHH/unit cytochrome c reductase). This observation, coupled with the variable decline in AHH induction with culture age, clearly shows that differences in inducible AHH in B cell lines cannot be assumed to reflect genetic differences of the donors.

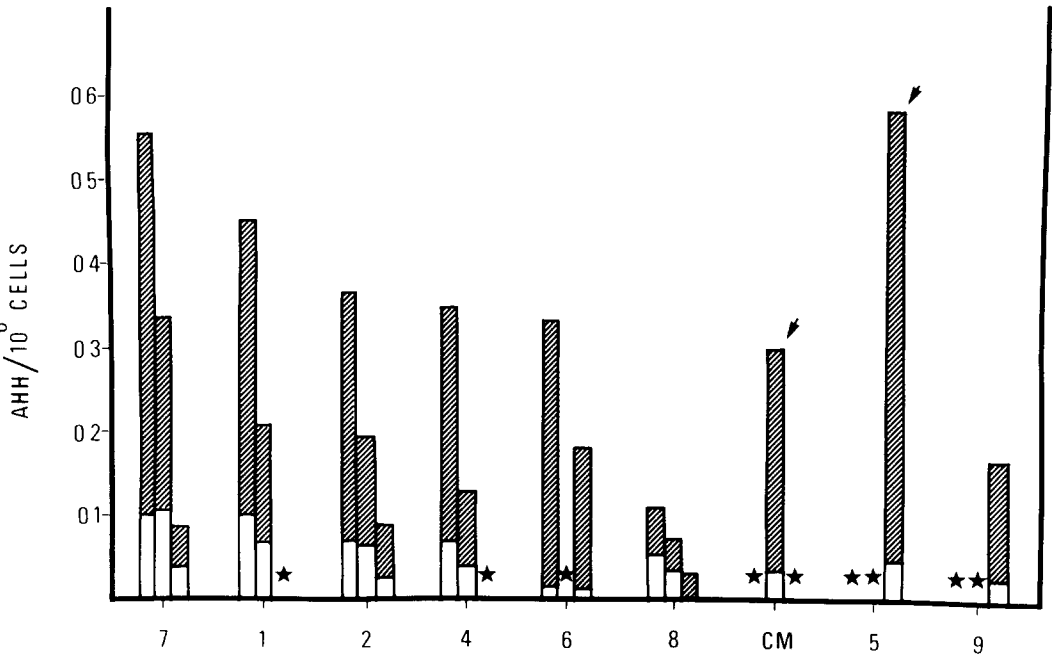

Fig. 1. AHH activity of newly established B lymphoblastoid lines during continuous culture. Basal (clear bars) and BA-induced (hatched bars) AHH levels (pmoles of 3-hydroxybenzo-(a)pyrene equivalents formed/min/10^6 cells) are shown for each line at 10, 26 and 68 days after establishment (★=not determined). Preparation and treatment of control and BA-induced (10 μM, 24 hr) cultures were as described in Refs. 9 and 10. AHH and cytochrome c reductase were assayed and cell number was determined by counting and direct determination of DNA (13) in the assay tubes. Values shown are the means of duplicate flasks, which differed by less than 20%. The arrows designate cultures in which an inducible cytochrome P_1-450 mRNA was demonstrated by Northern blot analysis. Mean cytochrome c reductase values (nmoles cytochrome c reduced/min/10^6 cells) for the lines on days 10, 26 and 68 were (mean (S.D.), N = 6): 6.0 (2.8), 7.7 (2.8) and 7.0 (0.5) respectively.

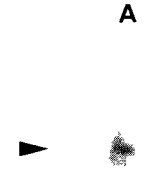

Fig. 2. Induction of P_1-450 mRNA. Total cellular RNA (10 μg) from line CM (see Fig. 1), induced with BA (lane A) and uninduced (lane B), was subjected to Northern blot analysis. The length of the BA-inducible hybridizing P_1-450 mRNA species (arrow) was estimated to be 2.8 kb in separate experiments in which radioactive DNA size markers were run in parallel. Exposure of Fuji NIF RX X-ray film was for the equivalent of 9 days at -80° with an intensifying screen.

Northern blot analyses of RNA isolated from lines CM and 5 (arrows, Fig 1), using a probe derived from rat P-450c cDNA, revealed a single BA-inducible 2.8 kilobase (kb) RNA species (Fig. 2 and data not shown). The length of human P_1-450 mRNA is 2.6 to 2.8 kb (18,22). Since the probe shows strong homology to human P_1-450 mRNA (18,19), we conclude that the inducible 2.8 kb RNA corresponds to a human P-450 mRNA, most likely that for P_1-450. The 2.8 kb mRNA was undetectable in RNA from induced or uninduced cells of line 7 after 30 population doublings (data not shown), which is as expected, given the low induced AHH activity in these cells.

In conclusion, B lymphoblastoid lines with substantial initial levels of induced AHH associated with an inducible P_1-450 mRNA species can be readily obtained from most donors. But, since expression of AHH in these lines is age dependent and may be heterogenous at the cellular level, the extent of AHH induction cannot be assumed to represent the AHH genetic status of the donor. The mechanism(s) responsible for the rapid loss of AHH inducibility during passage of the lines remains to be determined.

Acknowledgements-Supported by the Medical Research Council, the FCAR and the FRSQ. We thank Dr A. Okey (Hospital for Sick Children, Toronto) for advice, Dr. R. Roy for line CM, and E. Lemay and M. Gagnon for preparing the manuscript.

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