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THE *Ah* RECEPTOR, CYTOCHROME P450IA1 mRNA INDUCTION, AND ARYL HYDROCARBON HYDROXYLASE IN A HUMAN LYMPHOBLASTOID CELL LINE

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Abstract—The immunosuppressive and carcinogenic effects of aryl hydrocarbons such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 3-methylcholanthrene (MC) on B lymphocytes of adult rodents and the induction of cytochrome P450IA1 and aryl hydrocarbon hydroxylase (AHH) in human mitogen-activated lymphocytes and B-lymphoblastoid cell lines are believed to be mediated by the *Ah* receptor. However, there has not been a direct demonstration or characterization of the *Ah* receptor in defined populations of any of these cells. We report here the detection and characterization of an abundant, high-affinity B lymphocyte *Ah* receptor in the AHH-inducible human B lymphoblastoid cell line BCR-5. Our results represent the first characterization of a human lymphocyte receptor in a well-defined lymphocyte population. Sucrose density gradient analysis of BCR-5 cytosols incubated with [^{3}H]TCDD revealed a characteristic 9 S specific binding peak. The maximum concentration of *Ah* receptor was about 200 fmol/mg protein. Specific binding to the *Ah* receptor was also detected with [^{3}H]MC and, to a lesser extent, with [^{3}H]benzo[*a*]pyrene. The apparent binding affinity (K_d) for [^{3}H]TCDD (determined by saturation analyses) was about 5 nM. A specific [^{3}H]TCDD–*Ah* receptor complex which sedimented at 5 S was extracted from nuclei of BCR-5 cells incubated at 37° with [^{3}H]TCDD. The *Ah* receptor of BCR-5 cells is thus similar in characteristics to that identified in other cell lines. When BCR-5 cells were exposed in culture for 24 hr to increasing concentrations of benz[*a*]anthracene there was a concentration-dependent increase in induction and a good correlation ($r = 0.98$) between the level of induced AHH activity and the relative abundance of cytochrome P450IA1 mRNA. The human B lymphoblastoid cell line BCR-5, therefore, has a complete regulatory mechanism for *Ah* receptor-mediated induction of cytochrome P450IA1 that is essentially the same as that which has been well established in many rodent species. The accessibility of human blood lymphocytes and the ease of establishment of B lymphoblastoid cell lines from any donor provide a source of pure cultures of human B lymphocytes which can be grown continuously *in vitro* for the study of mechanisms related to *Ah* receptor-mediated cytochrome P450IA1 induction, immunosuppression and carcinogenesis.

Lymphocytes are potential *in vivo* targets for the toxic and carcinogenic effects of environmental chemicals. Experimental exposure of rodents to polycyclic aromatic hydrocarbons and polychlorinated biphenyls such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD¶) can result in immunotoxicity and immunosuppression [1–4], as well as leukemias and lymphomas [5, 6]. Benzo[*a*]pyrene (BP) diol-epoxide DNA adducts are present in lymphocytes of workers exposed to aromatic hydrocarbons [7, 8], and exposure of farm workers to chlorophenoxy herbicides is associated with an increased incidence of non-Hodgkin's lymphoma [9]. Human mitogen-activated peripheral blood lymphocytes (a mixture of T and B cells) and B lymphoblastoid cell lines (LCL), derived from circulating B lymphocytes by immortalization with the Epstein–Barr virus, possess aryl hydrocarbon-inducible cytochrome P450IA1 mRNA and aryl hydrocarbon hydroxylase (AHH) activity [10–13]. Thus, in addition to being potential targets for direct-acting carcinogens, human lymphocytes are capable of converting pre-carcinogenic hydrocarbons to their reactive forms [14].

The immunosuppressive effects of TCDD, and aromatic hydrocarbon-mediated carcinogenesis and immunosuppression in rodents, as well as the induction of cytochrome P450IA1 and AHH activity by TCDD and other aromatic hydrocarbons, are believed to be mediated by interaction of the ligand with a cytosolic receptor protein, the *Ah* receptor [1–3, 5]. Although exposure of perinatal mice to

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¶ Abbreviations: AHH, aryl hydrocarbon hydroxylase; BA, benz[*a*]anthracene; BP, benzo[*a*]pyrene; BSA, bovine serum albumin; HBSS, Hanks' balanced salt solution; HEPES, 4-(hydroxyethyl)-1-piperazineethanesulfonic acid; LCL, lymphoblastoid cell lines; MC, 3-methylcholanthrene; P450IA1, the form of cytochrome P450 previously known as P_1-450 in the mouse, P450c in rats, and form 6 in rabbits (AHH is a major catalytic activity of P450IA1); TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; and TCDF, 2,3,7,8-tetrachlorodibenzofuran.

TCDD results in thymic atrophy and suppression of T cell-mediated immune responses [15], the main immunosuppressive effect of TCDD on adult mice is the selective suppression of B cell-mediated humoral immunity via inhibition of the differentiation of B lymphocytes to antibody-secreting cells [16–18]. Studies of mouse strains having a genetically determined difference in the *Ah* receptor [3, 4], as well as structure/activity correlations [16], have suggested that binding of the immunosuppressive agent to a postulated B lymphocyte *Ah* receptor [18] is an essential event for this inhibition.

The *Ah* receptor has been studied and characterized in rodents [19, 20], it has been described recently in human tissues [21] and in human tumor cell lines [22, 23], and low concentrations of *Ah* receptor have been reported recently in human leukocytes [24]. There has been, however, no direct demonstration or characterization of a B lymphocyte *Ah* receptor in any species. We have shown that most newly established LCL have relatively high levels of benz[*a*]anthracene (BA)-inducible AHH activity [10]. Using one of our LCL which has sustained a high level of BA-induced P450IA1 mRNA and AHH activity during several months in continuous culture, we demonstrate here the presence of an abundant, high-affinity human B lymphocyte cytosolic *Ah* receptor.

MATERIALS AND METHODS

Chemicals. [^{3}H]TCDD (35 Ci/mmol) and nonradioactive 2,3,7,8-tetrachlorodibenzofuran (TCDF) were gifts from Dr. S. Safe (Texas A & M University). [^{3}H]BP (92 Ci/mmol) was obtained from Dupont Canada-NEN Research Products (Lachine, Quebec). [^{3}H]MC (generally labeled, 37 Ci/mmol) was from the Amersham Corp. (Oakville, Ontario). TCDD and TCDF are toxic substances and were handled with extreme care, as described by Poland and Glover [25]. Sucrose (density gradient grade) was from Beckman Instruments (Toronto, Ontario); HEPES was from the Calbiochem-Behring Corp. (La Jolla, CA); nonradioactive BA was obtained from Aldrich (Milwaukee, WI); nonradioactive BP, molybdate (sodium salt), dithiothreitol, bovine serum albumin (BSA), and catalase were obtained from the Sigma Chemical Co. (St. Louis, MO); dimethyl sulfoxide, glycerol, charcoal (Norit A), EDTA and all other routine chemicals were obtained from the Fisher Scientific Co. (Toronto, Ontario).

Buffers. The main buffer for cytosol preparation was HEGDM [25 mM HEPES, 3 mM EDTA, 2 mM dithiothreitol, 10% (v/v) glycerol and 20 mM sodium molybdate]. HEGDK buffer is HEGDM without molybdate but with the addition of 0.5 M potassium chloride. HEDM is a hypotonic buffer consisting of HEDGM but without the glycerol. HE2GDM is HEDM containing 20% (v/v) glycerol. All buffers were adjusted to pH 7.4 at room temperature.

Cell culture. The human B lymphoblastoid cell line 5 (BCR-5), which has maintained a high level of AHH induction during several months of continuous culture, was established by Epstein–Barr virus-transformation of peripheral blood lymphocytes from a normal donor [10]. A sample which had been frozen in liquid nitrogen 26 days (about 14 population doublings) after establishment served as the initial innoculum for these studies. Stock cultures were maintained by tri-weekly dilutions in fresh medium. The medium used was RPMI-1640 containing 25 mM HEPES, supplemented with 10% (v/v) fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin and 2 mM L-glutamine. Cultures were incubated at 37° in a humidified atmosphere of 5% CO_2. For the receptor studies, mass cultures (150 mL in 185 cm^2 flasks) were inoculated at a density of 4×10^5 cells/mL and were sub-divided every 2 days by centrifugation (10 min, 200 *g*) and suspension of the cells in fresh medium. Once sufficient cells (about 2×10^9) were obtained, samples were removed and transferred to 25 cm^2 flasks for incubation with BA and subsequent determination of AHH activity. The remaining cells were suspended in fresh medium at a concentration of 7×10^5 cells/mL, incubated for another 24 hr, and then processed to obtain the cytosol fraction. The viability of all cultures was checked by Trypan blue dye exclusion and was from 86 to 93% (mean 89%).

Cytosol preparation. The cytosol preparations were prepared essentially as described by Harper *et al.* [22]. All procedures were carried out at 4°. Cells (about 2×10^9) were recovered from log-phase mass cultures by centrifugation (200 *g*, 10 min) and washed three times with HBSS (pH 7.4); the pellet was suspended in 1 to 1.5 mL hypotonic HEDM buffer. The suspension was sonicated until about 90% of the cells were broken (estimated by phase-contrast microscopy). The homogenate was then diluted with an equal volume of HE2GDM buffer and centrifuged at 12,000 *g* for 4 min in a Beckman microfuge. The resulting supernatant was centrifuged at 105,000 *g* for 1 hr and the supernatant (cytosol) rapidly frozen and stored in liquid nitrogen until analysis. The protein content of the cytosol fractions (16–24 mg/mL; 30–40 mg/10^9 cells) was determined by the method of Bradford [26], using BSA as a standard.

Preparation of nuclear extract. If a nuclear extract was to be prepared from BCR-5 cells exposed to [^{3}H]TCDD in culture, the cell homogenate was centrifuged for 4 min at 10,000 *g* in a Beckman microfuge. The resulting pellet was washed 2–3 times with HEGDM buffer, incubated for 1 hr with HEGDK buffer at 4° to extract the nuclear receptor, and then centrifuged at 105,000 *g* for 1 hr. The resulting supernatant is termed the nuclear extract. We previously have shown that nuclei prepared in this manner from other cell lines are not contaminated with cytosol [27].

Preparation of cytosol for analysis by velocity sedimentation. The procedure used was essentially that of Denison *et al.* [19]. Briefly, cytosols to be analyzed for *Ah* receptor were incubated with [^{3}H]TCDD (or other [^{3}H]ligand as indicated in the text and figure legends), in the absence or presence of a 100-fold molar excess of competitor. All incubations were for 2 hr at 4°. After incubation unbound radioligand was removed by adding the cytosols to a pellet of dextran-coated charcoal (0.1 mg

charcoal/mg cytosolic protein) and incubating for 15 min at 4°; charcoal was removed by centrifugation at 4000 *g*.

Velocity sedimentation on sucrose gradients. Samples were analyzed by density gradient centrifugation using a vertical tube rotor technique as described by Tsui and Okey [28]. Aliquots were layered onto linear 10–30% sucrose gradients that had been prepared in the same buffer in which the cytosol or nuclear extract had been prepared. Gradients were centrifuged for 2 hr at 372,000 g_{av}. After centrifugation, twenty-five fractions (200 μL each) were collected and the radioactivity in each fraction was determined by liquid scintillation counting and was corrected for counting efficiency. BSA and catalase, labeled with [^{14}C]formaldehyde as previously described [19], were included in each gradient as internal sedimentation markers.

Induction and assay of AHH. Induction with BA was performed on cells in the logarithmic phase of growth essentially as described by Freedman *et al.* [11, 29]. Duplicate 25 cm^2 flasks containing 6–7 × 10^6 cells in 10 mL of medium were incubated for 24 hr. BA (10 μM final concentration unless otherwise specified) dissolved in acetone was then added and the incubation continued for another 24 hr. Control cells were exposed to acetone alone. The flasks were then placed on ice and the cells recovered by centrifugation (200 *g*, 15 min), washed with HBSS (200 *g*, 5 min) and suspended in 0.2 mL of a hypotonic buffer [30]. The samples were rapidly frozen and stored at −80° until analysis. AHH was assayed by a modification [30] of the method of Nebert and Gelboin [31] using recrystallized BP as substrate. From 5 to 10 × 10^6 cells per reaction flask were incubated at 37° for 35 min. The number of cells in the reaction flasks was estimated from the DNA content (duplicate determinations per flask) of the aqueous phase remaining after the hexane–acetone extraction step. DNA was measured by the method of Burton [32], using calf thymus DNA as the standard. One unit of AHH activity is defined as the amount of enzyme catalyzing the production of fluorescence equivalent to that of 1 pmol 3-hydroxybenzo[*a*]pyrene/min.

Northern analysis of cytochrome P540IA1 mRNA. Cytoplasmic RNA was isolated by the method of Maniatis *et al.* [33]. Fifteen micrograms of each sample was denatured [65°/5 min in 2.0 M formaldehyde, 50% (v/v) formamide] and electrophoresed in a 0.8% agarose denaturing gel (gel, 2.0 M formaldehyde; running buffer, 1.0 M formaldehyde). After electrophoresis, the RNA was transferred to a nylon filter, hybridized and autoradiographed as described by Labbé *et al.* [34]. The hybridization probe used (pHP$_1$450-3′) was an α-^{32}P-dCTP-labeled 1.05 kb 3′ fragment [35] of human P450IA1 cDNA obtained from the American Type Culture Collection. The probe was used at 1.5 × 10^6 cpm/mL. To serve as a length marker, 15 μg of total liver RNA from an Aroclor-treated rat was electrophoresed on the same gel and the corresponding portion of the filter was hybridized with a 0.47 kb *Hin*dIII-*Nco*I 3′ fragment of a rat liver P450IIB1 cDNA [36, 37]. The relative intensity of the P450IA1 bands of the autoradiogram was determined by densitometry scanning (Chromoscan 3, Joyce Loebl, UK).

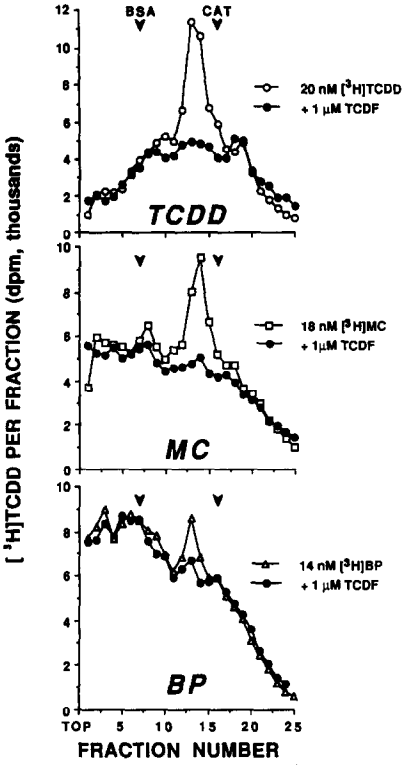

Fig. 1. Sucrose density gradient profiles demonstrating *Ah* receptor as detected by [^{3}H]TCDD, [^{3}H]MC and [^{3}H]BP in cytosol from BCR-5 cells. BCR-5 cytosol prepared in HEGDM buffer was incubated with [^{3}H]TCDD (○), [^{3}H]MC (□) or [^{3}H]BP (△) in the absence or presence (●) of a 100-fold excess of the competitor TCDF. After treatment with dextran-coated charcoal, samples were analyzed by velocity sedimentation on sucrose gradients. [^{14}C]Formaldehyde-labeled BSA (4.4 S) and [^{14}C]-formaldehyde labeled catalase (11.3 S) were added to the gradients as internal sedimentation markets.

RESULTS

Detection of Ah *receptor in cytosol from human B lymphoblastoid cells. Ah* receptor is detected by sucrose density gradient analysis of cytosol samples incubated with [^{3}H]TCDD. Chracteristically, a specific peak of radioactivity is detected in the 9 S region of the sucrose gradient. Specificity of binding is determined by comparing the radioactive profiles from gradients of cytosols incubated with [^{3}H]TCDD in the presence of a 100-fold excess of a competitor such as TCDF to profiles from cytosols incubated with [^{3}H]TCDD alone. Figure 1 (top panel) shows a typical profile for *Ah* receptor as detected by [^{3}H]TCDD in cytosol prepared from BCR-5. There was 145 fmol/mg cytosol protein of *Ah* receptor in the 9 S region of the gradient.

Ah receptor was also detected with nonhalogenated compounds such as BP and MC. As seen in Fig. 1, [^{3}H]MC (middle panel) and [^{3}H]BP (lower panel) each detected a specific binding peak in the 9 S

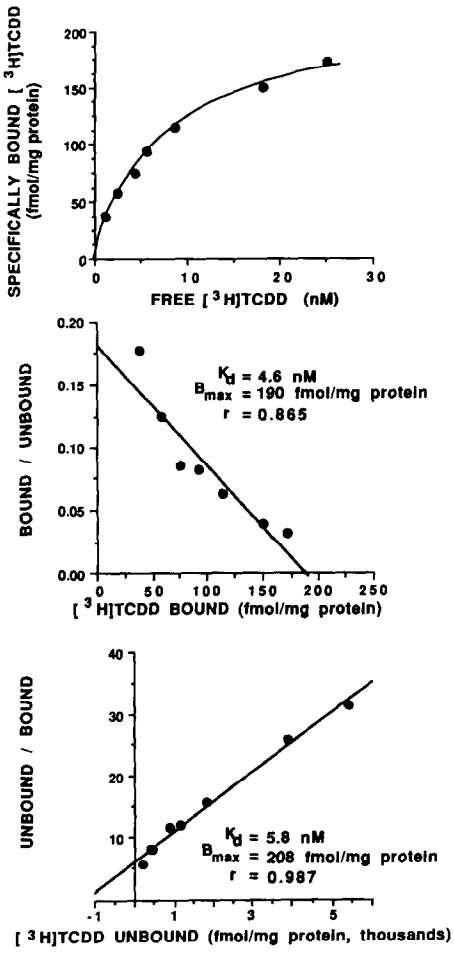

Fig. 2. Saturation analysis and determination of apparent affinity of binding of [^{3}H]TCDD in cytosols from BCR-5 cells. Cytosol aliquots (about 5 mg protein/mL) prepared in HEGDM buffer were incubated at 4° for 2 hr with [^{3}H]TCDD at concentrations ranging from 2 to 40 nM. Specific binding in the 9 S peak was determined for each sample by sucrose density gradient analysis. The Scatchard plot (middle panel) and Woolf plot (lower panel) were derived from data shown in the saturation plot (top panel). Binding parameters shown for the Scatchard and Woolf plots were calculated by least squares linear regression.

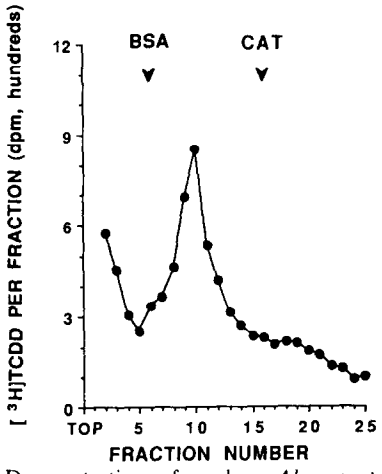

Fig. 3. Demonstration of nuclear *Ah* receptor–ligand complex extracted from cells incubated in culture with [^{3}H]TCDD. BCR-5 cells were incubated in culture for 2 hr at 37° with 2 nM [^{3}H]TCDD. Nuclear extracts were prepared and analyzed by velocity sedimentation on sucrose gradients. [^{14}C]Formaldehyde-labeled BSA (4.4 S) and [^{14}C]formaldehyde-labeled catalase (11.3 S) were added to the gradients as internal sedimentation markers. The concentration of binding sites in the nucleus was 85 fmol/mg nuclear extract protein.

region in cytosol from BCR-5 cells. No additional binding peaks were observed in other regions of the gradient. The amount of *Ah* receptor detected was 175 fmol/mg cytosol protein with [^{3}H]MC, very similar to the amount detected by [^{3}H]TCDD, whereas 20 fmol/mg cytosol protein was detected with [^{3}H]BP in the same cytosol preparation.

Estimation of the apparent affinity of [^{3}H]*TCDD binding to the* Ah *receptor.* The apparent affinity of [^{3}H]TCDD for the *Ah* receptor was estimated by incubating cytosol with various concentrations of ligand and determining the specific binding in the 9 S region by sucrose gradient density analysis for each concentration. As seen in Fig. 2, the *Ah* receptor appears to be saturated near 25 nM. The binding data were analyzed further by Scatchard and Woolf plot analyses (Fig. 2, middle and lower panels). The apparent K_d was computed as 4.6 nM and the B_{max} was 190 fmol/mg protein from the Scatchard plot; similarly the K_d was 5.8 nM and the B_{max} was 208 fmol/mg protein from the Woolf plot analysis.

Identification of nuclear-associated [^{3}H]*TCDD–receptor complex.* Binding of ligand to the cytosolic receptor results in a transformation of the cytosolic receptor–ligand complex to a nuclear binding protein. Nuclear-associated receptor can be extracted from cell nuclei by incubation with 0.5 M KCl and subsequently identified by sucrose gradient analysis. The nuclear-associated receptor–ligand complex sediments at 5–6 S as compared to the cytosolic receptor–ligand complex which sediments at 9 S. Figure 3 shows the gradient profile obtained when the nuclear extract from BCR-5 cells incubated in culture with [^{3}H]TCDD was analyzed by velocity sedimentation on sucrose gradients. There is a [^{3}H]TCDD binding peak in the 5 S region (fractions 6 to 11) of the gradient typical of nuclear-associated *Ah* receptor. The concentration of binding sites in the nucleus was 85 fmol/mg nuclear extract protein.

Induction of cytochrome P450IA1 mRNA and AHH activity. Log-phase cultures of BCR-5 were exposed to different concentrations of BA for 24 hr. A portion of each culture was then analyzed for AHH activity and the remainder was analyzed for cytochrome P450IA1 mRNA relative abundance. The autoradiogram (Fig. 4) shows the presence of an mRNA of about 3.0 kb. The amount of mRNA detected increased with the concentration of BA.

AHH activity increased with increasing concentrations of BA, up to an observed maximum at 10 μM (Fig. 5). The estimated concentration of BA required for half-maximal AHH activity (EC_{50}) was

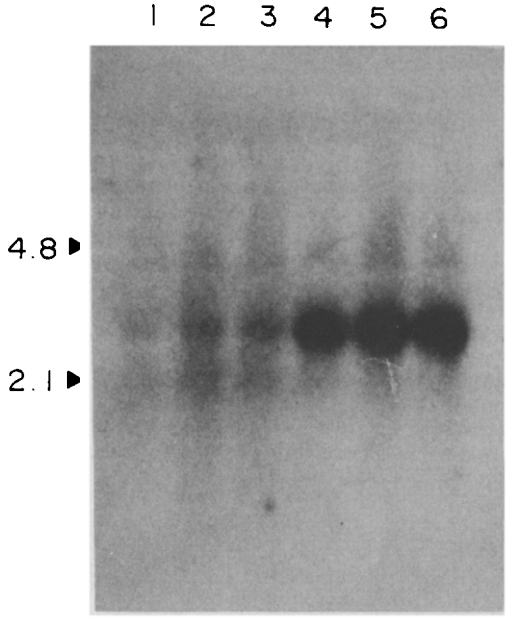

Fig. 4. BA induction of cytochrome P450IA1 mRNA. Cytoplasmic RNA was isolated from the control and BA-induced cultures described in the legend to Fig. 5. and subjected to Northern blot analysis using a human P450IA1 cDNA 3′ fragment as probe. The size markers (arrowheads) show the position (on the same gel) of the rat liver P450IIB1/P450IIB2 mRNAs (2.1 kb) [37] and the minor P450IIB1 form (4.8 kb) [36, 37]. Lanes 1–6: 15 μg each of cytoplasmic RNA from cells exposed to 0, 0.1, 1.0, 5.0, 10 and 30 μM BA.

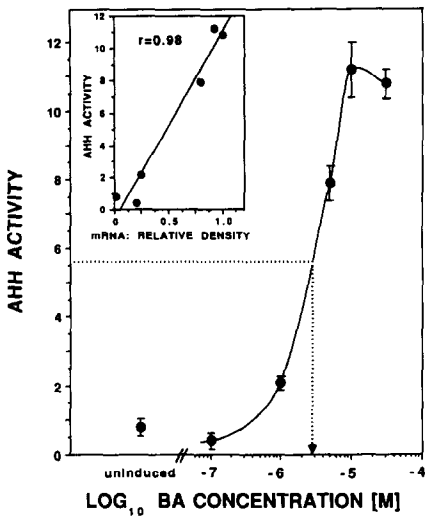

Fig. 5. Log-concentration response curve for AHH induction of BCR-5 cells by BA. Duplicate 50 mL log-phase cultures (7×10^5 cells/mL) were incubated for 24 hr with the concentrations of BA shown on the abscissa. An 8-mL portion of each culture was then removed and assayed for AHH activity, and the remaining 42 mL of each set of duplicates were combined for Northern blot analysis of cytochrome P450IA1 mRNA (see Fig. 4). AHH values shown (ordinate) are the means and range (vertical bars) of duplicate cultures. The activity is expressed as units per 8-mL sample. The assay flasks contained an average of $5.4 \pm 0.8 \times 10^6$ cell equivalents ($\pm$ SE, N = 11) based on their DNA content (see Materials and Methods). The basal (uninduced) AHH level for this experiment was 0.77 units per 8-mL sample. Inset: Correlation between induced AHH activity and relative abundance of P450IA1 mRNA. The relative intensity of the bands shown in Fig. 4 was determined by densitometry with the darkest band (30 μM BA, lane 6) set as 1.0. The linear regression line was calculated by the least squares method.

3×10^{-6} M. On the basis of the DNA content of the reaction flasks, the activity was 0.16 units/10^6 cells for uninduced cells and 2.2 units/10^6 cells for maximally induced cells. These values are typical of several experiments on different batches of BCR-5 cells. There was a good correlation ($r = 0.98$) between the level of BA-induced AHH activity and relative mRNA abundance (inset, Fig. 5).

DISCUSSION

The *Ah* receptor mediates the induction of cytochrome P450IA1 by compounds such as 3-methylcholanthrene (MC) and TCDD [1, 2]. The mechanism of action is thought to be similar to that of steroid hormones [38]. A cytosolic receptor protein, the *Ah* receptor, binds TCDD with high affinity and low capacity. Following binding of TCDD, the ligand–receptor complex undergoes a transformation such that the complex acquires high affinity for DNA and interacts with dioxin responsive elements located in the 5′ flanking region of the P450IA1 gene. This enhances P450IA1 mRNA synthesis [27, 39–45]. AHH is one of the best characterized activities associated with P450IA1.

Until recently, attempts to identify the *Ah* receptor in human tissues have met with little success. Manchester *et al.* [21] described a modified assay procedure for determining the *Ah* receptor levels in human placenta. The major features of this assay were the inclusion of 20 mM sodium molybdate in the homogenizing buffer to stabilize the receptor and a significant reduction in the amount of dextran-coated charcoal that was used to adsorb nonspecifically bound or free radioligand. Although sodium molybdate was found to have only a partial stabilizing effect on rodent *Ah* receptor, it was found to be essential for stabilizing the *Ah* receptor in human placenta [21]. Similarly, for the *Ah* receptor in the human BCR-5 cells, inclusion of 20 mM sodium molybdate and a reduction in the amount of charcoal facilitated detection of relatively high levels of *Ah* receptor in this B lymphoblastoid cell line.

The maximum concentration of *Ah* receptor detected with the modified assay in BCR-5 cell cytosol was about 200 fmol/mg protein. Recently Gillner *et al.* [24] reported the identification of *Ah* receptor in human leukocytes isolated from peripheral blood. Because of the low amount of specific binding of [^{3}H]TCDD in samples from individual donors, leukocytes of several donors had to be combined. The maximum amount of specific [^{3}H]TCDD binding to the combined leukocyte cytosols was 42 fmol/mg protein as detected by electrofocusing on polyacrylamide gels [24].

Similarly, Greenlee *et al.* [46] have reported an *Ah* receptor concentration of only about 30 fmol/mg in thymic lymphocytes as compared to cultured thymic epithelium (about 90 fmol/mg protein). The higher receptor levels detected in our assay may be due to the fact that LCL are blastoid cells and are thus comparable in structure and metabolic activity to mitogen-activated lymphocytes rather than to the unactivated thymus or peripheral blood leukocytes used in the above studies [24, 46]. Circulating lymphocytes have little or no inducible AHH activity unless they are first activated *in vitro* by mitogens to produce blast cells [13]. Whether the level of *Ah* receptor in unactivated lymphocytes increases during mitogenic activation is unknown.

It is also possible that the higher *Ah* receptor level of BCR-5 reflects a real difference between B and T lymphocytes. Epstein–Barr virus-immortalized LCL are pure cultures of B lymphocytes [47], whereas both thymus and peripheral blood lymphocytes are mainly T cells [48]. It should be noted here that unfractionated peripheral blood leukocyte suspensions prepared by the method used by Gillner *et al.* [24] typically contain 60–80% T cells, less than 10% B cells, and from 20–40% monocytes [49, 50]. Since freshly isolated blood monocytes have BA-inducible AHH levels which are, on the average, five times higher than mitogen-activated lymphocytes isolated from the same donor [51], it is possible that the *Ah* receptor detected by Gillner *et al.* in combined leukocyte cytosols originated from monocytes rather than from lymphocytes. Recent data from our laboratory indicate that human tonsils contain high levels of *Ah* receptor [52]; the lymphocyte population in tonsils is about 80% B cells and 20% T cells in contrast to peripheral blood in which the ratio of B cells to T cells is almost the reverse. The high receptor levels in tonsils suggest, again, that B cells have a high concentration of *Ah* receptor. Although there has not yet been a direct comparison of the relative levels of *Ah* receptor in T and B lymphocytes, a higher level in B cells (with a consequent increase in AHH inducibility) would render them more susceptible to the immunosuppressive and carcinogenic efforts of xenobiotics. This would be consistent with the observations that TCDD preferentially inhibits B cell function in adult mice [16–18], that treatment of mice with low doses of MC induces mostly B cell leukemias and lymphomas [6], and that human exposure to chlorophenoxy herbicides seems to be exclusively associated with non-Hodgkin's lymphomas [9] which are mostly (over 90%) B cell tumors [53]. The human population, however, is extremely heterogeneous, and the wide range in *Ah* receptor concentrations between the 200 fmol/mg reported here and the 30–40 fmol/mg reported elsewhere [24, 46] also could be the result of genetically determined differences in the *Ah* receptor protein in the human population.

The relatively high concentration of Ah receptor detected in tbe BCR-5 cells allowed characterization of the kinetics of ligand binding to the *Ah* receptor. Apparent binding affinities were calculated from saturation data by both Scatchard and Woolf plot analyses. The apparent K_d of about 5 nM agrees very well with the recently reported values obtained for other human cell lines, namely the adenocarcinoma LS 180 cell (about 5 nM)* and the liver hepatoma HEPG2 (about 10 nM) [23]. In general, the human *Ah* receptor appears to have a lower affinity for TCDD as compared to the rodent receptor (about 1 nM when determined using the same assay conditions).

The data presented here clearly demonstrate the presence, in a human B lymphoblastoid cell line, of *Ah* receptor which is similar in characteristics to that identified in other human cell lines. The presence of the *Ah* receptor of itself is not sufficient for AHH induction. The induction mechanism is a multistep process involving transformation of the receptor–ligand complex to a nuclear binding component resulting in the induction of AHH activity. This whole mechanism appears to be functional in BCR-5 cells since not only were high levels of *Ah* receptor detectable but it was also possible to extract and identify nuclear associated receptor–ligand complex and to detect substantial amounts of P450IA1 mRNA and corresponding levels of AHH activity after exposure to BA. The human B lymphoblastoid cell line BCR-5, therefore, has a complete regulatory mechanism for *Ah* receptor-mediated induction of cytochrome P450IA1 that is essentially the same as the mechanism that has been well established in many rodent species. Allelic differences at the murine *Ah* locus are responsible for marked individual variations in the response to aromatic hydrocarbon-induced carcinogenesis, toxicity and teratogenesis [54], and it is possible that genetic variation in the *Ah* receptor also exists in humans. Because of their accessibility and their potential as *in vivo* targets for aromatic hydrocarbon-mediated carcinogenesis and toxicity, lymphocytes (and B-LCL established therefrom) provide a useful experimental system for the investigation of possible genetically determined differences in the human *Ah* receptor.

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