EXPRESSION OF THE AH RECEPTOR IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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The Ah receptor is a cytoplasmic high affinity binding protein for 2,3,7,8-tetrachlordibenzo-p-dioxin (TCDD)[1]. In addition to TCDD, a number of its structural analogs such as the polychlorinated dibenzofurans also interact with the Ah receptor and produce the same spectrum of responses as TCDD in animal and cell models. The potency of these compounds is strongly correlated with binding affinity to the Ah receptor and it is generally accepted that most, if not all, TCDD's effects require binding to the Ah receptor. These effects include teratogenicity, carcinogenicity, immunotoxicity and a variety of biochemical effects involving drug-metabolizing enzymes and growth factor pathways [2,3]. Some of these effects have been observed in humans exposed accidentally or occupationally to TCDD or the PCDFs. However, there appears to be great inter-individual variation in the response of humans to TCDD. Our studies are designed to determine if variations in Ah receptor levels could explain inter-individual differences in the human response. If this is the case, Ah receptor levels could be used as a biomarker of susceptibility for TCDD exposure. We have therefore investigated optimal conditions for expression of the Ah receptor in peripheral blood lymphocytes, a tissue which is readily available for epidemiological studies. Ah receptor levels were quantitated from individuals using a sensitive photoaffinity labeling procedure using the TCDD analog I^{125}-2-azido-3-iodo-7,8-dibromodibenzo-p-dioxin [4]. The results demonstrate that the Ah receptor is expressed in human peripheral blood lymphocytes and that optimal expression occurs following 3 days of culture in the presence of mitogens.

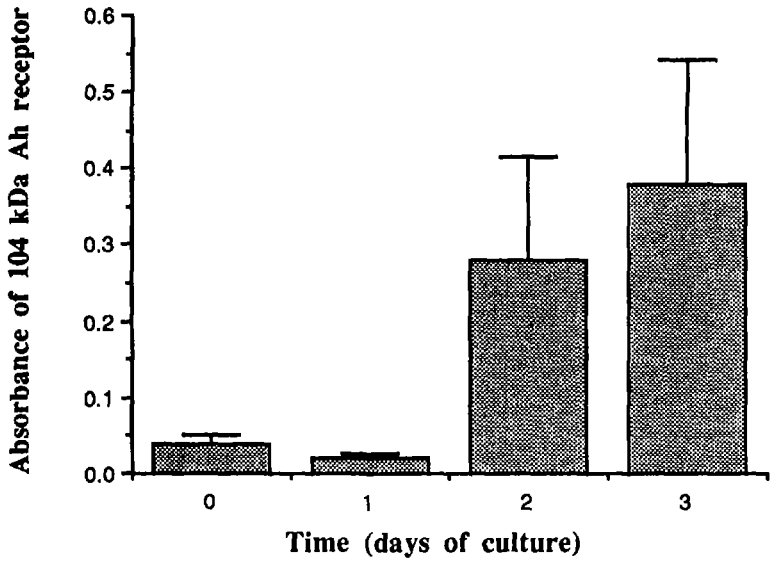
Materials and Methods:

Blood was drawn from healthy volunteers from North Carolina and peripheral blood lymphocytes purified using the Accuspin System (Sigma Chemical Co.). Cells were cultured in RPMI 1640 containing 10% Fetal Calf Serum at 1 x 10^6 cells/ml in the presence of optimal concentrations of the mitogens PHA and PWM. Cells were cultured for various time periods and cytosol fractions isolated by sonicating the cells in Hepes buffer containing 20 mM $NaMO_4$ and centrifugation at 100,000 X g for 30 minutes. Cytosols were assayed for protein with the Pierce Coomassie protein assay and all samples adjusted to 100 µg/ml. Cytosols were photoaffinity labeled with I^{125}-2-azido-3-iodo-7,8-dibromodibenzo-p-dioxin as previously described by Poland et al [4]. The samples were electrophoresed in 7.5% homogeneous polyacrylamide gels, the gels were dried and autoradiographed. Specific binding was detected in a 104 kDa band as demonstrated by inhibition of binding in the presence of a 200 fold molar excess of 2,3,7,8-tetrachlordibenzofuran The autoradiographs were scanned with an LKB laser densitometer and absorbance of the 104 kDa band analyzed.

Results and Discussion:

Shown below is the time course of expression of the 104 kDa Ah receptor protein in 3 normal individuals. Ah receptor protein was barely detectable in freshly isolated cells as well as in cells that were in culture for 1 day. By day 2 the expression of the receptor increased dramatically and by day 3 optimal expression was observed.

Time Course of Ah Receptor Expression in Cultured Human Lymphocytes



In addition to the toxic effects of TCDD, it produces a number of biochemical effects such as induction of CYP1A1, down regulation of binding activity of the estrogen and epidermal growth factor (EGF) receptors and changes in cytokine pathways [5,6]. These effects could suggest that the Ah receptor may play a role in cell cycle regulation. In human peripheral blood lymphocytes optimal expression of the receptor occurs at day 3 when the cells are actively dividing. We are investigating further if there is a relationship of Ah receptor expression and progression through the cell cycle in lymphocytes.

We are proceeding to investigate inter-individual variation in expression of the receptor in a cohort of 36 normal individuals. This cohort contains individuals that can be assigned into 3 groups of high, low and intermediate responders based on their induction of CYP1A1 enzyme activity following in vitro exposure to TCDD which has also been observed by others[7,8,9]. It will be interesting to determine if Ah receptor levels are responsible for the inter-individual variation observed in CYP1A1 induction. Furthermore, epidemiological evidence also suggest large inter-individual differences in human responsiveness to dioxin exposure, in that some individuals exposed to equivalent levels of TCDD in the Seveso exposure incident developed chloracne while other individuals did not. The reason for these

inter-individual differences in susceptibility may be due to variation in receptor number or receptor affinity if the receptor is the rate limiting event in the final biological response. We are currently investigating receptor expression in human populations that have been exposed to TCDD and other related compounds to determine if their is a relationship of receptor expression to biological responses observed in humans.

References:

1 Poland A, Knutson J. 2,3,7,8-Tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: Examination of the mechanism of toxicity. *Annu. Rev. Pharmacol. Toxicol.* 1982;22:517-54.
2 Silbergeld E, Gasiewicz T. Dioxins and the Ah receptor. *Amer. J. Indust. Med.* 1989;16:455-74.
3 Zeise L, Huff J, Salmon A, Hooper N. Human risks from 2,3,7,8-tetrachlorodibenzo-p-dioxin and hexachlorodibenzo-p-dioxins. *Adv. Mod. Environ. Toxicol.* 1990; 17:293-342.
4 Poland A, Glover E, Ebetino F, Kende A. Photoaffinity labeling of the Ah receptor. *J. Biol. Chem.* 1986; 261:6352-65.
5 Clark G, Tritscher A, Maronpot R, Foley J, Lucier G. Tumor promotion by TCDD in female rats. In: Gallo MA, Scheuplein RJ, Vander Heijden KA, eds. *Banbury Report 35: Biological Basis for Risk Assessment of Dioxins and related compounds.*Cold Spring Harbor Laboratory Press, 1991:389-404.
6 Clark G, Taylor M, Tritscher A, Lucier G. Tumor necrosis factor involvement in 2,3,7,8-tetrachlordibenzo-p-dioxin-mediated endotoxin hypersensitivity in C57BL/6J mice congenic at the Ah locus. *Toxicol. Appl. Pharmacol.* 1991;111:422-31.
7 Clark GC, Tritscher AM, Bell DA, Lucier GW. Integrative approach for evaluating species and interindividual differences in responsiveness to dioxins and structural analogs. *Env. Health Perspec.*1992; 98: in press.
8 Kouri R, Ratrie H. Aryl hydrocarbon hydroxylase induction in human lymphocyte cultures by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Life Sciences* 1974;15:1585-1594.
9 Jaiswal A, Gonzalez F, Nebert D. Human P1-450 gene sequence and correlation of mRNA with genetic differences in benzo[a]pyrene metabolism. *Nucleic Acids Research* 1985;13:4503-20.