PROLIFERATION OF SPLENIC LYMPHOCYTES IS INHIBITED MORE STRONGLY BY 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN IN C57BL/10 (Ah^+Ah^+) MICE THAN IN DBA/2 (Ah^-Ah^-) MICE

A. L. Pavlyak, S. M. Wielgosz, and E. Hüttner

UDC 616.411-008.953.2-02:[547.91:665.6.033.25]-092.9-07

KEY WORDS: lymphocyte proliferation; spleen; TCDD.

Pure lines of mice differ greatly in their ability to induce hydroxylases of polycyclic aromatic hydrocarbons (PAH) or benz(a)pyrene (BP). Hybrids between mice of the C57BL and DBA/2 lines inherit ability to induce BP hydroxylase (BPH) as a dominant, nonsex-linked trait [6]. This gene (Ah) is located in chromosome 17 of mice [5]. It plays the role of derepressor of transcription and synthesis of three isoforms of cytochrome P-450 (P_1-450, P_2-450 and P_3-450) [10], whose genes are located in mouse chromosome 9 [12]. BPH induction is controlled by an Ah-receptor, the product of the Ah locus, which binds the inducer and transports it into the nucleus. Among inducers of the Ah locus and the family of three cytochrome P-450 genes there are carcinogens – such as 3-methylcholanthrene, but the strongest of them is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In the presence of TCDD in the liver cytosol of mice sensitive to induction of BPH hydroxylase (C57BL, Ah^+Ah^+), 10-15 times more TCDD is bound with the Ah receptor than in the cytosol of mice not inducing BPH (DBA/2, Ah^-Ah^-) [2].

Depending on the method of application of the carcinogen, increased sensitivity to carcinogens of the PAH group is exhibited either in C57BL mice (carcinoma of the skin following percutaneous application of PAH) [4] or in DBA/2 mice (aplasia of bone marrow or leukemia after administration of PAH by the gastric route) [7, 8].

Besides genetic differences in the Ah locus, these tissue-specific differences in the action of carcinogens of the PAH gruop are affected also by tissue differences in expression of the Ah genes, which determined metabolic activation of PAH. TCDD is not only an inducer of BP hydroxylase and a teratogen [3], but it also inhibits proliferation of thymocytes in C57BL mice [1]. The aim of this investigation was to study differences between lines of mice differing in alleles of the Ah locus, with respect to inhibition of proliferation of splenic lymphocytes under the influence of TCDD.

EXPERIMENTAL METHOD

Male C57BL/10 and DBA/2 mice weighing 30 g were used. Splenic lymphocytes were obtained by Klingerman's method [9]. A suspension of lymphocytes with a density of 10^6 cells/ml was cultured in 200-ml samples on Titertek micropanels in Eagle's medium with the addition of 15% embryonic calf serum and mitogen (concanavalin A or phytohemagglutinin – PHA) in the presence of 5% CO_2 and at 37°C. After culture for 24 h, TCDD dissolved in dimethylsulfoxide in a concentration of 10^{-6} M was added in a volume equivalent to 1% of the volume of the culture. ^{3}H-Thymidine (0.5 mCi per culture, from UVVVR, Prague, specific activity 20 Ci/mmole) was added 67 h after the beginning of incubation for 3 h. The cells were adsorbed on glass fiber filters. Radioactivity of the dried filters was measured on a scintillation counter.

EXPERIMENTAL RESULTS

The effect of TCDD on proliferation of splenic lymphocytes of C57BL and DBA/2 mice was studied. In cultures stimulated to proliferation by con A, proliferation of lymphocytes from

Institute of Human Genetics, Polish Academy of Sciences, Poznan. Central Institute of Genetics and Study of Agricultural Crops, Academy of Sciences of the GDR, Gatersleben. (Presented by Academician of the Academy of Medical Sciences of the USSR, N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 3, pp. 331-333, March, 1989. Original article submitted May 30, 1988.

0007-4888/89/0003-0365$12.50

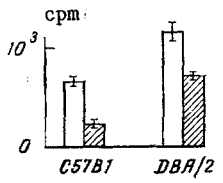

Fig. 1

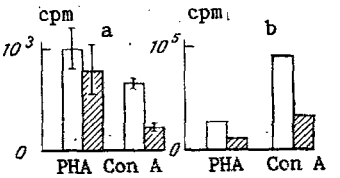

Fig. 2

Fig. 1. Effect of alleles of Ah gene on inhibition of proliferation of splenic lymphocytes, stimulated by con A. C57BL (Ah^+Ah^+) and DBA/2 (Ah^-Ah^-) mice were used. Here and in Fig. 2: unshaded columns – control cultures, shaded – treatment with 10^{-8} M TCDD.

Fig. 2. Effect of TCDD on proliferation of splenic lymphocytes of C57BL mice (a) compared with proliferation of thymocytes (according to Greenlee [1]) (b).

C57BL mice was inhibited by the action of TCDD by 65%. This effect was much less in cultures of DBA/2 mouse lymphocytes: proliferation was inhibited by 42% (Fig. 1).

No effect of TCDD on proliferation was found in lymphocyte cultures stimulated to proliferation by PHA in either line of mice. The results of experiments on C57BL mouse cells are shown in Fig. 2a.

Greenlee and coworkers [1] described inhibition of proliferation fo C57BL mouse thymocytes in the presence of TCDD (Fig. 2b) and explained this effect by the pleiotropic action of genes of the Ah locus. Inhibition of thymocyte proliferation also was observed in cultures stimulated by PHA. However, these cells were cultured by the above-mentioned workers with the use of a layer of "nurse" cells, thereby changing the conditions of proliferation. They likewise did not conduct experiments on Ah^-Ah^- mice or on tissues distinguished by the presence of an Ah receptor (TCDD).

We found that lymphocyte proliferation was inhibited in the presence of TCDD by a lesser degree in Ah^- mice (DBA/2) than in Ah^+ mice (C57BL). This confirms the mechanism of action of TCDD on lymphocyte proliferation through the Ah receptor. This function of the Ah receptor is additional to its role in the regulation of cytochrome P-450 synthesis.

Our observations apply both to interlinear and intertissue differences in sensitivity to carcinogens of the PHA group. We showed that DBA/2 (Ah^-Ah^-) mice were more resistant to inhibition of proliferation by TCDD (Fig. 1). This is in agreement with their resistance to the teratogenic effects of TCDD [8, 11]. lymphocytes stimulated by PHA did not exhibit inhibition of proliferation in the presence of TCDD (Fig. 2a). PHA may perhaps stimulate proliferation of cells distinguished by weaker activity of the Ah gene among splenic lymphocytes.

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