Biochemical Pharmacology, Vol. 27, pp.149-151.

BENZO[a]PYRENE-INITIATED LEUKEMIA IN MICE
Association with Allelic Differences at the Ah Locus

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(Received 14 September 1978; accepted 2 October 1978)

The murine Ah locus regulates the induction of more than 20 mono-oxygenase "activities" and associated cytochromes P_1-450 and P-448 (and probably several other forms of P-450) by 3-methylcholanthrene and many other polycyclic aromatic hydrocarbons. Regulation of responsiveness probably involves several alleles at more than one locus, but differences between C57BL/6 (responsive, Ah^b) and DBA/2 (nonresponsive, Ah^d) mice can be almost completely explained by the difference at the Ah locus. Heterozygotes (Ah^b/Ah^d) are responsive, but other genetic crosses between appropriate inbred strains can result in the expression of additive inheritance or a situation in which the lack of responsiveness is dominant. Responsiveness occurs not only in liver but also in numerous nonhepatic tissues such as lung, kidney, bowel, skin, lymph nodes, retinal pigmented epithelium of the eye, brain, ovary, uterus, mammary gland, and bone marrow (reviewed in Refs. 1 and 2).

Numerous cancerogenic and toxicologic phenomena in mice have been shown to be associated with allelic differences at the Ah locus, presumably due to quantitative and/or qualitative differences in the metabolism of carcinogens or drugs by increased amounts of P_1-450 (and perhaps other forms of P-450) in many tissues. For example, compared with Ah^d/Ah^d individuals, Ah^b/Ah^b homozygotes and Ah^b/Ah^d heterozygotes have a greater susceptibility to 3-methylcholanthrene (MC)- and benzo[a]pyrene (BP)-initiated subcutaneous sarcomas and MC-initiated lung tumors (reviewed in Ref. 1). On the contrary, large doses of oral BP (100-125 mg/kg/day) produce aplastic anemia in Ah^d/Ah^d mice, whereas the Ah^b/Ah^b and Ah^b/Ah^d individuals--who are more susceptible to solid tumors at the site of MC or BP inoculation--are extremely resistant to oral BP-induced bone marrow toxicity (3). This phenomenon is probably explainable by the "first-pass elimination" effect: the relatively high degree of aryl hydrocarbon hydroxylase induction in the bowel (400- to 800-fold) and liver (2- to 3-fold) of Ah^b/Ah^b and Ah^b/Ah^d mice results in the rapid metabolism of BP to innocuous products excreted in the feces and urine, when compared with the hydroxylase induction of less than 70-fold in the bowel and no induction in the liver of Ah^d/Ah^d mice (4). More BP actually enters and becomes covalently bound in the bone marrow of Ah^d/Ah^d mice than of Ah^b/Ah^b or Ah^b/Ah^d mice, and this covalent binding is inhibited by oral α-naphthoflavone (ANF)--suggesting that BP toxicity in these susceptible individuals is in fact mediated by P_1-450 in the bone marrow (J. S. Felton, R. C. Levitt, N. M. Jensen and D. W. Nebert, manuscript in preparation).

Massive doses of 100-125 mg BP ingested/kg/day produce bone marrow toxicity and death in 100 per cent of Ah^d/Ah^d mice in less than 4 weeks; no responsive Ah^b/Ah^b or Ah^b/Ah^d mouse develops aplastic anemia even when this dose is continued for 6 months (3). Because these

are such large doses of BP, we wondered how small a dose of oral BP would still cause an effect associated with the Ah locus.

Figure 1 shows the results of groups of 30 Ah^d/Ah^d or Ah^b/Ah^d mice which received estimated doses of 12 (left) or 6 (center) mg BP/kg/day. Differences in weight gain attributed to allelic differences at the Ah locus were detectable. To our surprise, however, the mice which became ill and began dying did not have hypoplastic or aplastic bone marrow but rather developed hematopoietic neoplasms, especially of the lymph nodes, spleen and thymus. No increased incidence of leukemia or differences in weight gain between Ah^d/Ah^d and Ah^b/Ah^d mice were found at estimated doses of 1.2 mg BP/kg/day in the diet for 240 days (data not illustrated).

When ANF was added to the diet at a dose 20 times greater than that of BP (right), the incidence of leukemia was prevented almost completely and the general health of the Ah^d/Ah^d mice remained as good as Ah^b/Ah^d mice receiving 12 mg BP/kg/day. These data suggest that ANF-sensitive metabolism of BP--presumably cytochrome P_1-450--in the bone marrow of Ah^d/Ah^d individuals is responsible for producing the reticuloendothelial malignancies. As is the case with the higher dose of BP (3, 4), we believe that these results represent "first-pass elimination" kinetics. A very recent study on Ah^d/Ah^d mice being more susceptible than Ah^b/Ah^d mice to leukemia produced by percutaneously applied MC (7) can probably also be explained on the basis of "first-pass elimination" kinetics. Obviously the presence or

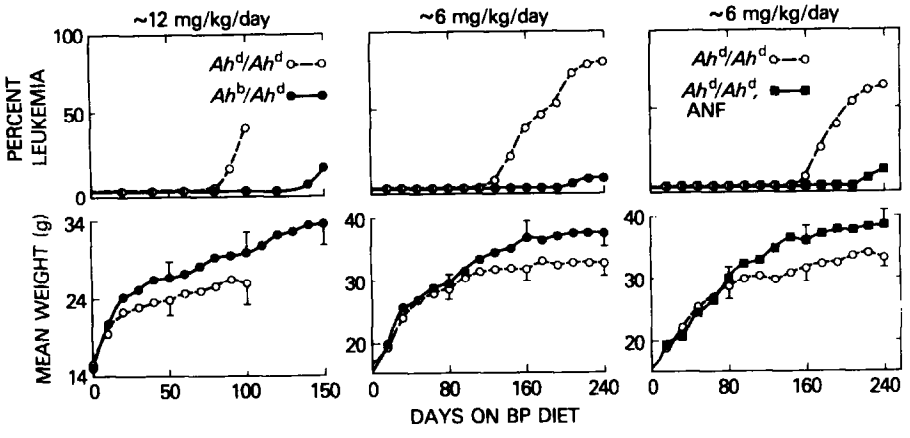

Fig. 1. Per cent incidence of leukemia and mean weight gain for groups of 30 Ah^d/Ah^d or Ah^b/Ah^d mice receiving oral BP at about 12 or 6 mg/kg/day. Each symbol represents the mean of 30 (or less, if some had died) mice in the group; brackets represent standard deviations. Weanlings from the (C57BL/6N)(DBA/2N)F_1 x DBA/2N backcross were phenotyped by the zoxazolamine paralysis test, as described previously (5). Ten days later the BP diet, prepared as described previously (3), was begun. In the case of ANF (right), approximately 120 mg ANF/kg/day was included with the 6 mg BP/kg/day. Wasted animals were killed and studied when it was judged that they probably would not live more than 1-2 days longer. We are grateful to Drs. Lawrence Corash, Michael M. Orlando and Alan S. Rabson for their advice about performing autopsies and examining histological sections of lymph nodes, spleen, thymus, bone marrow, kidney and liver. Whole blood counts were not especially helpful in the diagnosis of hematopoietic tumors. Lymphocytic leukemias, apparent stem-cell leukemias, and reticulum-cell neoplasms were all scored as "leukemia," according to the classification and description by Murphy (6). At an estimated 12 mg BP/kg/day (left), all Ah^d/Ah^d mice died before 110 days on the diet; none of the starting 30 Ah^b/Ah^d had died by day 100, and three had died after 150 days. At an estimated 6 mg BP/kg/day (center), 24 of the starting 30 Ah^d/Ah^d mice and two of the starting 30 Ah^b/Ah^d mice had died after 240 days on the diet. At an estimated 6 mg BP/kg/day (right), 19 of the starting 30 not receiving ANF had died and four of the starting 30 receiving ANF had died after 240 days on the diet.

absence of murine leukemia virus expressed by the various inbred strains (8) will modify the response elicited by P_1-450-mediated metabolism of polycyclic hydrocarbons under the control of the Ah locus.

There is evidence for the Ah locus in the human, and several reports suggest a correlation between aromatic hydrocarbon responsiveness and an increased incidence in certain types of solid tumors such as bronchogenic carcinoma and laryngeal carcinoma (reviewed in Refs. 1, 2 and 9). It would be interesting to examine the possibility that "low responsive" families have an increased incidence of leukemia whereas "high responsive" families have an increased incidence of solid tumors and other neoplasms. Ingestion of charcoal-cooked foods (10) and cigarette smoking (11-13) are both known to induce drug-metabolizing enzyme activities in the human.

In summary, at oral BP doses estimated to be between 6 and 12 mg per kg body weight per day, leukemia develops 100 or more days later in the nonresponsive Ah^d/Ah^d homozygous mouse but not in the responsive Ah^b/Ah^d heterozygote. These results may be explained on the basis of "first-pass elimination" kinetics. An excess of dietary ANF 20 times greater than the amount of BP prevents these hematopoietic neoplasms. These data suggest that ANF-sensitive metabolism of BP--presumably cytochrome P_1-450 in the bone marrow of Ah^d/Ah^d individuals--is responsible for causing the leukemia.

Acknowledgement--The expert secretarial assistance of Ms. Ingrid E. Jordan is greatly appreciated.

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