Aryl Hydrocarbon Hydroxylase Activity in Subpopulations of Peripheral Blood Mononuclear Cells

James R. Jett, John D. Stobo, and Harold L. Moses

ABSTRACT

Peripheral blood mononuclear cells (PMC), isolated by density gradient techniques with Ficoll-Hypaque, contain T- and B-lymphocytes and monocytes. Aryl hydrocarbon hydroxylase (AHH) activity was measured in PMC subfractions consisting of T-lymphocyte-enriched, T-lymphocyte-depleted, and monocyte-depleted populations. The T-cell-enriched populations consistently showed enhancement of AHH activity with both the fluorometric and radiometric technique when compared to the total PMC population. This enhanced AHH activity was observed when the T-cell-enriched populations were isolated either before or after 96 hr of lymphocyte culture, by the sheep red blood cell rosette method, or by the nylon wool column technique before lymphocyte culture. T-cell-depleted populations (B-cell enriched) obtained by sheep red blood cell rosette method had diminished AHH activity.

Monocytes were shown to contribute to the total PMC AHH activity through an indirect technique by first depleting the monocytes from PMC with the carbonyl iron method. The monocyte-depleted populations had less AHH activity than did the total PMC population after both 24 and 96 hr of culture. The greatest amount of AHH activity was present in PMC populations with their native number of monocytes when cultured for 96 hr in the presence of mitogens.

INTRODUCTION

AHH (2), a membrane-bound microsomal mixed-function oxygenase, may be one of the primary factors in human susceptibility to carcinogenesis. AHH functions in metabolizing polycyclic aromatic hydrocarbons to epoxides (6, 25) that may bind to DNA and other macromolecules, be changed to other products such as phenols and dihydrodiols, or be conjugated to glutathione (26). Some of the metabolites, such as the dihydrodiols, may recycle through the AHH system to produce secondary metabolites such as the diol-epoxide, which may be even more mutagenic and carcinogenic than is a primary oxide (15, 22).

Interest in AHH has arisen as a possible practical clinical assay to determine inherited capability to metabolize polycyclic aromatic hydrocarbons and to indicate predisposition to developing bronchogenic carcinoma (17). Evidence has been presented that AHH inducibility as reflected by the inducibility ratio (ratio of 3-ethylcholanthrene induced to uninduced base-line levels of AHH activity in mitogen-stimulated PMC) is an inherited trait (1, 16). The AHH inducibility ratio has also been reported to be elevated in mitogen-stimulated PMC from patients with carcinoma of the lung (17) and larynx (28) as compared to control populations. G handicraft (7) have reported elevated induced AHH activity in lung cancer patients. However, conflicting data have been reported suggesting that AHH inducibility may not be elevated in patients with lung cancer (12, 23), in contrast to the original report of Kellermann et al. (17). In addition it has been shown that AHH activity is related to the degree of mitogen stimulation of lymphocytes and that patients with lung cancer have a diminished mitogenic response (12).

In most studies PMC have been isolated by a density gradient technique involving polysucrose-metrizoate (3). PMC obtained by this method contain both lymphocytes and monocytes (19). The lymphocyte population is known to contain both thymus-derived cells and bursa equivalent-derived cells (13). All of the studies on AHH and PMC have related the activity to a base line of total cell number, DNA content, or protein content. If the different cell populations in PMC do not have equal AHH activity, variations in cell populations in PMC can cause alterations in AHH activity independent of the genetically determined capability for metabolizing polycyclic aromatic hydrocarbons.

Bast et al. (2) have shown that isolated peripheral blood monocytes have measurable AHH levels without prior mitogen exposure and that AHH activity can be induced 6.5- to 37-fold by benzanthracene. However, the relative contribution of monocytes to the AHH activity in mitogen-stimulated total PMC populations after 96 hr in culture is not known.

In the present study we have investigated the relative contribution of the 3 major cell populations in PMC to the total AHH activity measured with both radiometric and fluorometric assays. This was accomplished by decreasing the heterogeneity of the PMC and determining the AHH activity in the more homogenous cell populations. Preparations enriched with T-cells clearly show greater AHH activity than did the native PMC population, while preparations enriched with B-cells exhibited a diminished AHH activity. Monocytes were shown to influence induced AHH activity after both 24 and 96 hr in culture. The data indicate that T-lymphocytes provide a major contribution to the AHH activity measured in mitogen-stimulated total PMC after 96 hr in culture.
MATERIALS AND METHODS

Lymphocyte Culture. Venous blood was collected in sterile heparinized tubes, and PMC were isolated by Hypaque-Ficoll gradient centrifugation as previously described (3, 5). The PMC, as well as the subpopulations of PMC, described below were cultured by the same method as previously reported (12). All cultures, except where otherwise stated, were in the presence of 1% phytohemagglutinin and 1% pokeweed mitogen. One lot of fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.; Lot C762518) was used for all fluorometric assays, and one lot of fetal calf serum (Reheis, Phoenix, Ariz.; Lot L15412) was used for all radiometric assays (1).

Preparation of PMC Subpopulations. After isolation of PMC by the Hypaque-Ficoll procedure, populations of cells enriched for T- or B-lymphocytes were obtained by the SRBC rosette technique as described previously (13, 27). Another method used to obtain a T-cell-enriched population consisted of passing the PMC over columns prepared by packing plastic syringes with washed nylon wool (LP-1, Leukopak; Fenwal Laboratories, Morton Grove, Ill.) equilibrated with 10% heat-inactivated fetal calf serum at 37°C. The effluent cells consisted of an enriched T-cell population (14).

Monocytes were depleted from PMC by incubation of the mononuclear cells with carbonyl iron (Technicon Instruments Corp., Tarrytown, N. Y.) followed by passage of cells through a magnetic field (27). Effluent populations obtained by this method were evaluated for their relative frequency of phagocytes following ingestion of latex particles (27) (Dow Chemical Co., Indianapolis, Ind.) and for their relative frequency of nonlymphocyte mononuclear cells demonstrated by staining for cytoplasmic esterase (18).

Radio metric Assay for AHH Activity. The radio metric assay for AHH used in this study was the same as previously reported (12). After 72 hr of culture, cells were induced with unlabeled BP (1 μg/ml) (Eastman Kodak Co., Rochester, N. Y.). Twenty-four hr later the cells were harvested, counted, and assayed. Base line samples of 1.5 ml, containing 7.5 × 10⁸ cells, were taken immediately, and the reaction was stopped with 1 ml of acetone. The remaining cells were incubated in assay medium for 8 hr at 37°C. At 8 hr, samples were assayed (Reheis, Phoenix, Ariz.; Lot L15412) was used for all radiometric assays (1).

Fluorometric Assay for AHH Activity. The fluorometric assay for AHH used in this study was the same as that previously described (16, 20), except that unlabeled BP (1 μg/ml) was used to induce AHH activity instead of 3-methylcholanthrene. The BP was added to one-half of the cells after 72 hr in culture, and the induced and uninduced levels of AHH activity were determined 24 hr later as previously described (16, 17). All samples were run in triplicate, and the fluorescence was corrected to 10⁶ cell equivalent. Fluorescence was then converted to fmoles from a standard curve derived in our laboratory with the use of 3-hydroxybenzo(a)pyrene (obtained from the IIT Research Institute, Chicago, Ill., through the National Cancer Institute Carcinogenesis Research Program). The AHH activity is expressed as units/10⁶ cells. An AHH unit is defined as the amount of enzyme catalyzing, per min at 37°C, the formation of hydroxylated products causing fluorescence equivalent to that of 1 fmoles of 3-hydroxybenzo(a)pyrene.

RESULTS

T-Cell-enriched Populations Separated by SRBC Rosette before Culture. The mononuclear cell population separated by the SRBC rosette technique has been shown to contain approximately 92% SRBC rosette-forming cells (T-cells) (27), while total PMC populations contain approximately 70% T-cells. These T-cell-enriched populations also contain approximately 1 to 2% immunoglobulin-bearing cells and 2 to 4% esterase-positive cells (27).

In one set of experiments, T-cell populations were obtained by the SRBC rosette technique before the lymphocytes were cultured. The T-cell-enriched populations were then cultured in the presence of variable concentrations of phytohemagglutinin (0.5, 1, and 2%), since T-enriched populations may require a concentration of phytohemagglutinin for optimum blastogenesis different from that of total PMC. With the 1 and 2% concentrations of phytohemagglutinin, the T-cell-enriched populations showed an enhancement of AHH activity over the total PMC population from the same patient cultured at the same concentrations of phytohemagglutinin as determined by the radiometric procedure (Table 1). At the 0.5 concentration of phytohemagglutinin, the AHH activity of the T-cell-enriched population was slightly less than that in the total PMC.

T-Cell-enriched Population Separated by Nylon Wool Column before Culture. In another set of experiments, non-T-cells were removed from the total PMC by allowing them to adhere to nylon wool (14). The nonadherent T-cell-enriched population was again shown to have elevated AHH activity as determined by the radiometric method relative to the total PMC. In this experiment, the induced AHH level of the PMC population was 985 cpm/2.5 × 10⁶ cells, whereas the T-cell-enriched population exhibited a 15% greater AHH activity.

T-Cell-enriched Populations Separated by SRBC Rosettes after 96 Hr in Culture. Since T-cell function has been shown to be influenced by monocytes and their products (21), T-cell-enriched populations were separated after 96 hr in culture to examine the influence of cocultivation with monocytes on T-cell AHH activity. With both the radiometric and fluorometric assays, AHH activity was found to be

<table>
<thead>
<tr>
<th>% phytohemagglutinin</th>
<th>Total PMC</th>
<th>T-enriched</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>836⁶</td>
<td>703 (−16)³</td>
</tr>
<tr>
<td>1.0</td>
<td>942</td>
<td>1302 (38)</td>
</tr>
<tr>
<td>2.0</td>
<td>848</td>
<td>1177 (38)</td>
</tr>
</tbody>
</table>

⁶ AHH activity, as determined by radiometric procedure, is expressed as cpm/2.5 × 10⁶ cells.
³ Numbers in parentheses, percentage of enhancement of activity in T-cell-enriched cultures as compared to total PMC.
enhanced in the T-cell-enriched populations as compared to the total PMC (Table 2). The T-cell-enriched population exhibited AHH activity that was 18 to 98% greater than that in the total PMC with the radiometric assay. Both basal (uninduced) and induced levels of AHH were greater in the T-lymphocyte-enriched cells as determined by the fluorometric assay (Table 2).

**T-Cell-depleted Populations Separated by SRBC Rosettes after 96 Hr of Culture.** The T-cell-depleted populations were not assayed in all experiments involving SRBC rosette separation of lymphocytes because of the variable low yields of non-T-cells with this procedure. However, in those experiments in which sufficient cells were present for assay, the T-depleted cells had low levels of AHH activity relative to the total PMC or T-enriched populations as determined by both assay procedures (Table 2).

**Cell Populations Depleted of Monocytes before Culture.** The cell population in the effluent, with the carbonyl iron technique, was found to contain 0 to 1% phagocytic cells following exposure to latex particles as compared to 20 to 22% phagocytic cells in the PMC population. The monocyte-depleted cell population had 6 to 7% esterase-positive cells as compared with 25 to 27% in the PMC.

The PMC-containing monocytes were shown to have significantly higher AHH activity (radiometric assay) than did the monocyte-depleted cell population after 24 hr in culture (Chart 1). AHH activity was still diminished in the monocyte-depleted population as compared to the PMC after 96 hr in culture. Mitogen stimulation of monocyte-depleted cell populations caused an increase in AHH activity after 96 hr. Without mitogens, the AHH activity was diminished slightly at 96 hr from those levels observed at 24 hr in both cell populations. The AHH activity in PMC with monocytes was 2.5 times greater than that in the monocyte-depleted population at 96 hr without mitogens. These data indicate that the monocytes do contribute to the AHH activity observed after 96 hr in culture in both mitogen-stimulated and nonstimulated populations.

Mitogen stimulation had a significant effect only after 96 hr of culture, with both cell populations, and the mitogen-stimulated activity was approximately 2-fold that observed without mitogen stimulation. The highest levels of AHH activity were present in PMC populations with their native number of monocytes when cultured for 96 hr in the presence of mitogens (Chart 1).

**DISCUSSION**

In a number of studies AHH activity has been assayed in attempts to determine inherent capabilities for metabolizing polycyclic aromatic hydrocarbons. These studies utilized mitogen-stimulated PMC after 96 hr in culture, and the AHH activity was related to the total number of cells present. PMC, isolated by Ficoll-Hypaque sedimentation, contain at least 3 major populations of cells, monocytes, B-lymphocytes, and T-lymphocytes. Data in this paper strongly suggest that the T-lymphocytes contribute a major amount of the AHH activity found in mitogen-stimulated total PMC. Both radiometric and fluorometric assays for AHH activity were used in these experiments, which demonstrated that T-lymphocyte-enriched cells had higher AHH activity, both basal and induced, than did the total PMC population or T-lymphocyte-depleted cells. The only situation in which T-cell-enriched populations of cells did not show greater activity than did the total PMC was with the use of 0.5% phytohemagglutinin in T-enriched cells separated by SRBC before culture. This result probably reflects diminished blastogenesis in the T-cell-enriched population relative to the total PMC due to the different phytohemagglutinin dose-response curve observed in T-enriched populations relative to the total PMC. The degree of mitogen stimulation has been shown to influence the level of AHH activity (11, 12).

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**Table 2**

<table>
<thead>
<tr>
<th>Cell population</th>
<th>AHH specific activity</th>
<th>Water-soluble metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base-line</td>
<td>Induced</td>
</tr>
<tr>
<td>1. Total PMC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-enriched</td>
<td>125</td>
<td>175</td>
</tr>
<tr>
<td>T-depleted</td>
<td>320</td>
<td>470</td>
</tr>
<tr>
<td>2. T-enriched</td>
<td>56.7</td>
<td>360 (10)</td>
</tr>
<tr>
<td>T-depleted</td>
<td>86 (32)</td>
<td>155</td>
</tr>
<tr>
<td>3. Total PMC</td>
<td>175</td>
<td>110</td>
</tr>
<tr>
<td>T-enriched</td>
<td>320</td>
<td>175</td>
</tr>
<tr>
<td>T-depleted</td>
<td>327</td>
<td>56.7</td>
</tr>
<tr>
<td>4. T-enriched</td>
<td>360 (10)</td>
<td>175</td>
</tr>
<tr>
<td>T-depleted</td>
<td>327</td>
<td>56.7</td>
</tr>
</tbody>
</table>

- Units of AHH activity per 10⁶ cells as determined by the fluorometric assay in uninduced cells (base line) or cells induced with BP (1.0 μg/ml) for 24 hr.
- Ratio of induced to base-line activity.
- AHH activity as determined by the radiometric assay expressed as cpm/2.5 x 10⁶ cells.
- Numbers in parentheses, percentage of enhancement of AHH activity in T-cell-enriched populations as compared to PMC.
- Assay not done.
Gurtoo et al. (8, 9) have previously suggested that T-cells are the primary responding cells in the PMC based on their measurement of AH activity in B-cells and T-cells of leukemia. In our study measurement of AH activity was carried out on nonmalignant T-lymphocyte-enriched and B-lymphocyte-enriched populations, and it should more accurately reflect the normal situation. The data show that mitogen-stimulated T-enriched cells have more AH activity on a per cell basis than do T-depleted cell populations. The T-lymphocytes are the most numerous cells in total PMC populations (approximately 70%): they would, therefore, have the great majority of the AH activity measured in the total PMC population.

Since Bast et al. (2) have reported that monocytes contain significant inducible AH activity even without mitogen stimulation, experiments were carried out to determine the relative contribution of monocytes to the AH activity measured in PMC. The results of these experiments indicate that monocytes do contribute to the total AH activity measured by the radiometric assay at both 24 and 96 hr in culture. This contribution could be due to high levels of metabolism by monocytes or indirectly through augmentation of T-cell AH activity by monocytes. A requirement for monocytes for in vitro T-cell viability and reactivity to mitogens has been reported (21). The possibility of a toxic effect of the carbonyl iron or manipulations associated with this technique on nonmonocytic cells cannot be excluded totally; however, the nonmonocyte cells obtained by this technique were viable as determined by dye exclusion.

The results of these studies indicate that the subpopulations of PMC contain variable amounts of induced and basal AH activity with the T-lymphocyte contributing a major amount of the activity in mitogen-stimulated PMC after 96 hr in culture. As shown in this and previous studies (4, 12, 29), the degree of mitogen stimulation of PMC also influences AH activity in these cells. In addition, mitogen stimulation of lymphocytes has been shown to be decreased in patients with lung cancer (10, 24). The data presented here and in previous reports (11, 12) suggest that studies on AH activity in patients with different cancers should take into account the relative composition of PMC and the degree of mitogen stimulation.

REFERENCES


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