Monoclonal antibodies to cytochrome P-450 immunopurify a 45-kDa protein from a human lymphoblastoid cell line

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Monoclonal antibodies (MAbs) to rat liver cytochromes P-450 have previously been used for successful immunopurification of cytochromes P-450 from animal tissues. We now report application of this MAb-based immunopurification technique to the human lymphoblastoid AHH-1 cell line. Immunopurification carried out with 3 different MAbs each yielded a 45-kDa polypeptide. The purified protein contains an MAb-specific epitope present on cytochromes P-450, and may therefore be a human cytochrome P-450.

Cytochrome P-450 *Aryl hydrocarbon hydroxylase* *Monoclonal antibody* *Immunopurification* *Epitope* *Lymphoblastoid cell*

1. INTRODUCTION

The cytochromes P-450 metabolize a variety of xenobiotic and endobiotic compounds, including drugs, carcinogens, and steroids [1–3]. These enzymes are present in multiple forms [4] which catalyze reactions that convert these substrates to a variety of possible products, including detoxified metabolites and toxic or carcinogenic compounds. This multiplicity thus far has limited progress in the detection and identification of specific isozymes in a tissue by conventional methods, although the capability to distinguish and measure individual P-450s is essential for a variety of studies, including those relating the role of individual P-450s to metabolism of specific substrates.

As an approach to the problem of distinguishing closely related isozymes, monoclonal antibodies (MAbs) to different P-450s have been prepared [5–10]. The MAbs have thus far proven useful for the detection and identification of P-450s by enzyme inhibition assay [11,12], radioimmunoassay [13,14], and immunopurification studies [14,15].

The MAb-based techniques have detected MAb-specific P-450s in individual human tissues and cells, including lymphocytes [11]. The cells previously examined, however, may have consisted of different cell populations which are heterogeneous in their P-450 content. Examination of individual cloned cell lines for P-450 content should reveal the pattern of P-450 expression in individual cells. The recently derived AHH-1 cell line possesses P-450-dependent monoxygenase activities [16]. We have examined the P-450 in these cells by carrying out MAb-directed immunopurifications, proven successful for several animal tissues [14,15], on AHH-1 cell extracts. The MAbs used were prepared to purified 3-methylcholanthrene (MC)- and phenobarbital (PB)-induced rat liver cytochrome P-450. Three of the MAbs we tested specifically extract from solubilized cell extracts a 45-kDa protein which is putatively a human P-450.

2. MATERIALS AND METHODS

AHH-1 cells were cultured in RPMI medium 1640 supplemented with 5% horse serum (both

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from Gibco) and maintained in constant exponential growth by daily dilution to 4×10^5 cells/ml as described [16]. They were induced by a 24 h pretreatment with 2 μg/ml benz[*a*]anthracene (BA) (Sigma).

For radiolabeling of cell protein, the cells were incubated overnight in the presence of 1 mCi [^{35}S]Met (Amersham, 60 Ci/mmol). Cells were centrifuged, washed with phosphate-buffered saline (PBS), and resuspended in 0.05 M Tris-HCl (pH 8.0) to 10^8 cells/ml. They were then solubilized with 0.05 M Tris-HCl (pH 7.5), 0.5 M NaCl, 1 mM EDTA, 1% 3-(3-cholamidopropyldimethylammonio)-1-propanesulfonate (Calbiochem), followed by 30 min incubation at 4°C and centrifugation in an Eppendorf microfuge to remove insoluble matter. The solubilized cell extracts thus obtained were used for immunopurification studies.

The MAbs used were 1-7-1 and 1-31-2 to MC-induced P-450 [8] and 2-66-3 and 4-7-1 to PB-induced P-450 [10]. As a control for nonspecific binding, anti-lysozyme HyHel-9 (obtained from Dr S. Smith-Gill, NCI) was used. These MAbs were purified from mouse ascites fluid and covalently linked to Sepharose as described [14,15]. Solubilized cell extracts were then incubated with each of the Sepharose-MAb matrices to adsorb MAb-specific P-450. After extensively washing the resin [15], adsorbed protein was eluted with 0.05 M Tris-HCl (pH 6.8), 2% SDS, and subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) [17]. Immunopurified proteins were visualized by autoradiography.

3. RESULTS

AHH-1 cells display P-450-dependent mixed-function oxygenase activities that are induced by BA treatment [16]. Since MAb-directed immunopurification has been successfully used to prepare a variety of cytochromes P-450 from different animal tissues and species [14,15], we attempted to isolate the P-450 present in these cells. Since the MAb specificity of the P-450s in these cells was unknown, we carried out immunopurifications using 4 MAbs to two different rat liver P-450s.

The SDS-PAGE of proteins immunopurified from AHH cells grown in the presence of [^{35}S]Met is shown in fig.1. The whole cell extract has a range of radiolabeled proteins (lane 1). Immunopurification carried out with each of MAbs 1-7-1, 4-7-1 and 1-31-2 yielded a 45-kDa protein (lanes 4–9). The intensity of this band was similar for both basal and BA-induced cells. The binding by MAbs 1-7-1 and 1-31-2 is perhaps not surprising since these were prepared to, and are specific for rat liver MC-induced P-450 [14], which has aryl hydrocarbon hydroxylase (AHH) activity, while the AHH activity of these cells suggests they also possess a similar AHH-active, MC-inducible

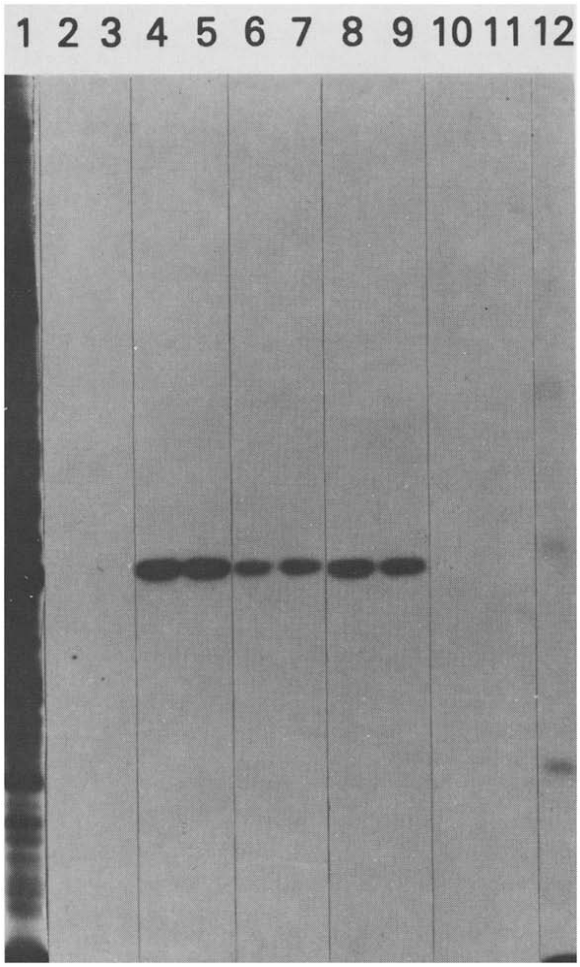

Fig.1. SDS-PAGE of ^{35}S-labeled proteins from AHH-1 cell extracts. Lane 1 contains whole cell extract. Remaining lanes contain protein immunoabsorbed to Sepharose-MAb HyHel-9 (lanes 2,3), MAb 1-7-1 (lanes 4,5), MAb 4-7-1 (lanes 6,7), MAb 1-31-2 (lanes 8,9), MAb 2-66-3 (lanes 10,11). Even- and odd-numbered lanes are BA-induced and basal cells, respectively. Lane 12 contains molecular mass markers (96, 67, 45, 32 kDa).

P-450. However, the MAb 4-7-1, prepared to the relatively AHH-inactive PB-induced rat liver cytochrome P-450, also yielded the 45-kDa polypeptide. The observation that MAbs to both MC and PB-induced P-450s recognize a protein of the same M_r in AHH-1 cells thus suggests that these cells have a 45-kDa protein which is epitopically related to these P-450s.

Immunoadsorption of the 45-kDa polypeptide by MAbs 1-7-1, 1-31-2, and 4-7-1 is specific since with two other MAbs, HyHel-9 and 2-66-3, no adsorbed polypeptides were observed (lanes 2,3,10, 11). HyHel-9 is specific for lysozyme, an unrelated protein, while 2-66-3 recognizes an epitope on PB-induced rat liver P-450.

4. DISCUSSION

We have shown that 3 MAbs which recognize specific P-450s in PB- or MC-induced rat liver also recognize a protein from AHH-1 cells. That this protein may be a P-450 is suggested by the observation that it contains 3 different epitopes which are present on rat liver P-450s. For a single epitope on a P-450 to be also present on an unrelated protein is unlikely, although possible. For example an MAb to streptococci has recently been shown to bind myosin as well [18]. However, the presence of 3 distinct P-450-specific epitopes on an unrelated protein is even more improbable.

Owing to its low level in AHH-1 cells, we have only been able to detect this protein when radiolabeled with [^{35}S]Met, and have been unable to otherwise characterize the isolated protein as a P-450. When cells were incubated with amino-[^{14}C]levulinic acid, a heme precursor, the activity of incorporated heme in cellular protein was too low for unambiguous autoradiographic identification of any purified heme protein. There is also an insufficient quantity of this protein for a reduced-CO difference spectrum.

The intensities of the bands obtained from extracts of control and BA-induced cells were similar, indicating that the protein we have isolated is noninducible by BA. Since BA induces AHH activity about 10-fold [16], whether the isolated protein is responsible for AHH activity is unclear, although several possibilities exist. First, the purified protein may not be responsible for AHH activity, and AHH-active P-450s are not purified by any of the MAbs under our experimental conditions; for example, they may bind to MAb relatively weakly and be removed during the extensive washings of the immunoabsorbent. A second possibility is that the purified protein may indeed be the major P-450 responsible for AHH activity, but its level is unaffected by BA; in this instance BA treatment might elevate AHH by causing an allosteric or other modification in P-450 structure to yield a more active form, or by increasing the level of a cofactor required for expression of activity.

The putative P-450 we have purified has a molecular mass of 45 kDa. This value is relatively low compared to that of other P-450s purified from animal sources, which have a range of 48–56 kDa [4], and to that of human P-450s isolated from liver [19,20] and placenta [21] which also have a molecular mass in this range. Thus, whether the molecular mass we observed is typical for certain types of human P-450, or is unique for the enzyme from lymphoblast cells is not clear at present.

Although an individual cell has the potential to express a number of P-450s, whether each individual cell in a population expresses a single P-450 or a range of isozymes are expressed is unclear. Expression of P-450 is influenced by a wide variety of factors and complex regulatory mechanisms which are poorly characterized at present. Cloned cell lines in a controlled environment provide a system with relatively uniform properties for studying the cellular and molecular aspects of P-450 regulation. The AHH-1 cell line is suitable for such studies, using MAbs to examine the P-450 content under various experimental conditions. This report exemplifies such a study in that it demonstrates that (a) AHH-1 cells produce a protein, putatively a P-450, which contains the epitopes for 3 different MAbs made to P-450s, and (b) expression of this putative P-450 is not induced by BA-treatment.

Immunopurification as well as other MAb-based methodologies can be extended to include other MAbs, and thus to characterize the entire range of P-450s and other cellular proteins on the basis of their interaction with a library of MAbs. Such a multidimensional approach should serve to provide much useful information in phenotyping MAb-defined P-450s in human tissues and cells.

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