THE APPLICATION OF MONOCLONAL ANTIBODIES FOR STUDIES ON CYTOCHROME P-450

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1. INTRODUCTION

The mixed function oxygenase system metabolizes numerous exogenous and endogenous compounds in higher organisms. These include xenobiotics such as drugs, carcinogens, and environmental pollutants as well as endobiotics such as steroids and prostaglandins /1-5/. The substrate specificity of this system resides in the cytochromes P-450 which are the primary enzymatic interface between humans and a wide variety of chemical compounds. The many compounds encountered by these enzymes are metabolized to either nontoxic products or toxic metabolites, depending on the type and amount of cytochrome P-450 isozymes present. The wide range of xenobiotics and endobiotics which are encountered by higher organisms requires that the organism possess a sufficient number of appropriate cytochromes P-450 that can adequately metabolize the far ranging spectrum of different substrates. The presence of a large number of cytochromes P-450 /5/ satisfies this requirement. Thus there are multiple forms of cytochrome P-450, of which some are constitutive while others are inducible by different classes of inducers. The catalytic activities of the individual isozymes typically exhibit unique yet broad positional, regio-, and stereo-selectivity profiles toward different substrates. A single cytochrome P-450 may therefore catalyze multiple reactions, while a single substrate may be processed by several cytochromes P-450.

The multiplicity of cytochrome P-450 results in complex metabolic pathways and kinetics for reactions in which numerous substrates are simultaneously processed into a variety of metabolites. The cytochromes P-450 catalyze beneficial reactions that detoxify harmful substrates as well as detrimental reactions that yield toxic metabolites or activate substrates to mutagens or carcinogens. The balance between detoxification and activation, the actual spectrum of metabolites, and the quantity and kinetics of appearance of metabolites is governed by the type and amount of cytochromes P-450 present. This distribution of individual cytochromes P-450, or phenotype, is regulated by genetic and environmental factors and is also a function of age, sex, developmental stage and nutritional state. The distribution of cytochromes P-450 may vary among tissues, species, and individuals, and is a major determinant of the ultimate fate of compounds encountered by an organism. Detection, identification and characterization of the individual cytochrome P-450 isozymes is therefore a primary objective in studies of drug metabolism in higher organisms and has implications for

studies in many fields, such as pharmacology, toxicology, carcinogenesis, and environmental science. Cytochrome P-450 multiplicity has accordingly been investigated in numerous purification, genetic, enzyme activity, and immunological studies /5/. Progress has been limited, however, by the isozymic nature of the system, and the limited sensitivity or resolution of the methods employed in distinguishing closely related cytochromes P-450.

2. MONOCLONAL ANTIBODIES

A new approach to the multiplicity problem involves the use of monoclonal antibodies (MAbs) to cytochromes P-450. Monoclonal antibodies are extremely useful tools for the study of structurally related proteins /6,7/ and are particularly suitable for investigating isozymic systems. Whereas polyclonal antibodies are a heterogeneous mixture containing a population of antibodies to a variety of epitopes (antigenic determinants), a MAb is a chemically defined, homogeneous reagent. Its exquisite specificity derives from a unique hypervariable region that is complementary to and thus recognizes a specific epitope on the protein. The epitope is either conformational, which is defined as one that is dependant on the spatial conformation of the protein, or it is sequential, depending only on the primary amino acid sequence. In each case the antigenic determinants are topographic, i.e. composed of structures on the protein surface.

a. The Origin and Nature of Monoclonal Antibodies

Monoclonal antibodies are produced by the hybridoma technology described by Kohler and Milstein /8/. The hybridoma technology grew out of the clonal selection hypothesis of Burnet and colleagues /9/ which states that each antibody forming cell or B-lymphocyte and its progeny is committed to the production of a single type of antibody molecule which recognizes and binds to a single epitope. An animal can produce millions of antibodies in response to the various antigens encountered, of which only a small fraction of B-lymphocytes respond to any given antigen. Since most antigens have many determinants, the B-cells collectively produce a large number of antibodies which recognize different epitopes on the antigen. Conventional antisera to a given antigen are consequently polyclonal, heterogeneous, and vary with each immunization. In "hybridoma" technology /6,8/ myeloma tumor cells

are fused with the B-lymphocytes of the spleens of mice immunized with a particular antigen. The fused cells or "hybridomas" combine the characteristics of the B-lymphocytes, which individually are committed to the production of a specific antibody, and the immortality of the myeloma tumor cells. The individual hybridomas are cloned and screened for the clones which produce the desired MAb. The selected clone is usually subcloned two to three times to assure its monoclonality. These hybridomas are then grown *in vitro* in a defined medium where they produce the chemically defined MAb, or they are injected into the peritoneal cavity of appropriate recipient mice where they grow as ascites tumors which yield large quantities of the MAb. In our laboratory, the entire procedure of immunization, hybridization, selection, subcloning three times and growth in cell culture or as ascites fluid, requires approximately seven months /10-13/. MAbs are additionally advantageous as a continuing research tool owing to their availability: hybridoma cell lines are potentially immortal if cryopreserved properly and limitless amounts of desired MAbs may therefore be obtained when needed.

b. Monoclonal Antibodies to Cytochrome P-450

MAbs can be used to immunochemically define cytochromes P-450, employing various MAb-based methodologies. Since the number of possible epitopes and the nature of their interaction with the MAbs vary widely, the use of MAbs offers an approach of unrivalled specificity for characterizing isozymes such as the cytochromes P-450.

There are several basic reasons for employing MAb methods for exploration of isozymic systems typified by the cytochromes P-450. The discovery and characterization of new cytochrome P-450 isozymes from a variety of sources has been a continuing process. The number of known forms has continued to increase. Currently there are at least 15-20 known forms. At this time, with our very limited knowledge of the genetics of the cytochrome P-450 system, speculation as to the total number of possible cytochrome P-450 gene products is premature. Individual cytochromes P-450 may be characterized either in the purified state or while in a tissue in the presence of other cytochromes P-450, in which case one must be capable of measuring some specific attribute of the cytochrome P-450 of interest that is not present in the other cytochromes P-450. The fundamental objective of characterizing structurally and/or functionally discrete forms of cytochrome P-450 is

an experimentally elusive problem, owing to the limited resolution of the methods employed in relation to the diversity of the cytochromes P-450.

The most commonly employed classification systems for distinguishing cytochromes P-450 have serious limitations. A spectral definition of cytochromes P-450 is inadequate since numerous distinct cytochromes P-450 are known to exhibit the same spectral maximum in the 450 nm region. Classification of cytochromes P-450 by their catalytic activities is inadequate because various isozymes have overlapping substrate selectivity and reactivity. The most common classification schemes are based on mobility in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The disadvantage of this method is that since it relies primarily on molecular size and not amino acid sequence, it does not distinguish between isozymes of similar molecular weight. In addition the limited resolution of the method (500-1000 daltons) does not allow for the separation of a large number of isozymes within the molecular weight range of the cytochromes P-450.

Immunologic identification of cytochrome P-450 isozymes is potentially a high resolution means for classifying and thereby establishing a new taxonomic system for cytochromes P-450, since the number of different antibodies, epitopes, and types of interaction between them are large and can be precisely defined. The major deficiency of polyclonal antibodies is their heterogeneity. In a polyclonal antibody preparation there are multiple antibodies to multiple epitopes, as well as different antibodies which exhibit diverse binding properties to the same epitope. Monoclonal antibodies, however, are specific reagents that target defined epitopes on the P-450 surface. MAb specificity is dictated by the identity of the hypervariable residues in its binding site, of which there are many variations. The epitopes on a cytochrome P-450 span its entire surface and may overlap to yield a continuum of epitopes. The potential for matching large numbers of MAbs with epitopes is therefore great and affords high resolution to procedures that utilize MAbs. The only practical limiting factor is the time and effort applied to MAb preparation. Nevertheless, there exists the potential of generating large libraries of MAbs to cytochromes P-450.

It must be emphasized that MAbs are specific probes for epitopes, and not for individual isozymes. Several cytochromes P-450 may thus interact with a single MAb if they all possess the MAb-specific epitope. On the other hand, if an epitope is uniquely present in a particular isozyme, an MAb to that epitope is also specific for this isozyme. It is

likely, therefore, that a sufficiently large repertoire of MAbs would include different types, among which would be those that are highly specific for individual isozymes, those that are generally specific for most or all cytochromes P-450, and those of limited specificity that recognize relatively few isozymes.

The MAb-cytochrome P-450 interaction may be probed in a number of ways. The most fundamental description of this macromolecular association is the affinity constant under a defined set of conditions. Formation of the classical lattice of polyclonal antibody-antigen complex with its concomitant precipitation ought strictly not to appear when an MAb reacts with a nonrepetitive epitope on a monomeric antigen since each antigen molecule interacts with only one MAb and each MAb binds only one or two antigen molecules. Some cytochrome P-450, however, are multimeric in the commonly used solvents; for these P-450s one may envisage multiple crosslinkings of these multimers by the divalent MAb, followed by precipitation. Such nonclassical immunoprecipitation occurs only if the cytochrome P-450 is of a type that polymerizes, and its MAb-specific epitope is accessible to the MAb and not on an intermolecular interface. Characterization of macromolecular association phenomena therefore provides some elementary information on the nature of the inter-cytochrome P-450 and MAb-cytochrome P-450 interactions.

The above discussion provides a partial framework for the study of MAb-cytochrome P-450 interactions. One of the requirements for characterization of cytochromes P-450 by MAb based methods is the availability of a wide array of MAbs. Since production of MAbs to cytochrome P-450 is a relatively recent development generalizations cannot yet be made. Instead, with the limited number of MAbs available at present different laboratories are probing the limits of MAb based methods in cytochrome P-450 research.

A number of MAbs to liver microsomal cytochromes P-450 have been reported. These include series of MAbs to the rabbit LM2 /10,11, 14,15/, LM4 /10,11/, and form 1 /16/ isozymes, and to the rat liver cytochromes P-450 induced by 3-methylcholantrene (MC) /12,17/ and phenobarbital (PB) /13,18/. MAbs are prepared by the hybridoma method /8,19/, using spleen cells from mice immunized with purified cytochrome P-450. Hybridomas are then screened via immunoassay to identify those that secrete MAbs to the cytochrome P-450 antigen. Stable clones are maintained in culture or frozen, and are identified in further studies by a reference number for the microtiter wells from

which they were cloned. Small amounts of MAbs may be obtained from the culture medium while larger quantities of MAbs are obtained from ascites fluid. For some studies the ascites fluid can be used directly while for other studies the MAb in the ascites fluid is further purified by ammonium sulfate precipitation and/or ion-exchange chromatography. Usually 1-10 mg of MAb is obtained per ml ascitic fluid.

Since a number of MAbs are normally obtained to each antigen it is generally useful to establish a preliminary classification, based on the interaction of MAb with cytochrome P-450, prior to extensive experimental use. In our laboratory we initially determine whether a newly prepared MAb selected by a radioimmunoassay is positive or negative in each of two tests: 1) inhibition of an easily measured catalytic activity such as aryl hydrocarbon hydroxylase (AHH); 2) precipitation of cytochrome P-450. Although an MAb reacting with a nonrepetitive epitope on an antigen does not produce a classical antibody-antigen precipitating complex, an immunoprecipitate may nevertheless form in the absence of a detergent which dissociates cytochrome P-450 multimers /12,17/. Three classes of MAbs that bind cytochrome P-450 are thus obtained: 1) inhibiting and precipitating; 2) noninhibition and precipitating; 3) neither precipitating nor inhibiting. A list of MAbs that is representative of these three classes is presented in Table 1.

TABLE 1. Classes of Monoclonal Antibodies to Different Cytochromes P-450

Cytochrome P-450	MAb	Immuno-Precipitation	AHH Inhibition
Rabbit LM_2	1-26-11	+	+
	1-31-1	+	–
	1-31-4	–	–
Rabbit LM_4	1-1-8	–	–
	1-4-3	–	–
Rat MC-induced	1-7-1	+	+
	1-36-1	+	–
	1-43-1	–	–
Rat PB-induced	2-66-3	–	+
	2-8-1	–	–
	1-48-9	–	–

The cytochromes P-450 collectively catalyze the oxidation of literally thousands of xenobiotic and endobiotic substrates including a large variety of different classes of chemicals. One of the essential questions concerning cytochrome P-450 function relates to our ability to measure the contribution of unique forms or classes of cytochromes P-450 to the total metabolism of an individual substrate in different tissues and individuals. Available methods do not permit such a measurement and current understanding of the substrate and product specificity of unique forms of cytochrome P-450 are derived only after prior purification and isolation of the unique cytochrome P-450. The purified cytochrome P-450 is then reconstituted with cofactors and enzymes of the mixed-function oxidase complex and its substrate and product specificity is measured. The latter methods have yielded useful information on cytochrome P-450 specificity but do not add much to our understanding of the contribution of the particular cytochrome P-450 to total tissue metabolism. This approach also has severe limitations imposed by the requirement for purification of the cytochrome P-450, a difficult task especially for the minor forms and those in extra-hepatic tissues. Furthermore, the reconstituted system may not adequately reflect the *in situ* activity of the cytochromes P-450 located in the endoplasmic reticulum of the intact cell.

3. INHIBITION OF CATALYTIC ACTIVITY BY MONOCLONAL ANTIBODIES

Monoclonal antibodies that modulate P-450 dependent catalytic activities target and hence identify the isozymes responsible for specific reactions. A primary test for characterization of a newly prepared MAb is often its effect on enzyme activity. A number of inhibitory MAbs have been reported by several laboratories and are represented by the MAbs in Table 2. MAbs that inhibit cytochrome P-450 dependent activities of both the purified enzyme as well as of tissue microsomes are listed.

The influence of MAb binding on catalytic activity of cytochrome P-450 may be a function of the conformational changes induced in the enzyme. A spectrum of effects on substrate selectivity and reactivity may be observed when activity modulating experiments are carried out with a series of MAbs. Among the possibilities are: (1) the MAb has no effect if it binds an epitope removed from and noninteracting with the active site; (2) the MAb modulates activity by either a) binding an

TABLE 2. Some Monoclonal Antibodies that Inhibit Catalytic Activity

MAb (antigen)	Activity Inhibited	Enzyme Source	Reference
1-7-1 (rat MC-P-450)	AHH, ECD	rat liver, lung, kidney, human placenta, lymphocytes	/20/ /23/
	2-acetylaminofluorene hydroxylase	rat liver	/22/
C8 (rat P-450c)	AHH	rat liver	/17/
2-66-3 (rat PB-P-450)	AHH, ECD	rat liver	/13/
1-26-11 (rabbit LM2)	AHH	rabbit P-450 LM2	/11/
1-14-3 (rabbit LM4)	AHH	rabbit P-450 LM4	/11/
2A2 (rat PB-P-450)	AHH, ECD, aldrin epoxidase, pregnenalone 16-α-hydroxylase	rat liver	/18/
MBS 106 (rabbit PB-P-450)	AHH	rabbit liver	/15/
1F11 (rabbit P-450 1)	progesterone 21-hydroxylase	rabbit liver	/16/

epitope on or near the active site or b) acting as an allosteric effector by binding an epitope removed from the active site. One should bear in mind that bound MAb may affect activity not only altering the conformation of the active site but also by virtue of its large size, sterically hindering access of reductive, substrate, or essential cofactors to the active site. Conversely, the presence of substances that bind cytochrome P-450 may interfere with binding of MAb. For example, an MAb may completely inhibit an activity of a cytochrome P-450 in the purified state but might not inhibit its activity if the same cytochrome P-450 is yet in its microsomal membrane state and the MAb-specific epitope is enclosed within the membrane, inaccessible to the MAb. The observed degree of inhibition of microsomal activity should therefore be viewed as a minimum value since under a different set of conditions, such as in solubilized tissue preparations, an inaccessible epitope may become exposed to the MAb and will be inhibited to a greater extent. MAb inhibition experiments on tissues therefore more accurately measures the sensitivity of particular cytochrome P-450 dependent reactions to an MAb rather than the actual content of an MAb-specific cytochrome P-450.

Monoclonal antibodies that completely inhibit the enzyme activity of the epitope containing cytochrome P-450 to which they bind are

extraordinarily useful for reaction phenotyping. "Reaction phenotyping" is the identification and quantification of the contribution of individual or classes of cytochromes P-450 to the total metabolism of a specific cytochrome P-450 substrate in a tissue. The inhibitory MAb is added to the tissue preparation and its degree of inhibition of the reaction is a measure of the minimum contribution of the MAb sensitive cytochrome P-450 to the total reaction of the tissue. The observed inhibition reflects only the minimum contribution of the sensitive cytochromes P-450 since in some instances the MAb sensitive epitope of the cytochrome P-450 may be bound or inserted in the membrane structure and inaccessible to the MAb. If this is the case, a solubilization of the microsomes is required prior to MAb-inhibition measurements. After solubilization, the inhibition by the MAb measures the total contribution of the MAb-sensitive cytochrome P-450 to the total reaction.

Reaction phenotyping of cytochromes P-450 can be applied to analysis of a variety of reactions which may be direct or indirect results of cytochrome P-450 activity. These include: 1) substrate disappearance, 2) substrate and product specificity with respect to the formation of different metabolites, 3) indirect action phenomena which are a result of cytochrome P-450 catalyzed formation of reactive metabolites. The formation of these reactive metabolites may result in a) metabolite binding to macromolecules such as protein and DNA, b) cell toxicity, c) mutagenicity, and d) carcinogenicity. The addition of an inhibitory MAb to any of the above assay systems will in each case qualitatively and quantitatively define the contribution of the MAb specific cytochrome P-450 to the total activity measured.

a. Reaction Phenotyping of Substrate Specificity

One of our most useful MAbs is MAb 1-7-1, made to MC-induced rat liver cytochrome P-450 (MC-P-450), which is positive for binding, immunoprecipitation and inhibition of two cytochrome P-450 dependent catalytic activities: AHH, which measures the oxidation of benzo(a)-pyrene (BP) to phenols; and ethoxycoumarin deethylase (ECD), which measures the deethylation of this drug. As shown in Table 3, MAb 1-7-1 inhibits both these activities of purified MC-P-450 by 92%, and of microsomes from MC-treated rats by 75% and 71% /12/. The inhibitable fraction of the total AHH and ECD activities of MC-microsomes is therefore catalyzed by cytochromes P-450 recognized by MAb 1-7-1.

Both AHH and ECD also derive from either the same cytochrome P-450 or from cytochromes P-450 which are different but share a common epitope. However, MAb 1-7-1 does not significantly inhibit other cytochromes P-450, as shown by its lack of inhibition of activities of microsomes from control and PB induced rats. The AHH and ECD of these microsomes therefore resides in cytochromes P-450 other than the type inhibited by MAb 1-7-1.

TABLE 3. Inhibition of AHH and ECD by MAb 1-7-1

	AHH		ECD	
Enzyme Source	Control	% Inhibition	Control	% Inhibition
Purified MC-P-450*	1177	92	110	92
MC-Microsomes**	1170	75	5280	71
PB-Microsomes	600	0	1266	8
Control Microsomes	367	0	815	0

* pmol/min/nmol; ** pmol/min/mg for microsomal preparations

In addition to defining the cytochromes P-450 responsible for specific reactions, MAbs are useful probes for determining interspecies differences in cytochrome P-450 reaction phenotype. The effect of MAb 1-7-1 on liver AHH and ECD of four different species exposed to different inducers was examined /20/ and is shown in Table 4. Rats are highly induced by MC for both AHH and ECD, and induced by PB to a lesser extent. The MAb 1-7-1 sensitive P-450 contributes 75% of the total activity of MC-induced rat liver but contributes little or none of the AHH or ECD activity of liver from control or PB treated rats. With the highly inducible C57 mouse strain, 88% of MC-induced liver AHH activity but only 39% of ECD activity derives from cytochromes P-450 sensitive to MAb 1-7-1. Sixty one percent of ECD thus derives from cytochromes P-450 that are insensitive to MAb 1-7-1. Liver ECD of control and induced guinea pigs and hamsters differs from that of C57 mice and rats in that it is not inhibited by MAb 1-7-1, and therefore entirely catalyzed by cytochromes P-450 that are insensitive to MAb 1-7-1. With regard to AHH, the MC-induced activity is partially derived from MAb-sensitive cytochromes P-450 in all species. The AHH of control and PB-treated animals are not affected by MAb 1-7-1, except for control guinea pigs in which 25% of AHH results from an MAb 1-7-1-sensitive cytochrome P-450.

TABLE 4. MAb 1-7-1 Inhibition of Hepatic AHH and ECD of Different Species and Strains

		AHH(pmol/min/mg)		ECD (nmol/min/mg)	
Species	Induction	Control	% Inhibition	Control	% Inhibition
Rat (Sprague-Dawley)	none	367	0	.82	0
	PB	600	0	1.27	8
	MC	1170	75	5.28	71
Mouse (C_{57}BL/6)	none	88	1	6.4	12
	PB	128	2	7.0	17
	MC	5716	88	21.9	39
Mouse (DBA/2)	none	70	0	4.0	11
	PB	51	0	4.7	25
	MC	501	3	16.9	0
Guinea Pig (NIH Outbred)	none	282	25	2.0	0
	PB	703	0	2.9	0
	MC	1642	47	5.3	0
Hamster (Golden Syrian)	none	100	0	8.7	0
	PB	192	0	18.0	5
	MC	387	51	22.1	0

In addition to examining interspecies differences, intertissue comparisons can be made with activity inhibition experiments. Such a study has been performed with the liver, lung and kidney microsomes of several species /20/. Table 5 shows the results obtained with rat tissues. The AHH of liver from untreated rats is unaffected by MAb 1-7-1, whereas that of lung is substantially inhibited (42%). Lung therefore has a basal cytochrome P-450 that has the MAb 1-7-1 specific epitope and which is not present in liver. Upon PB treatment, this MAb-specific cytochrome P-450 is also present since AHH is inhibited by 24%. Such a PB-inducible and MAb-inhibitable (39%) cytochrome P-450 also appears in kidney. Upon MC treatment all tissues are similarly inhibited to a great extent, indicating that the major induced cytochrome P-450 that catalyzes AHH is of the type recognized by MAb 1-7-1.

The ECD of both control and PB-treated liver, lung, and kidney indicate that the ECD-active cytochromes P-450 in either of these tissues are insensitive to MAb 1-7-1, in contrast to the AHH-active cytochromes P-450. With MC-treated rats, while most ECD of liver and kidney was inhibited, the degree of inhibition was less than that observed with AHH measurements. In addition considerably less inhibition of lung ECD than AHH (38% vs 78%) was observed. These data demonstrate

that ECD of MC-treated rats consist of two types of cytochrome P-450: MAb-sensitive and MAb-insensitive. Such experiments also illustrate how MAbs can be employed to reaction phenotype tissue cytochrome P-450 content.

TABLE 5. AHH and ECD of Rat Tissues and Their Inhibition by MAb 1-7-1 (Values in Parenthesis Indicate Percent Inhibition by MAb 1-7-1)

	Liver	Lung	Kidney
		AHH (pmol/min/mg)	
Control	229(0)	7.7(42)	1.8(11)
PB	141(9)	3.2(24)	2.3(39)
MC	2099(81)	53.2(78)	151.3(87)
		ECD (nmol/min/mg)	
Control	1.06(0)	0.16(0)	0.01(0)
PB	2.73(0)	0.10(0)	0.02(0)
MC	8.37(65)	0.24(38)	0.27(70)

In one study the inhibitory effect of an MAb to rabbit liver cytochrome P-450 3b was measured on the rabbit liver microsomal 16α- and 6β-hydroxylation of progesterone /21/. This MAb inhibits these activities in catalytically distinct, variant forms of this isozyme. The MAb inhibited 40-70% of the 16α-hydroxylase activity of either New Zealand White or IIIVO/J liver microsomes. The MAb did not inhibit 6β-hydroxylation by microsomes from IIIVO/J microsomes but this activity was inhibited in microsomes from New Zealand White rabbits. The different inhibition patterns observed with both strains correlated with the effect of the MAb on the activities of the P-450 3b variant purified from each strain. Thus the use of an MAb provides information relating the activity of a specific cytochrome P-450 isozyme to the activity of the whole tissue, and suggests its utility in such future phenotyping studies.

b. Reaction Phenotyping for Product Specificity

The utility of MAbs in defining the product specificity of cytochromes P-450 is demonstrated in a study of the influence of MAb 1-7-1-7-1 on the metabolism of the carcinogenic aromatic amine 2-acetylaminofluorene (AAF) by both purified cytochrome P-450 and rat liver microsomes /22/. Oxidation of AAF occurs at several positions on this

compound to yield multiple products. The MAb inhibited formation of the 7-OH-, 5-OH-, 3-OH-, 1-OH-, and N-OH- derivatives of AAF. The hydroxylase activities responsible for these products thus reside in one or more cytochromes P-450 with the MAb 1-7-1 specific epitope. Formation of the 9-OH- product was not inhibited and this metabolite is therefore generated either by a cytochrome P-450 not sensitive to MAb 1-7-1, or by a mechanism independent of that involved in formation of the other metabolites. These experiments thus demonstrate the unique capability of MAbs to define the cytochromes P-450 responsible for site specific reactions.

c. Reaction Phenotyping of Human Cytochrome P-450

A major goal of cytochrome P-450 research is the understanding of the basis for individual variability in drug and carcinogen metabolism in humans. MAbs may play a role in such studies by providing a means to phenotype individual human tissues. Such reaction phenotyping can be achieved by either MAbs that are raised to cytochromes P-450 from human tissues, or by MAbs to cytochromes P-450 from animal tissues that cross-react with human cytochromes P-450. We have obtained MAbs that recognize polycyclic hydrocarbon-induced cytochromes P-450 in rat liver that have a common epitope with cytochromes P-450 that are present in several mammalian species including humans /23, 24/. These MAbs were used to reaction phenotype several cytochrome P-450 catalyzed activities in different human tissues.

The influence of MAb 1-7-1 on the control and hydrocarbon inducible AHH and ECD of several human tissues was studied /23/, and the results are depicted in Figure 1. Both AHH and ECD are elevated in the placenta of women who smoke cigarettes. ECD in non-smokers is generally present at lower levels than in smokers, while AHH is not detectable in non-smokers. At saturating levels of MAb 1-7-1 more than 90% of placental AHH is inhibited whereas the ECD is only inhibited by about 40%. None of the AHH or ECD activity in normal and induced monocytes is inhibited by the MAb 1-7-1, indicating that the cytochromes P-450 responsible for these activities in monocytes is distinct from the MAb-sensitive cytochrome P-450 in placenta. MAb 1-7-1 inhibits about 50% of the AHH and ECD of control and induced lymphocytes, suggesting that they both contain forms of cytochrome P-450 with common epitopes and that a type of cytochrome P-450 in induced lymphocytes is also present in basal cells. Human liver behaves like monocytes

in that neither the AHH nor ECD activity are inhibited by MAb 1-7-1 and thus are catalyzed by a cytochrome P-450 distinct from the MAb 1-7-1 sensitive cytochrome P-450.

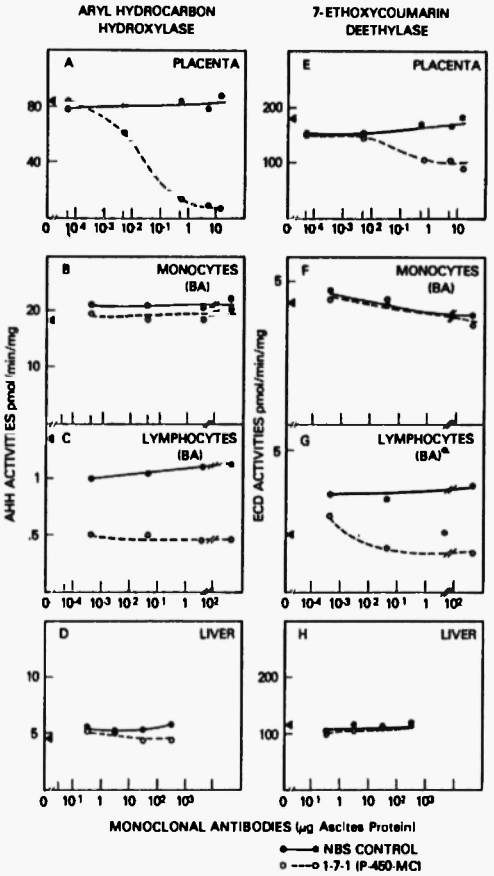

Figure 1: Effective of monoclonal antibody 1-7-1 on the AHH and ECD activities of different human tissues.

Thus, the MAbs have: (i) identified cytochromes P-450 with a common antigenic site in placenta, lymphocytes, and human cells in culture; (ii) identified two forms of hydrocarbon-induced cytochromes P-450 in human lymphocytes, at least one of which has a common epitope with

a cytochrome P-450 of placenta and with a cytochrome P-450 form present in uninduced lymphocytes; (iii) identified two forms of cytochrome P-450 responsible for smoking-induced ECD activity in placenta, one of which is also responsible for AHH activity; (iv) shown that the cytochromes P-450 of liver, basal, and BaA-induced monocytes are different from the MAb-sensitive cytochromes P-450 of placenta and lymphocytes; (v) quantitated in several human tissues the percentages of control and inducible AHH and ECD that are dependent on MAb-sensitive P-450. The results obtained with the MAb analysis of human tissue demonstrate the value of MAbs for defining antigenic site relatedness for different enzymatic functions of cytochromes P-450 and for identifying and quantifying the amount of a specific enzyme activity in a tissue dependent on specific cytochromes P-450. The study with human tissues may be a prototype for the use of MAbs for phenotyping and mapping of cytochromes P-450 responsible for specific metabolic reactions in humans and thus may be useful in determining the relationship of cytochrome P-450 phenotype to individual difference in drug metabolism and carcinogen susceptibility.

The finding that multiple forms of cytochromes P-450 are responsible for placenta and lymphocyte AHH and ECD suggests the use of MAbs as diagnostic tools in future studies for phenotyping humans. The MAbs can be useful for population studies, which are required for "biochemical or molecular epidemiology" /25/, identifying differences in ethnic populations /26/ or pharmacoanthropology /27/ or predictive identification of hypersusceptible individuals /28/. Figure 2 shows a population study of the effects of MAb 1-7-1 on both AHH and ECD of women who smoke cigarettes /24/. Each dot represents a placenta from a single individual and the data shows that in most smokers more than 80% of the AHH is catalyzed by an MAb sensitive cytochrome P-450. Inhibition of ECD is more variable than AHH inhibition, indicating the presence of a second cytochrome P-450 that is ECD-active and which is not inhibited by MAb 1-7-1. The ECD thus derives from at least two forms of cytochrome P-450, one or more insensitive and one or more sensitive to MAb 1-7-1. This is further demonstrated in Figure 3 which shows the effect of MAb 1-7-1 on the ECD of non-smokers and smokers. Little or no inhibition was observed for non-smokers, while the ECD level for smokers correlated with the degree of inhibition. Our studies thus indicate that there are two forms of ECD-active placental cytochrome P-450, and MAb 1-7-1 can be used to measure the ECD contributed by both the inducible and non-inducible forms of cytochromes

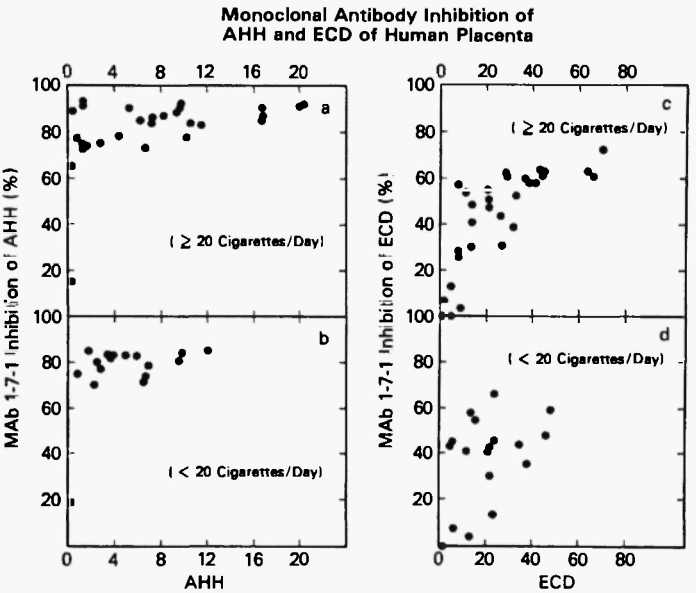

Figure 2. Monoclonal antibody 1-7-1 inhibition of AHH and ECD of placenta from women who smoke cigarettes (each point represents a single placenta).

P-450 in tissues from different individuals. Such studies demonstrate how MAbs add a new dimension of assay for the role of specific cytochromes P-450 in catalyzing specific metabolic reactions.

d. Cytochrome P-450 Catalyzed Protein and DNA Adduct Formation, Toxicity, Mutagenicity, and Carcinogenicity

Microsomes and appropriate cofactors incubated with xenobiotics result in the conversion of the xenobiotics to metabolites that are reactive and that may covalently bind to proteins /29,30/, or DNA /31,32/. This *in vitro* formation of adducts to macromolecules such as to protein or DNA may reflect the mechanisms engaged in the *in vivo* formation of xenobiotics bound to protein or DNA adducts. These reactions are believed to be responsible for xenobiotic induced toxicity /30/, mutagenicity, and carcinogenicity /33,34/. The formation of BP-DNA adducts may /31,32/ be a paradigm for the numerous adducts of xenobiotic to protein or DNA *in vivo*. As specific inhibitors to certain individual or classes of cytochromes P-450 MAbs will be useful in defining the role of the specific cytochromes P-450 to the formation of

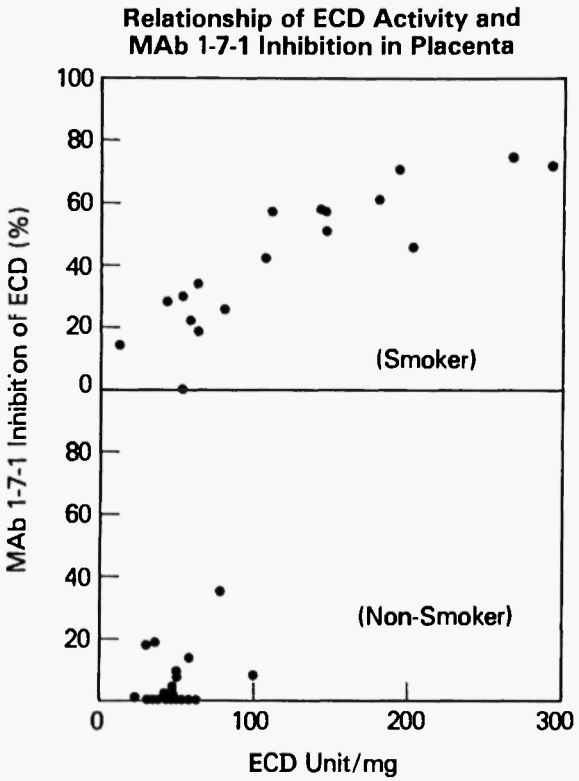

Figure 3. Monoclonal antibody 1-7-1 inhibition of placental ECD from smokers and non-smokers. (Each point represents a single placenta.)

individual adducts. Thus, the MAbs may be useful in defining the role of specific cytochromes P-450 to the toxic, mutagenic, or carcinogenic activity of individual xenobiotics.

The microsome catalyzed activation of BP to metabolites binding to DNA /31,32/ is the system used for the activation of xenobiotics to their mutagenic forms as tested in the Ames mutagen detection system /33,34/. A large preponderance of all known mutagens require metabolic activation. In most cases the activation system is essentially a result of cytochrome P-450 catalysis. Thus, the cytochromes P-450 are the primary biological agents of mutagen activation. Currently, the role of individual cytochromes P-450 in the activation of the numerous mutagenic agents is virtually unknown. A library of MAbs that inhibit

individual and classes of cytochromes P-450 may be useful for defining the role of each type of cytochrome P-450 in the activation of specific mutagens. MAbs have been successfully used for this purpose in a study /35/ which examined the effects of specific inhibitory MAbs to PB-P-450 and MC-P-450 on mutagen activation by liver microsomes from C57BL/6 and DBA/2 mice. The study defined the role of the MAb specific cytochromes P-450 for the activation of AAF, aflatoxin, nitrosomorpholine and BP-7,8 diol to mutagenic metabolites in control, MC, PB, and pregnenolone 16α-carbonitrile treated mice. Each MAb-defined form contributed to the mutagenicity of aflatoxin, AAF and BP-diol to different extents in the livers from the differently pre-treated rats. In the case of the nitrosomorpholine there was a large stimulation of mutagenic activity indicating that the MAb sensitive cytochrome P-450 was likely involved in nitrosomorpholine detoxification. The inhibition of this cytochrome P-450 by the MAb resulted in the nitrosomorpholine being more available to those other cytochromes P-450 engaged in its activation to mutagenic forms.

e. Regulation of Cytochrome P-450 Expression under Different Nutritional Conditions

Enzyme inhibitory MAbs have been used to phenotype the effect of dietary components on the expression of rat intestinal cytochromes P-450 which are active for ECD and AHH. The appreciable activities in the small intestine of rats fed cholesterol high diets was found due to an MAb 1-7-1 sensitive cytochrome P-450 /36/. This cytochrome P-450 was found in the small intestine of control and PB-treated rats as well as in MC-treated rats. In contrast, the livers of the same animals showed sensitivity to MAb 1-7-1 inhibition of AHH and ECD only when they were pretreated with MC, but no inhibition was observed in control or PB-treated rats. Thus the MAb 1-7-1 detected an intestinal cytochrome P-450 with an epitope in common with the MC-induced liver cytochrome P-450. The MAb also identified cytochromes P-450 with common epitopes whose presence are diet sensitive.

f. Cytochrome P-450 Expression in Cell Culture

MAbs have also been successfully used to identify the specific type of cytochrome P-450 that is expressed in various cells grown *in vitro* and exposed to different types of inducers. In one study /37/ the MAb

1-7-1 (to MC-P-450) and MAb 2-66-3 (to PB-P-450) were used to study the cytochromes P-450 expressed in nine differentiated or de-differentiated cell lines developed from rat hepatoma cells. The cells were reaction phenotyped for cytochromes P-450 responsible for aldrin epoxidase, ECD and AHH. This analysis showed the extent of expression of the MAb sensitive cytochromes P-450 in the different cells. Both types of cytochrome P-450, those induced by MC as well as those induced by PB, were expressed to different extents in the different mouse cell lines.

4. RADIOIMMUNOASSAY WITH MONOCLONAL ANTIBODIES

In addition to analyzing tissues for the amount of cytochromes P-450 contributing to different xenobiotic reactions, radioimmunoassay (RIA) methods have been developed which directly measure MAb-defined cytochrome P-450 content /38/. With this type of assay it should be possible to quantitatively determine in a tissue the amount of cytochromes P-450 containing the epitopes recognized by specific MAbs. Since RIA detects cytochrome P-450 independent of catalytic activity, it is useful for detecting cytochrome P-450 forms that are unstable and easily lose their enzymatic activity upon tissue processing.

In competitive RIA, the wells are coated with a standard preparation of microsomes or purified cytochrome P-450. The RIA is based on [^{35}S]Met labeled MAb. The [^{35}S]MAb is mixed with the sample containing an unknown amount of the cytochrome P-450, and the mixture is added to the well where the unknown competes with the cytochrome P-450 coated on the well for binding to [^{35}S] MAb. Results are expressed as percent binding at different levels of competing antigen. Figure 4 shows results with a competitive RIA using [^{35}S] MAb 1-7-1 and liver microsomes from MC-treated rats to coat the wells. Liver microsomes from MC-induced rats compete most effectively for the MAb while the lesser competition (rightward shift) in curve by control and PB microsomes indicate that their content of MAb 1-7-1-sensitive cytochrome P-450 is one-fiftieth that present in MC-microsomes. The RIA is relatively cytochrome P-450 specific since the control antigens (serum albumin and cytochrome C) do not compete for binding to [^{35}S] MAb 1-7-1.

Cytochrome P-450 in liver, lung, and kidney microsomes from several MC-induced animals were also examined by competitive RIA with MAb 1-7-1 /39/. Their microsomes competed to different degrees with

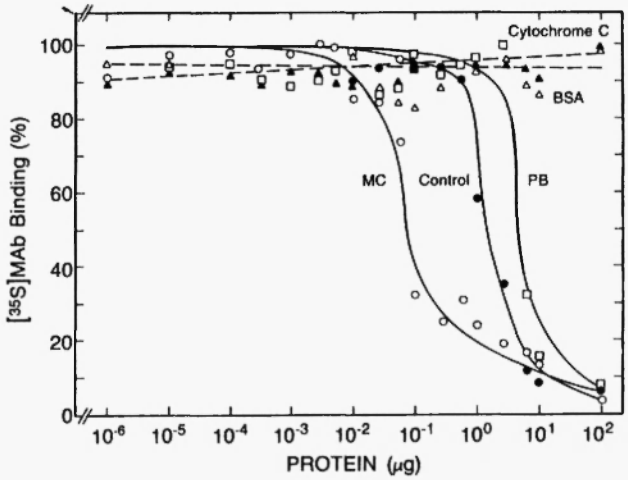

Figure 4. Competitive radioimmunoassay for cytochromes P-450 in the liver microsomes of control, PB, and MC-treated rats, using [^{35}S] MAb 1-7-1, specific for MC-induced P-450.

the well-coated liver microsomes of MC-treated rats for binding to this MAb (Figure 5). Binding of [^{35}S] MAb 1-7-1 to the well was reduced by competing liver microsomes from MC-treated rats, with a 50% reduction (I_{50}) of the binding occurring with 0.042 μg of microsomal proteins. MC-treated C_{57}BL/6 mouse liver microsomes also effectively

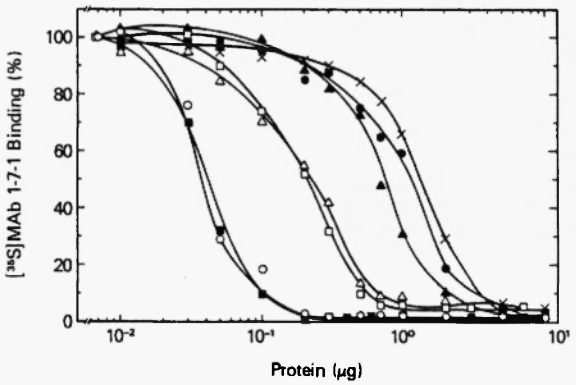

Figure 5. Competitive RIA for MC-microsomes using [^{35}S]-MAb 1-7-1. MC-rat liver microsomes (○); MC-C_{57}BL/6 mouse liver microsomes (■); MC-guinea pig liver micosomes (△); MC-DBA/2 mouse liver microsomes (□); MC-hamster liver microsomes (▲); MC-rat lung microsomes (●). and MC-rat kidney microsomes (X).

competed for binding, whereas liver microsomes from MC-treated DBA/2 mice, a strain that is relatively non-inducible for AHH activity, only moderately competed for the binding. Extrahepatic tissue microsomes of MC-treated guinea pigs and hamsters competed least effectively for the binding to [^{35}S] MAb 1-7-1. The effectiveness of microsomes in competing for the binding decreased in the following order: liver microsomes of MC-treated rats (I_{50} = 0.042 μg); liver microsomes of MC-C_{57}BL/6 mice (I_{50} = 0.050 μg); liver microsomes of MC-treated guinea pigs (I_{50} = 0.21 μg); liver microsomes of MC-treated DBA/2 mice (I_{50} = 0.23 μg); liver microsomes of MC-treated hamsters (I_{50} = 0.78 μg); lung microsomes of MC-treated rats (I_{50} = 1.02 μg); and kidney microsomes of MC-treated rats (I_{50} = 1.04 μg); The differential competition reflects the existence of different levels of cytochrome P-450 with MAb-specific epitopes in these microsomal preparations.

The competitive RIA method has also been applied with additional MAbs to rat liver MC-P-450, including MAb 1-31-2 /39/. In addition, an RIA based on MAb 2-66-3 to rat liver PB-P-450 has also successfully detected greater levels of epitope-specific cytochromes P-450 in liver microsomes from PB-treated rats than in liver microsomes from untreated or MC-induced rats /38/.

Competitive RIA has also been successfully applied to the detection of human cytochrome P-450. Individual placenta from women who smoked cigarettes displayed higher levels of MAb 1-7-1 specific cytochrome P-450 than placenta from nonsmokers /40/. This result is consistent with the higher level of AHH and of the greater inhibition of AHH by MAb 1-7-1 in placenta from smokers /24/. The RIA also detected the elevation of MAb 1-7-1 specific cytochrome P-450 in lymphocytes /40/ and cloned lymphoblastoid AHH-1 cells /41/ treated with the AHH inducer benz(a)anthracene.

The experiments described thus far were performed by competitive RIA. An additional approach for evaluating MAb-specific cytochromes P-450 is with a two-site immunoradiometric assay, which employs two MAbs. The first MAb is coated on the microtiter wells, and the subsequently added antigen binds to the wells. A labeled second MAb is then added and its binding to the wells is measured. If the two MAbs are directed toward independent epitopes, the second MAb will bind to the wells. If both MAbs are directed to the same or overlapping epitopes, little or no binding of the second MAb will be observed. The two-site assay was applied using rabbit P-450 1 /16/. The results demonstrated that three MAbs to this isozyme bind to three distinct, nonoverlapping

epitopes. This approach to relating the epitope specificities of panels of MAbs should prove useful in defining various isozymes in terms of epitope structure.

The RIA method is generally applicable to a range of MAbs. The method is efficient and many samples can be simultaneously analyzed. The information obtained on specific cytochrome P-450 content of a sample is limited only by the available number of MAbs. This method has several advantages over enzyme inhibition assays. Firstly, the MAb used need not be of a type that inhibits enzyme activity. Secondly, RIA can detect cytochromes P-450 that are labile with respect to activity /40/, since successful detection only requires structural integrity of the limited portion of the molecule that includes the epitope, and is not dependent on the integrity of the active site.

The RIA for cytochromes P-450 with MAb-specific epitopes is at present semiquantitative except for a narrow, linear region of the standard competition curve. However, improvements are continually in progress for increasing the quantitativeness as well as the sensitivity of the RIA.

In addition to RIA, nonradioisotopic methods have been applied to detection of cytochrome P-450, although thus far to a more limited extent. In particular, enzyme-linked immunoadsorbent assay (ELISA), was used to characterize a panel of nine MAbs to the rat hepatic P-450c isozyme /17/. Competitive ELISA, in which each peroxidase-labeled MAb competes with each unlabeled MAb, demonstrated that the MAbs were directed toward at least five spatially distinct epitopes on P-450c. In addition, three MAbs cross-reacted with the P-450d isozyme, indicating the immunochemical relatedness of P-450c and P-450d.

5. IMMUNOPURIFICATION USING MONOCLONAL ANTIBODIES

Immunoaffinity chromatography is a generally successful and widely used technique for the purification of numerous proteins. When a MAb is coupled to the solid-phase support, the method is uniquely suited for purification of MAb-defined isozymes such as the cytochromes P-450. Several such immunoadsorbents have been prepared, and when solubilized liver microsomes from rat /39,42/ or rabbit /14,16/ are applied to Sepharose-MAb, the resin selectively adsorbs a fraction of the total microsomal cytochrome P-450. The immunoadsorbed cytochromes P-450 are in the native state, based on the CO-reduced difference spectra /42/ or catalytic activity /15/. Elution of cytochrome P-450 from the

immunoadsorbents examined thus far, however, require denaturing conditions such as 0.1 M glycine /42/, acetic acid /14/, or sodium dodecyl sulfate /16/. Although catalytically inactive, the desorbed cytochrome P-450 isozymes may, however, be subjected to numerous structural analyses that do not require functional enzyme.

SDS-PAGE analysis of immunoadsorbed and subsequently eluted proteins provides a preliminary characterization of the purified isozymes based on the standard criterion of molecular weight and affords a rapid and substantial purification of cytochromes P-450. Since the specificity of the procedure is based on epitope content, more than one isozyme may be obtained if these are antigenically related by a common MAb-specific epitope. MAb-based immunopurification therefore purifies epitope-related, or MAb-defined classes of cytochromes P-450, where each class consists of one or more distinct enzymes.

Immunopurification has been applied, using MAbs to MC-P-450, to different tissues from MC-induced animals. In particular, immunoadsorptions with MAb 1-31-2 and MAb 1-7-1 using liver, lung, and kidney microsomes from rats, C_{57}BL/6 and DBA/2 mice, hamsters, and guinea pigs were carried out /39/. Figure 6 shows the SDS-PAGE analysis of proteins that were immunopurified from different tissues of MC-treated rats. Whereas Sepharose-MAb 1-7-1 binds two species of molecular weights 56,000 and 57,000 (lane 4), Sepharose MAb 1-31-2 binds only the high molecular weight species (lane 5) and control MAb 1-14-1 (made by hybridomas of spleens from nonimmunized mice) was not observed to bind any microsomal protein (lane 3). In addition both Sepharose-MAb 1-7-1 and MAb 1-31-2 bind only one protein of molecular weight 57,000 from lung microsomes (lanes 8 and 9) whereas control MAb 1-14-1 did not bind this protein (lane 7). With kidney microsomes, no protein was found to bind any of the three MAbs (lanes 11, 12 and 13). These observations demonstrate the capability of MAb-based procedures to readily detect cytochrome P-450 in different tissues.

A similar SDS-PAGE analysis of immunoaffinity purified liver microsomal cytochromes P-450 from different species was also carried out /39/. With C_{57}BL/6 mice, MAb 1-7-1 binds two proteins whose MWs are 56,000 and 57,000 respectively, while MAb 1-31-2 binds only the MW 57,000 species and the control MAb 1-14-1 did not bind any protein. These observations clearly demonstrate similarities between C_{57}BL/6 mouse and rat liver in that both high molecular weight species contain epitopes for the binding of MAb 1-7-1 and MAb 1-31-2, whereas

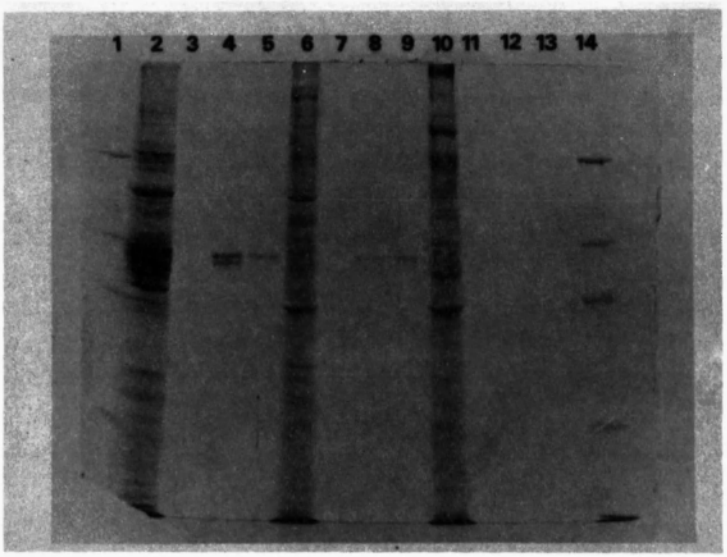

Figure 6. SDS-PAGE analysis of MC-rat microsomal proteins and proteins immunopurified with MAbs. Lanes 1 and 14 contain molecular weight standards (93K, 67K, 60K, 43K, 30K and 20K). Lane 2 contains liver microsomes; lanes 3-5 contain liver microsomal material adsorbed to Sepharose-bound MAb 1-14-1, 1-7-1, and 1-31-2, respectively. Lane 6 contains lung microsomes; lanes 7-9 contain lung microsomal material bound to Sepharose-MAb 1-14-1, 1-7-1, and 1-31-2, respectively. Lane 10 contains kidney microsomes; lanes 11-13 contain kidney microsomal material bound to Sepharose-bound 1-14-1, 1-7-1, and 1-31-2, respectively.

the low molecular weight species contain only the epitope for MAb 1-7-1. In liver from DBA/2 mice, one species of MW 56,000 was shown to bind MAb 1-7-1, while neither MAb 1-31-2 nor control MAb 1-14-1 bound any protein. Guinea pigs and hamsters, whose liver microsomal AHH activities are only moderately inducible by MC, also exhibited species dependent differences in MAb-specific cytochrome P-450. A polypeptide of MW 53,000 was purified from guinea pigs by immunoabsorption with Sepharose-MAb 1-7-1, whereas hamsters yielded a very faint band of MW 57,000. Sepharose-MAb 1-31-2 and control MAb 1-14-1 did not yield any detectable band for either species. The relative intensities of Coomassie blue staining of the immunopurified proteins generally correlates with their levels in the microsomes as detected by

RIA /39/. The immunoaffinity purification is thus useful for semi-quantitative as well as qualitative determination of MAb-specific cytochrome P-450.

Immunopurifications carried out with MAb 1-7-1 and liver microsomes from untreated rats did not yield detectable protein on SDS-PAGE /42/ which demonstrates that the proteins obtained from MC-treated rats were MC-induced. This procedure may therefore be useful for studies of cytochrome P-450 induction in different tissues.

Figure 7 shows the proteins purified from liver (lane 2 and 3) and lung (lane 4) microsomes of MC-rats, and livers of $MC_{57}BL/6$ mice (lanes 5 and 6), MC-DBA/2 mice (lane 7), MC-guinea pigs (lane 8) and MC-hamsters (lane 9). The apparent molecular weight range is 53,000-57,000 and these are tabulated in Table 6. The eight purified proteins contain heme as demonstrated by the appearance of a 420 nm peak in their reduced-CO difference spectra, a characteristic feature of denatured cytochrome P-450. Since cytochromes P-450 are very labile at low pH, the desorption procedure, using buffer at pH 3.0, inevitably converted cytochrome P-450 to cytochrome P-420.

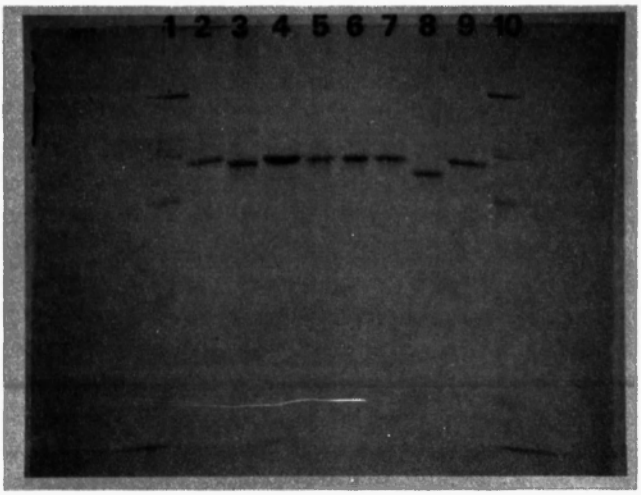

Figure 7. SDS-PAGE analysis of immunopurified proteins. Lanes 1 and 10 contain molecular weight standards. Lanes 2-9 contain about 2μg each of proteins purified from: MC-rat liver microsomes (MW 57,000 species in lane 2 and MW 56,000 species in lane 3), MC-rat lung microsomes (lane 4), MC-C_{57}BL/6 mouse liver microsomes (MW 57,000 species in lane 5 and MW 56,000 species in lane 5), MC-DBA/2 mouse liver microsomes (lane 7), MC-guinea pig liver microsomes (lane 8) and MC-hamster liver microsomes (lane 9).

TABLE 6. Purified cytochromes P-450

Species, Tissues	Molecular Weight of Cytochrome P-450 Purified with Sepharose-		
	MAb 1-7-1		MAb 1-31-2
rat liver	56,000	57,000	57,000
rat lung	57,000		57,000
C_{57}BL/6 mouse liver	56,000	57,000	57,000
DBA/2 mouse liver	56,000		N.D.
guinea pig liver	53,000		N.D.
hamster liver	57,000		N.D.

N.D. : None detectable

Several conclusions may be derived from the immunopurification data presented thus far and serve to exemplify the type of information gained by such studies: 1) MAbs to an epitope to a cytochrome P-450 from one species (rat) detect cytochromes P-450 with this epitope in different tissues and species; 2) a single MAb may identify more than one cytochrome P-450 in a tissue that contains its target epitope; 3) a single cytochrome P-450 may bind more than one MAb, demonstrating that it contains epitopes for all MAbs to which it binds.

Immunopurification carried out with three MAbs to rabbit P-450 form 1 and rabbit liver microsomes yielded results comparable to those described above /16/. While all three Sepharose-MAbs adsorbed P-450 form 1, MAb 1G11 additionally bound P-450 3b, indicating that these two cytochromes P-450 possess the MAb 1G11 specific epitope in common. MAb 2F5 interacted with P-450 form 1 as well as a species with electrophoretic mobility intermediate between P-450 forms 1 and 3b. Each Sepharose-MAb thus exhibits a unique pattern of adsorption for rabbit liver cytochromes P-450, which serves to antigenically relate these isozymes.

Immunoaffinity purified cytochromes P-450 have generally not been enzymatically characterized but have been rather analyzed by SDS-PAGE, since the procedure for desorption from the Sepharose-MAb denatures the cytochrome P-450. However, a successful measurement of AHH was performed for a purified rabbit P-450 in a reconstituted system immobilized on Sepharose-MAb /15/. This was feasible because the MAb MBS 105 used in this experiment was of a type that does not inhibit activity. As more such noninhibitory MAbs become available, such experiments will complement and greatly enhance the characterization of MAb-immunopurified cytochromes P-450 from different sources.

In addition to SDS-PAGE and activity measurements, the purified cytochromes P-450 are suitable for amino acid sequence analysis. Such data may be the only method that is capable of distinguishing closely related P-450 isozymes. The feasibility of using MAb-based immunopurification to provide sufficiently pure cytochrome P-450 for sequencing was demonstrated /43/ by sequence analysis of the ten NH_2-terminal amino acids of two forms of cytochrome P-450 from C_{57}BL/6 mice (56K and 57K), one form from DBA/2 mice (56K) and one form from guinea pigs (53K). The 56K polypeptides from rats, C_{57}BL/6 mice, and DBA/2 mice were shown to have identical NH_2-terminal sequences for the first ten amino acids. In this NH_2-terminal region, the 57K polypeptides from rats and C_{57}BL/6 mice are homologous to one another but are nonhomologous with the 56K polypeptides, while the 53K polypeptide from guinea pig has an NH_2-terminal sequence that is unique and nonhomologous with either of the other cytochromes P-450. MAb-based immunopurification thus provides cytochromes P-450 suitable for primary structural analysis with a fraction of the effort required in conventional purification procedures.

The use of Sepharose-bound MAbs provides an essentially one-step, effective and selective purification of cytochromes P-450 of different antigenic classes. When followed by SDS-PAGE, enzymatic analysis, or primary sequence analysis, this method should prove useful in screening samples for different types of cytochromes P-450. The extraordinary and precise specificity of MAbs renders them chemical reagents par excellence for the isolation and characterization of specific cytochromes P-450 from crude and heterogeneous biological preparations.

6. SUMMARY AND PERSPECTIVES

The cytochromes P-450 are the primary enzyme interface between higher organisms and xenobiotics such as environmental chemicals and drugs. Biochemical individuality in responsiveness to these agents is complex and relates to both environmental and genetic factors. The nature and complexity of problems relating to cytochrome P-450 multiplicity seems exquisitely susceptible to analysis by MAbs. Utilization of MAbs in conjunction with current methodologies offers entirely new approaches which should be generally useful in recognition of specific cytochrome P-450 isozymes. MAbs are thus tools which expand the power of existing methods. Their power lies in their applicability in conjunction with numerous techniques: a multiplier effect

results when a given method is applied with a panel of MAbs. MAbs thus promise to enhance the resolving power of established techniques to classify and characterize. For example, RIAs and immunopurifications based on two MAbs to MC-P-450 /39/ not only detect and purify specific isozymes, but serve to classify these isozymes on the basis of their interaction with the respective MAb.

In this review we have surveyed the types of studies feasible with MAbs that have been reported in the literature. Since numerous laboratories with varied interests are preparing MAbs, one may envisage additional future studies that were not previously feasible without the exquisite specificity of MAbs. These involve both laboratory and clinical studies.

MAbs promise to be useful tools for enzymological and biophysical studies of cytochrome P-450 structure-function relationships. It is plausible that at least some of the enzyme-inhibitory MAbs alter the structure of the active site, either in the heme moiety or in the protein conformation of the active site. Examination of these changes by spectral or chemical methods may reveal hitherto unknown aspects of mechanism of substrate binding and conversion of substrate to specific products.

The interactions between cytochrome P-450 and other cellular components may be probed with MAbs. The extent to which MAbs bind specific membrane-bound cytochromes P-450 indicates the degree to which various epitopes are internal or external to the membrane structure. Topographical relationships may be derived from this information. In the same manner the interaction of cytochrome P-450 with substrates, cofactors, or metabolically linked enzymes such as NADPH-cytochrome P-450 reductase or cytochrome b_5, may be evaluated by the influence of different MAbs on these intermolecular interactions.

We have already used MAbs to identify specific polypeptides formed by the translation of MC and PB induced rat liver mRNAs /44/. The MAbs can be powerful tools for cloning of individual cytochromes P-450. MAb based immunopurification procedures can be used to purify cytochromes P-450 whose NH_2-terminal amino acid sequences may be determined and compared to sequences derived from the analysis of nucleotide sequence of cloned cytochrome P-450 genes. Furthermore, a comparison of sequences generated by the two techniques can be very useful in analyzing the synthetic and degradative processing of cytochromes P-450. These may include such steps as cleavage of terminal amino acids or glycosidation. Thus, hybridoma technology and DNA

recombinant techniques can be important complementary methods for understanding the molecular biology of cytochrome P-450. These approaches can be used to genotype and phenotype differences in the structure and organization of cytochrome P-450 genes in different individuals and to measure their expression under a variety of conditions.

The MAbs will prove useful for identifying forms of cytochromes P-450 that are under regulational control by different nutritional and hormonal conditions during developmental stages and after exposure to inducers. For this purpose, a variety of MAb based methods can be used histologically by localizing specific cytochromes P-450 by light or electron microscopy linked with MAb-directed immunofluorescence, by qualitative or quantitative phenotyping by immunoassay, or by reaction phenotyping utilizing enzyme inhibitory MAbs.

MAb directed characterization of tissue phenotype for cytochromes P-450 should prove useful in defining the enzymatic basis for biochemical individuality. A phenotyping study of individuals who display a deficiency in the metabolism of a particular drug, for example, may reveal whether the presence or absence of a specific cytochrome P-450 is responsible for the observed metabolic effect. Pretesting of individual patients prior to treatment would therefore have predictive value in drug therapy.

A library of monoclonal antibodies to the different cytochromes P-450 may serve to prepare an atlas of specific cytochromes P-450 in different species, strains, tissues, and individuals. These MAbs may be useful for studying genetic control, phylogenetic evolution, and the changing distribution of cytochromes P-450 during fetal and sexual development, after exposure to different inducers, and in different nutritional states. Along with epidemiological, toxicological and pharmacological studies, MAbs may thus be useful in increasing our understanding of the role of cytochromes P-450 in individual differences in responsiveness to drugs and toxic agents and in susceptibility to carcinogenesis.

The value of MAbs in cytochrome P-450 research is clear. MAb-based methods are broadly applicable, however, for isozymic systems in general. Several of these, such as the glutathione, glucuronic acid, and sulfo-transferases are especially relevant to drug and carcinogen metabolism. These enzymes will surely be subjected to MAb-based analyses if we are to obtain a comprehensive view of drug and carcinogen metabolism.

ABBREVIATIONS

AAF	2-acetylaminofluorene
AHH	aryl hydrocarbon hydroxylase
BP	benzo(a)pyrene
ECD	7-ethoxycoumarin 0-deethylase
ELISA	enzyme-linked immunosorbent assay
MAb	monoclonal antibody
MC	3-methylcholanthrene
MC-P-450	major form of MC-induced rat liver microsomal cytochrome P-450
PB	phenobarbital
PB-P-450	major form of PB-induced rat liver microsomal cytochrome P-450
SDS-PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis

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