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Cytochrome P450 and Monoclonal Antibodies

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I. Introduction

The cytochrome P450 enzymes are the primary metabolic interface between higher organisms and xenobiotics as well as the primary agents for the metabolism of certain endobiotics. Thus, they play a key role in xenobiotic and endobiotic metabolic processing. A vast array of xenobiotics including drugs, carcinogens, mutagens, pesticides, and other environmental chemicals that are both manmade and natural products are metabolized by mammalian and lower organisms by the highly multiple forms of cytochrome P450 and metabolically related enzyme systems (Coon et al., 1980; Gelboin, 1980; Sato and Kato, 1982). The metabolically linked enzymes are the flavoprotein-linked reductases, hydratases catalyzing the hydrolysis of epoxides to dihydrodiols (Gelboin, 1980), and various transferases catalyzing synthetic reactions that conjugate the xenobiotic metabolites with glucuronic acid, sulfate, glutathione, acetyl coenzyme A or methyl donors (Jacoby, 1980). The cytochrome P450s exist in multiple isozymic forms (Coon et al., 1980; Gonzalez, 1989; Nebert et al., 1991), many of which may direct the flow of substrates into alternate metabolic pathways. Thus, cytochrome P450s are a paradigm for multiisozymic systems in which the distribution of isozymes govern substrate conversion into different metabolites and thus into alternate metabolic routes. These alternative metabolic routes may result in different and often opposing physiological and pathological consequences (Omenn and Gelboin, 1984; Sato and Kato, 1982; Sugimura et al., 1982) and be analyzed with MAbs† (Gelboin and Friedman, 1985). The cytochrome P450-containing mixed-function oxidase system is also respon-

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† Abbreviations: MAb, monoclonal antibody; MC, methylcholanthrene; RIA, radioimmunoassay; ELISA, enzyme-linked immunosorbent assay; AHH, aryl hydrocarbon hydroxylase; ECD, ethoxycoumarin deethylase; BP, benzo(a)pyrene; PB, phenobarbital; BNF, β-naphthoflavone; PCN, pregnenolone 16α-carbonitrile; EROD, ethoxyresorufin deethylase; ER, endoplasmic reticulum; AAF, 2-acetylaminofluorene; BA, benzyl alcohol; PROD, pentoxyresorufin O-deethylase; PGE_2, prostaglandin E; DDC, 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine; CYP, cytochrome P450.

sible for the metabolism of important classes of endobiotics which include all classes of steroids, fatty acids, bile acids, and prostaglandins (Sato and Kato, 1982).

Cytochrome P450-catalyzed metabolism has two facets that may be either beneficial or detrimental to the host organism, one route leading to metabolites that are detoxified by conjugation and safely excreted and the other to metabolites that are toxic, mutagenic, or carcinogenic (Miller, 1959; Coon et al., 1980; Sato and Kato, 1982; Sugimura et al., 1982). There are a large number of forms of constitutive and inducible cytochrome P450s. More than 200 cytochrome P450 genes have been identified in 27 gene families (Nebert et al., 1991; Nelson et al., 1993), and many of the cDNAs of these cytochrome P450s have been sequenced. The large multiplicity of cytochrome P450 forms, their widely heterogenous structure and phylogenetic distribution, and in many cases their poorly defined substrate and product specificity make difficult the determination of their in situ quantitative contribution to the metabolism of specific substrates. This difficulty is further complicated by the often considerable overlapping stereo- and regioselectivity for different substrates and products and their different activities with substrates at different concentrations. This, in turn, has prevented an understanding of the relationship between the genetic factors regulating cytochrome P450 phenotype and individual responsiveness and sensitivity to drugs and carcinogens. Also limited is our knowledge of the role of individual cytochrome P450s in endobiotic metabolism that may be related to physiological variation among individuals.

A. Monoclonal Antibodies: Production and Properties

MAbs are produced by the "hybridoma" technology (Kohler and Milstein, 1975). Their discovery is a milestone achievement of modern biology (Kohler and Milstein, 1975; Yelton and Scharff, 1981). The hybridoma technology grew out of the clonal selection hypothesis of Burnet and colleagues (Burnet, 1959), which states that each β-lymphocyte antibody-forming cell and its progeny is committed to the production of a single type of antibody molecule. The MAbs are chemically defined reagents that recognize a single antigenic determinant or "epitope" on a macromolecule (Kohler and Milstein, 1975). This specificity continues for the lifetime of the cell and the lifetime of its progeny. An animal may produce millions of antibodies in response to the various immunogens to which it is exposed. Only a small fraction of β-lymphocytes respond to any given immunogen. These replicate and differentiate into antibody-secreting cells. Because immunogens may have many determinants, the β-cells collectively produce a large number of different antibodies that collectively recognize different epitopes on the antigen. These are the polyclonal antibodies which recognize a multiplicity of epitopes reflecting the heterogeneity of antibodies in polyclonal antibodies. In this system any contaminant of the immunogen will find expression in the polyclonal antibody. The composition of polyclonal antibodies produced by the same immunogen will vary with each immunization, and they cannot be considered stable or pure reagents.

In terms of chemical precision, purity, specificity, and reproducibility for limitless generations of cells, MAbs are new and superior to polyclonal antibodies as a class of reagents. For the production of MAbs, myeloma tumor cells are fused with the isolated dissociated B-lymphocytes of the spleen from mice immunized with a particular immunogen. The fused cells or "hybridomas" exhibit unique and special character by combining the characteristics of the B-lymphocytes that are committed to the continuous and stable production of a specific antibody with the myeloma tumor cells imparting immortal properties to the hybridoma cells. The individual hybridomas are cloned and screened for the desired MAb. The screening is done by a variety of methods including binding assays, Western blots, and enzyme inhibition. The selected clone and its progeny produce identical antibodies directed to a single epitope. The selected clone is usually subcloned three times to assure monoclonality. These hybridomas grown in cultures in a defined medium produce the chemically defined MAbs free of contaminating cells and protein debris. Large amounts of MAbs can be obtained by injecting the hybridomas into the peritoneal cavity of appropriate recipient mice, where they grow as ascites tumors and produce large quantities of the specific MAb. The MAb-producing hybridomas are essentially immortal and can be frozen and stored or grown in culture indefinitely. Depending on their use and application, the MAbs from either culture fluid or ascites fluid can be easily purified with simple chromatographic procedures to yield purified MAbs. One such procedure has been described using an hydroxyapatite column (Stanker et al., 1985).

Other studies (Benjamin et al., 1984) have described the use of MAbs to study the surface and immunogenic domains of the well-defined proteins, myoglobin, lysozyme c, cytochrome *c*, and serum albumin as model antigens. The major conclusions were that most of the surface of a protein may be antigenic. These may include overlapping and multiple determinants. Antigenic sites are made of a three-dimensional complex of amino acids that require the native conformation of the protein for their integrity as antigens. On a given antigen, the subset of determinants that are antigenic varies among species and depends on the differences between the host cell protein and the antigen. Studies such as the latter can be applied to the study of cytochrome P450s and thus define the character of cytochrome P450 immunogenicity and its antigenic determinants. This may also yield information concerning cytochrome P450 active sites and mechanism of enzyme action.

MAbs are directed to conformational or sequential

epitopes of the antigen protein. Conformational determinants depend on the native spatial conformation of the protein, whereas sequential epitopes depend only on the sequence of amino acids. In each case, the antigenic determinants are topographic, i.e., composed of structures on the protein surface. MAbs to different types and positioned epitopes may help elucidate the topography of the cytochrome P450 antigen in relation to the microsomal membrane and identify sites that are immunodominant, i.e., those to which most of the immune response is directed.

B. Homogeneous Monoclonal Antibodies from Impure Antigens (Cytochrome P450s): Hybridoma Cloning as Cytochrome P450 Purification System

Each spleen B-lymphocyte of an immunized mouse is committed to the production of an epitope-specific MAb (Burnet, 1959; Kohler and Milstein, 1975). The MAbs can be instruments for cytochrome P450 detection (Cheng et al., 1984a,b; Gelboin and Friedman, 1985; Song et al., 1985a,b) (see section III) and purification of cytochrome P450s (Cheng et al., 1984a,b; Friedman et al., 1983; Gelboin and Friedman, 1985) (see section V). The cloning component of the hybridoma technology permits the use of impure antigen preparations because the cloning and assay will permit the isolation of the desired clone specific for a single epitope. The technique can be used for obtaining MAbs to proteins present in low concentrations or those that may be minor deviants of the primary protein in the immunogen preparation. However, unfractionated preparations, such as solubilized microsomes, are not useful as immunogen preparations because the fraction of hybridomas committed to a single epitope would be in low concentration, and it would be difficult to screen and detect the hybridoma producing the desired MAb.

C. Monoclonal Antibodies for Cytochrome P450 Research

The properties and molecular diversity of cytochrome P450s make them extraordinarily suitable for investigation with MAbs. The cytochrome P450s are present in multiple forms, and the levels of each cytochrome P450 may change in response to environmental and endogenous factors. The MAbs will prove useful for identifying and quantifying cytochrome P450 proteins that vary under different conditions, which include nutritional and hormonal states, developmental stages, age, sex, and inducer exposure. For this purpose a variety of MAb-based methods can be used (table 1). A full library of MAbs to cytochrome P450 could develop a cytochrome P450 taxonomy and would greatly enhance the progress of cytochrome P450 research. Table 1 shows the range of applications of MAbs to cytochrome P450 research (Gelboin and Friedman, 1985).

These include quantitative and qualitative detection of epitope-specific cytochrome P450s (section III), determination of epitope specificity for different cytochrome P450s, rapid and simple immunopurification (section V), immunohistochemistry (section IV) at the tissue, cellular, and subcellular levels, and reaction phenotyping (section VI), i.e., the determination of the quantitative catalytic contribution of each cytochrome P450 to the metabolism of a given substrate. The MAb technology and its biological potential make it a probe *par excellence* for cytochrome P450 research. MAbs to cytochrome P450s of the IgG, IgM, IgG2a, and IgG2b types have been isolated (Park et al., 1982, 1984, 1986a,b). The isolated MAbs to cytochrome P450s can be broadly classified in three categories. There are MAbs that bind epitope-specific cytochrome P450s but do not immunoprecipitate the cytochrome P450 protein or inhibit its enzymatic activity. A second category of MAb binds and immunoprecipitates the cytochrome P450 but does not inhibit its catalytic activity. A third class of MAbs exhibits all three activities, i.e., binds, immunoprecipitates, and inhibits the catalytic activity of the epitope-specific cytochrome P450. The measurement of binding can be accomplished with variations of RIA or ELISA or by Western blotting. MAbs have been reported that inhibit enzyme activity but do not give a Western blot (Park et al., 1986a,b). Also, MAbs to one class of cytochrome P450s unexpectedly have been found to cross-react with a cytochrome P450 from a different class (Park et al., 1986a,b). It is clear that many of the MAbs to cytochrome P450s are directed to different epitopes on the cytochrome P450 molecule because the binding by the MAb has different functional effects and, in some cases, several MAbs to the same cytochrome P450 show no competitive binding to the cytochrome P450 (Song et al., 1985a). The epitope relatedness of two different MAbs

TABLE 1
Monoclonal antibodies for P450 research

1) Detection
   - Qualitative, Quantitative: RIA, ELISA, Western Blot
2) Immunohistochemistry
   - Tissue
     - Intercellular
     - Intracellular
3) Immunopurification
   1) Single step, rapid
   2) Protein sequencing
   3) Epitope mapping
4) Reaction phenotyping
   - Inhibitory MAbs
     1) Single P-450 contribution to:
        - Substrate disappearance
        - Product formation, positional-, regio-, stereospecificity
     2) Adduct formation
     3) Adduct formation
     4) Transforming products
5) Toxic products
6) P450 Topography
7) Enzyme mechanism
8) Taxonomy

to the same cytochrome P450 can easily be determined by measuring the ability of one MAb to compete with the second MAb for a binding site on the cytochrome P450. A total lack of competition indicates unrelatedness of the epitopes on the cytochrome P450s that the two MAbs target. The degrees of competition for epitope sites indicate the overlap between epitopes; the extent of competition of the MAbs for their target epitopes is an indicator of epitope relatedness (Song et al., 1985a). A membrane-buried epitope can also be uncovered by membrane solubilization.

D. Monoclonal Antibodies and Epitope Specificity

The specificity of the MAb resides in its precise ability to recognize and bind a specific epitope on the surface of a cytochrome P450. Thus, an MAb may recognize only a single species of cytochrome P450 if it contains a unique epitope. In this case, the MAb would be specific for only a single isozymic form of cytochrome P450. An MAb that recognizes an epitope that is present in more than one form of cytochrome P450 will bind all of those forms of cytochrome P450 containing the common epitope. Thus, on theoretical grounds, a comprehensive MAb library may contain MAbs that recognize single unique forms of cytochrome P450, MAbs that recognize two or more forms, and possibly MAbs that recognize entire large classes of cytochrome P450s sharing epitopes common to all of the forms. Epitope specificity of an MAb is now generally tested by comparing their binding potential to different cytochrome P450s by ELISA or Western blots. These define epitope specificity within a narrow range because the number of cytochrome P450s in a single laboratory is generally relatively small. Thus, a full description of MAb specificity will be possible when a comprehensive library of cytochrome P450s becomes available. This may be accomplished with the use of cDNA-expressed cytochrome P450s as immunogens.

E. Complementary Monoclonal Antibody-directed "Reaction Phenotyping" and Cytochrome P450 cDNA Expression (fig. 1)

The cytochrome P450s collectively catalyze the oxidation of a multitude of xenobiotic and endobiotic substrates. One of the essential questions relating to cytochrome P450 function concerns the ability to measure the contribution of each individual form or class of cytochrome P450 to the metabolism of an individual substrate (Fujino et al., 1982). This measurement in different tissues, organs, and individuals would yield information concerning the metabolic routing by cytochrome P450s that determine their role in metabolism (fig. 1).

Recent advances in the molecular biology of cytochrome P450 have succeeded in the cloning of a large number of rodent and human cytochrome P450 cDNAs (Gonzalez, 1989; Nebert et al., 1991). Many of these cDNAs have been constructed into different vectors and

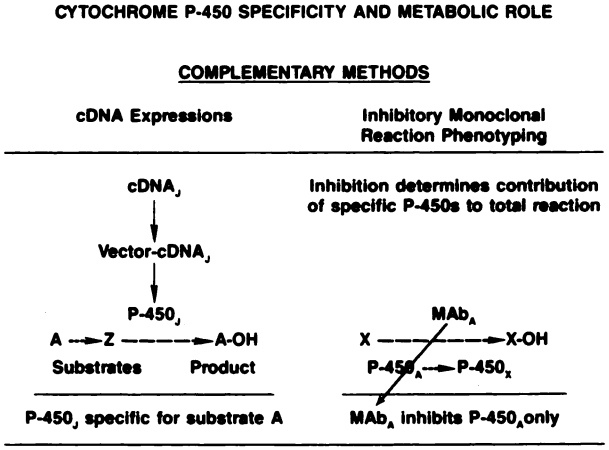

FIG. 1. Complementary use of cDNA expression and reaction phenotyping.

expressed as a cytochrome P450 protein from a single cDNA (Aoyama et al., 1989a,b, 1990; Battula et al., 1987; Crespi et al., 1989; Gonzalez, 1989). These expression systems have included yeast, Cos cells, vaccinia virus (Gonzalez, 1989), baculovirus (Summers and Smith, 1987), and human AHH-1 lymphoblastoid cells (Crespi et al., 1989). The expressed cytochrome P450s are characterized for their metabolic profiles and for their ability for mutagenic activation (Aoyama et al., 1989a,b, 1990; Crespi et al., 1989). Another possible use of cDNA expression is for the production of cytochrome P450s for use as immunogens in MAb production. The formation of individual expressed cytochrome P450s is a major advance in cytochrome P450 research, but it does not offer insight into the quantitative contribution of a cytochrome P450 to the metabolism of a given substrate in vivo. This is largely due to the inability to quantitate both the amount and activity of each cytochrome P450 present in situ. MAbs that inhibit the enzyme activity of specific epitope-containing cytochrome P450s are major complementary reagents to cDNA expression. They can determine the quantitative contribution of the epitope-specific cytochrome P450 to any reaction measured in a tissue preparation containing multiple cytochrome P450s. Thus, the amount of inhibition by an MAb is useful for defining the role of a single cytochrome P450 in xenobiotic and endobiotic metabolism. This technique, which we have called "reaction phenotyping," can be used quite successfully in conjunction with the cDNA cytochrome P450 expression for which substrate and product specificity of an individual cytochrome P450 can be defined. This is especially true when the MAb recognizes two cytochrome P450s and the cDNA expression shows that only one of the two cytochrome P450s is active for the reaction studied. Thus, characterizing functional specificity of each cytochrome P450 can easily be accomplished with cDNA expression, and the amount of contribution of a cytochrome P450 to specific metabolism can be determined with inhibitory MAbs (Fujino et al., 1982).

F. Monoclonal Antibody-directed Immunoassay of Cytochrome P450

The MAbs, being chemically defined reagents with extraordinarily high specificity for unique epitopes, can be the basis for both qualitative detection of cytochrome P450 in a tissue and quantitative measurement of epitope-defined individual or classes of cytochrome P450 (Cheng et al., 1984a,b; Song et al., 1984, 1985a,b) (table 1) (section 3). The type of immunoassay can be tailored to the particular application and can utilize either radiolabeled MAbs or the ELISA type utilizing as a detection system an appropriate enzyme-linked anti-IgG. In addition, a RIA can be utilized with anti-IgG ^{35}S- or ^{3}H-labeled MAb. With this detection system one may screen numerous unlabeled MAbs for their binding to cytochrome P450s. A variety of immunoassays have been developed, and the appropriate assay can be chosen for the individual problem being investigated. With the MAb-directed immunoassays, a tissue or organ of an individual can be measured for an MAb-defined content of cytochrome P450. Western blots are a detection method with which a large number of cytochrome P450s can be simultaneously detected with MAbs and analyzed for cross-reactivity.

MAbs are directed at single epitopes on the antigen cytochrome P450. The singularity of epitope-MAb combination gives the MAb its high specificity. It is also true, however, that the nature of the epitope cannot be experimentally predetermined; thus, the MAb cannot be "made to order." Thus, various features of the protein immunogen may govern MAb interaction with the various domains of cytochrome P450 as well as the cytochrome P450 active site. A large limitation of MAb-directed methods would prevail when the MAb target epitope was common to a large number of other cytochrome P450s. Another possible interference with MAb-based analysis can result if the cytochrome P450 epitope is buried in the membrane and is unavailable to the MAb. In some cases release of a hidden epitope can be accomplished with membrane solubilization. Interpretation of MAb-based immunoassays must also take into account the affinity of the MAb to the target cytochrome P450 as well as any possible steric hindrance. It is also possible that in a competitive immunoassay the amount of inhibition by the target cytochrome P450 may be mimicked by another cytochrome P450 (or other protein) that may share part of the epitope of the target cytochrome P450 protein. It is also not uncommon for an MAb giving a positive ELISA or RIA reaction to give a negative double-immunodiffusion analysis result. Also, an MAb inhibitory to the cytochrome P450 enzyme activity may not give a positive Western blot and those MAbs yielding a positive Western blot do not necessarily inhibit cytochrome P450 enzyme activity.

Reaction phenotyping (table 1) of cytochrome P450 can be applied to the analysis of a variety of reactions which may be either direct or indirect consequences of cytochrome P450 activity. These include (*a*) substrate disappearance, (*b*) product formation, stereochemical aspects of cytochrome P450 reactions, and (*c*) indirect action phenomena that are a result of cytochrome P450-catalyzed formation of reactive metabolites. These reactive metabolites may result in (*a*) metabolite binding to macromolecules such as protein (Miller, 1959) and DNA (Gelboin 1969), (*b*) cell toxicity, (*c*) mutagenicity (1), 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 epitope-specific cytochrome P450 to the total direct or indirect activity measured (Fujino et al., 1982, 1984a,b).

G. Cloning and Regulation of Cytochrome P450s and Cytochrome P450 Peptide Processing

The MAbs to individual and classes of cytochrome P450 may be useful to identify epitope-specific polypeptides formed by the translation of cytochrome P450-specific rat liver mRNAs. The MAbs can be powerful tools for cloning of individual cytochrome P450s. MAb-based immunopurification procedures can be used to purify cytochrome P450s whose amino-terminal sequence can be determined and compared to those derived from the analysis of the nucleotide sequence of cloned cytochrome P450 genes. A comparison of sequences generated by the two techniques can be very useful in analyzing the synthetic and degradative processing of cytochrome P450s. These may include metabolic processes that yield altered amino-terminal sequences by glycosidation or other peptide alterations. Thus, hybridoma technology and DNA recombinant techniques are important complementary methods for understanding the molecular biology of cytochrome P450s. These approaches can be used to characterize the structure and organization of cytochrome P450 genes in different individuals and to detect and measure cytochrome P450 expression under a variety of conditions. This knowledge may lead to better understanding of the physiological and pharmacological implications of different cytochrome P450 phenotypes.

H. Implications for Ecogenetics, Pharmacogenetics, and Risk Assessment

Humans are exposed to a variety of xenobiotics in foods, in the work place, in the general environment and to drugs administered therapeutically. Many of these xenobiotics are toxic, mutagenic, and carcinogenic. There is a large variation in responsiveness of individuals to these xenobiotics (Harris and Cerutti, 1982). For example, there are large differences among workers in their sensitivity when exposed to toxic substances in the work place; there also are highly variable cancer risks to individuals who are heavy smokers of cigarettes. There have been conflicting reports concerning the relationship of

AHH inducibility and the incidence of lung cancer in humans (Kellerman et al., 1973; Paigen et al., 1977). The MAbs can measure cytochrome P450 gene expression and thus offer new potential for precise phenotyping of humans for specific forms of cytochrome P450 and may thus be useful for resolving the question of the relationship of cytochrome P450s to hypersusceptibility to carcinogens.

Pharmacokinetic studies of drug and carcinogen metabolism in human tissues have described large differences among individuals (Harris and Cerutti, 1982; Kalow, 1982; Omenn, 1982). In some cases there are similarities in the metabolism of certain drugs and carcinogens. Thus, it may be possible to describe a carcinogen-susceptible human phenotype by certain patterns or rates of drug metabolism. This, however, is likely to be imprecise with respect to the contribution of specific forms of cytochrome P450 because multiple forms are active in vivo. An extraordinary polymorphism of human drug metabolism has been reported. Two drugs, debrisoquin and sparteine, are deficiently metabolized by 7 to 9% of the white population. The absence of a particular form of cytochrome P450 may be responsible for the genetic polymorphism in debrisoquin hydroxylation (Gonzalez, 1989). In vivo metabolic studies, however, express the sum total of a complex of processes that includes absorption, distribution, metabolism, and excretion. Nevertheless, the genetic polymorphism may be a powerful tool for the identification of specific phenotypes related to carcinogen susceptibility (Gelboin, 1977, 1983). The MAbs complement these techniques by permitting the direct assay of the expressed protein that may be under the control of both structural and regulational genes.

MAbs are also useful for detection and quantification of individual differences with respect to the amount of a carcinogen that has been activated and subsequently bound to DNA. This method of "quantifying" the formation of DNA-carcinogen adducts may also prove useful for determining exposure of individuals to carcinogens and, with certain defined conditions, for assessing the contribution of genetic factors to carcinogen metabolism and activation (Harris and Cerutti, 1982).

The cytochrome P450s are the primary interface between xenobiotics and higher organisms, and the profile of cytochrome P450s is likely to relate to drug and carcinogen sensitivity. Essential questions for the future are whether the cytochrome P450 phenotype can be precisely defined and whether specific phenotypes are associated with either or both the incidence of cancer induced by chemical carcinogens and rates of drug metabolism. In this review we discuss several approaches to utilizing MAbs for specific individual and classes of cytochrome P450. The MAb methods either singly or in combination permit the development of an atlas of cytochrome P450s responsible for specific xenobiotic and endobiotic reactions in tissues, species, and individuals. The MAb-directed methods can determine phylogenetic differences in cytochrome P450s and assess the changing composition of cytochrome P450 phenotype with sex, age, species, and embryonic development, under different hormonal and nutritional conditions and during exposure to natural and synthetic inducers. Epidemiological, toxicological, and pharmacological studies coupled with MAb-directed phenotyping of individuals may establish the relationship of an individual's cytochrome P450 phenotype profile and individual sensitivity to xenobiotics, such as drugs and carcinogens.

II. Library of Monoclonal Antibodies

The development of a comprehensive library of MAbs to individual cytochrome P450s would serve many purposes. Although the large number of cytochrome P450s, and the virtually infinite number of epitopes contained therein, makes a complete library an impractical goal, a comprehensive MAb library to epitopes on cytochrome P450 would serve the purposes of identifying those cytochrome P450s with epitopes unique to one form of cytochrome P450 and those cytochrome P450s sharing a common epitope. These MAbs could be used for identification, qualitative detection, and quantitative analyses, immunopurification, and immunohistochemical localization of individual cytochrome P450s. In addition, the MAbs can define cytochrome P450 topography and cytochrome P450 immunodominant areas, as well as help one to understand the mechanism of cytochrome P450 enzyme action. Finally, a most important use of the enzyme inhibitory MAbs is their ability to determine the quantitative contribution of each cytochrome P450 to the metabolism of specific substrates in situ.

MAbs made to different cytochrome P450s and reported by various laboratories are listed in table 2.

A major hybridoma study has produced a set of MAbs to CYP 1A1 and CYP 1A2 (Park et al., 1982). One of these, MAb 1–7–1, has been extensively used worldwide since it was produced in 1982. MAb 1–7–1 is a product of a somatic cell hybrid made from myeloma cells and spleen cells of BALB/c mice immunized with partially purified CYP 1A1 from rats treated with MC. The cytochrome P450 used as antigen was largely CYP IA1 but may have contained small amounts of CYP IA2. After immunization, 37 clones were produced that bound to CYP 1A1 as measured by RIA. More than 10 clones bound the CYP 1A1 to a very high degree, measured by RIA and immunoprecipitation. The precipitation was due to the binding of antibody resulting in enzyme aggregation. The MAb 1–7–1 was a strong inhibitor of CYP 1A1 enzyme activity.

Gel electrophoretic analysis showed that a single large microsomal protein band interacted with MAb 1–7–1 and was followed by a second weak band that was subsequently identified as CYP 1A2. Two other MAbs,

TABLE 2
MAbs to cytochrome P450

CYP Antigen	MAb	CYP immunogen	CYP trivial name	Common substrates	CYP specificity	MAb inhibitory activity	Reference
1A1/1A2	1-7-1	Rat 1A1/1A2	*c/d*	Benzopyrene (1A1)	1A1/1A2	+	Park et al., 1982
	1-7-1	1A1/1A2	*c/d*	7-Ethoxycoumarin (1A2)	1A1/1A2	+	Park et al., 1982
1A1	1-36-1	Rat 1A1	*c*	Benzopyrene	1A1	−	Park et al., 1982
	1-31-2	Rat 1A1	*c*	Benzopyrene	1A1	−	Park et al., 1982
1A1	1-12-3	Fish 1A1(E)	*e*	Benzopyrene	Fish 1A1	+	Stegeman et al., 1985
		(scup)		Ethoxycoumarin	Rat 1A1		Park et al., 1986a,b,c
1A1	MBS 106	Rabbit 1A1	P448	Benzopyrene	1A1	±	Boobis et al., 1981
	MBS 107	Rabbit 1A1	P448	Benzopyrene	1A1		Boobis et al., 1981
1A1	3/4/2	Rat 1A1	*c*	Benzopyrene	1A1		Sesardic et al., 1986
	12/2/3/2	Rat 1A1	*c*	Benzopyrene	1A1		Sesardic et al., 1986
	9/20/11	Rat 1A1	*c*	Benzopyrene	1A1		Sesardic et al., 1986
1A1	C1/C4	Rat 1A1	*c*	Benzopyrene	1A1	+	Thomas et al., 1986a,b
1A1/1A2	C2/C3	Rat 1A1/1A2	*c,d*	Acetanilide (1A2) Benzopyrene (1A1)	1A1/1A2	±	Thomas et al., 1984a,b
1A1	C8	Rat 1A1	*c*	Benzopyrene	1A1	+	Thomas et al., 1984a,b
1A2	Anti 1A2	peptides	*d*	Benzopyrene	1A1/1A2	−	Edwards et al., 1990
1A1/1A2	3/4/2	peptides	*c*	Benzopyrene	1A1		Edwards et al., 1988
1A2	antipeptide	peptide	*d*	Acetanilide	1A2	−	Myers et al., 1990
2B1/2B2	Anti PB CYP	Rat 2B1/2B2	*b*	Benzopyrene	2B1/2B2		Letawe-Goujon et al., 1984
2B1	B50	Rat 2B1	*b*	Testosterone	2B1/2C11	+	Letawe-Goujon et al., 1984
2B1/2B2	2-66-3	Rat 2B1/2B2/2B6	PB-4/PB-5	Benzopyrene (2B1/2B2)	2B1/2B2/2B6	+	Park et al., 1984
	2-8-1	Rat 2B1/2B2/2B6	PB-4/PB-5	Testosterone (2B1/2B2)	2B1/2B2/2B6	+	
	4-29-5	Rat 2B1/2B2/2B6	PB-4/PB-5	Benzphetamine (2B1/2B2)	2B1/2B2/2B6	+	
	4-7-1	Rat 2B1/2B2/2B6	PB-4/PB-5	Morphine (2B1/2B2)	2B1/2B2/2B6	+	
2B4	1-26-11	Rabbit 2B6	LM2	Benzopyrene	2B6	−	Park et al., 1981, 1980
1A2	1-14-3	Rabbit 1A2	LM4	Acetanilide	1A2	±	Park et al., 1980, 1981
2C3, 2C5	2F5	Rabbit form 1	*3b*	Progesterone	2C5	+	Reubi et al., 1984a,b
	1F11, 1G11				2C3 P450	+	
2C11	1-68-11	Rat 2C11	*2C*	Androstenedione	2C11, 2C12	+	Park et al., 1989
2C11/2C12	F3, F20, F22, F23, M16	Rat 2C11/2C12	16α, 15β, DEa	Testosterone	2C11/2C12	+	Morgan et al., 1987
2E1	1-91-3	Rat 2E1	*J*	Aniline	2E1	+	Ko et al., 1987
	1-98-1	Rat 2E1	*J*	Aniline	2E1	−	
3A4, 3A5	HP-4, HP-11	Human 3A4/3A5	PCN-E	Androstenedione	rat 3A1/3A2 human 3A4/3A5		Barnes et al., 1987

TABLE 2
Continued

CYP Antigen	MAb	CYP immunogen	CYP trivial name	Common substrates	CYP specificity	MAb inhibitory activity	Reference
3A4 (?)	13-7-6,	Human P450 S	P450 S	Androstene-dione	P450 S		Beaune et al., 1985
	13-1-13	Human P450 S	P450 S	Not measured	P450 S		Beaune et al., 1985
3A1/3A2	2-13-1	Rat 3A1/3A2	PCN-E	Androstene-dione	3A1/3A2		Park et al., 1986a,b,c
	2-3-2	Rat 3A2/3A2	PCN-E	Androstene-dione	3A1/3A2		Park et al., 1986a,b,c
P-19	MAb anti 55	Human placenta P-19	P450 arom	Androstene-dione	P-19	+	Mendelson et al., 1985
P-19	MAb 3-2 C2	Human placenta P-19	P450 arom	Androstene-dione	P-19	+	Yoshida and Osawa, 1991

MAb 1–36–1 and 1–31–2, obtained from the same immunization bound only the CYP 1A1 and not CYP 1A2. The latter two MAbs did not inhibit the activity of the major form, CYP 1A1, or the minor form, CYP 1A2.

The MAb 1–7–1 inhibited several activities of CYP 1A1 including ECD and AHH to varying degrees. The inhibitory effect of MAb 1–7–1 on the purified reconstituted CYP 1A1 for AHH was 90%. Further analysis of BP metabolism in the presence of the inhibitory MAb 1–7–1 (CYP 1A1/1A2) indicated that the inhibition occurred at all positions at which oxidation occurs in the BP molecule. The MAb 1–7–1 was also examined for its inhibitory effect on the activity of AHH and ECD in liver microsomes (section VI) (Fujino et al., 1984a,b). With both enzymes, the MAb 1–7–1 inhibited the activity of the AHH and ECD in microsomes from MC-induced rats by about 80 and 35%, respectively, indicating the percentage of enzyme activity of liver microsomes from MC-induced rats that is due to cytochrome P450 containing the epitope recognized by MAb 1–7–1. These results were the first to demonstrate the use of MAbs for reaction phenotyping which determines the amount of substrate metabolism or product formation that is the result of the catalytic action of an individual or class of cytochrome P450 inhibited by the MAb.

The amount of inhibition of enzyme activity indicates the quantitative contribution of the individual cytochrome P450 to the reaction. The noninhibited remainder of enzyme activity is due to cytochrome P450s other than those sensitive to the MAb and are lacking the target epitope. A further extension of these studies (section VI) showed that, in microsomes from control or PB-treated rats, the AHH and ECD were totally unaffected by the MAb 1–7–1, indicating that AHH and ECD activity in control or PB-treated microsomes contain cytochrome P450s that catalyze AHH and ECD activity and are different from the cytochrome P450 in the microsomes of MC-treated rats that is responsible for AHH and ECD and are inhibited by MAb 1–7–1. Thus, the cytochrome P450s catalyzing AHH and ECD in control and PB-induced microsomes do not contain the MAb 1–7–1 epitope. This study was the first to demonstrate that inhibitory MAbs can be used to determine precisely the quantitative contribution to enzyme activity by epitope-specific cytochrome P450s (Fujino et al., 1984a,b; Park et al., 1992).

MAbs were also prepared to rabbit liver cytochrome P450 and four MAbs were obtained against cytochrome P448. All four MAbs interacted with CYP 1A1 (P448) in intact microsomes and selectively immunoabsorbed the CYP 1A1 from the microsome preparation. One of the antibodies inhibited CYP 1A1-catalyzed AHH in a reconstituted system. A second antibody had no inhibitory effect on enzyme activity, and two of the MAbs stimulated enzyme activity. The increase in enzyme activity caused by MAbs is sometimes observed, and, although the molecular mechanism of this increase is not known, it is possible that the MAb distorts the active site in a manner that stimulates substrate conversion to product (Boobis et al., 1981).

Two of the major cytochrome P450s involved in polycyclic aromatic hydrocarbon metabolism and the metabolism of other common carcinogens are CYP 1A1 and CYP 1A2. These cytochrome P450s have a high degree of sequence homology and have been difficult to distinguish immunologically. Using both binding and immunoprecipitation assays, some MAbs prepared to CYP 1A1 and CYP 1A2 were found to share common epitopes, and, thus, it has been difficult to distinguish whether the MAb inhibitory activity was due to inhibition of CYP 1A1 or CYP 1A2 or both. The MAb 1–7–1 (Cheng et al., 1984a,b) detected two protein bands which stained both CYP 1A1 and CYP 1A2. Cross-reactivity of some of the MAbs was indicated by their inhibition of hamster, guinea pig, and C57 black/6J mouse enzyme activity. No cross-reactivity was found with the DBA/2J mouse. In this mouse strain, cytochrome CYP 1A1 was not detected. Another study showed that purified rabbit cytochrome CYP 1A2 (LM4) and CYP 1A2 (LM6) are immunologically related to the rat CYP 1A1 and CYP 1A2 (Thomas et al., 1984a,b).

In another study MAbs were raised to rat liver CYP 1A1 and CYP 1A2 and rabbit liver CYP 1A1 (form 4). Six MAbs were studied, and each reacted strongly with a homologous antigen and with a microsome fraction enriched with that antigen following treatment of the animal with inducing agents. Several of the antibodies also showed cross-reactivity either within or between the species. A combination of ELISA, Western blotting, and RIA analysis showed that each of the antibodies reacted with a different epitope. Proteolytic digestion of antigen, followed by Western blotting of the peptide fragments, enabled the antibodies to be distinguished. Epitope mapping may help characterize the different isozymes of the cytochrome P450 (Sesardic et al., 1986).

In a study focussed on CYP 1A1, 10 hybridomas were selected that produced antibodies to CYP IA1. These were purified from ascites fluid, and their immunoglobulin type was determined. One was an IgG2b, and the remainder were in the IgG1 subclass. An ELISA demonstrated that the antibodies were directed against at least five different epitopes on the CYP 1A1. This epitope distinction was further confirmed by immunoprecipitation analysis. Three of the MAbs cross-reacted strongly with the same epitope on cytochrome CYP 1A2, another cytochrome P450 enzyme induced by MC in the livers of rats. A study of the cross-reactivity of the MAbs generated from CYP 1A1 found that these MAbs to CYP IA1 did not cross-react with CYP 2A1, 2B1, 2B2, 2A2, G, or 2C11, as measured by Western blots. One of the MAbs was a potent inhibitor of the enzyme activity of 1A1 in a reconstituted system. A previous study (Park et al., 1982) isolated MAbs to CYP 1A1 that cross-reacted strongly with CYP 1A2 and exhibited enzyme inhibitory activity toward both. The close homology of CYP 1A1 and CYP 1A2 has been a serious obstacle to reaction phenotyping of 1A2 in the presence of 1A1 (see below). Thus, some MAbs to 1A1 that also bind or inhibit the enzyme activity of 1A2 can cause serious interference when distinguishing between the amount of enzyme activity contributed by 1A1 and 1A2. There are current efforts being made to obtain MAbs that are specific for 1A2 (Edwards et al., 1991).

Four inhibitory MAbs have been produced to partially purified CYP 2B1/2B2 from PB-induced rat liver (Park et al., 1984). The antigen preparation may have also contained CYP 2B6 in addition to the major CYP 2B1/2B2. In two experiments, MAbs of the IGM and IgG1 types were produced, and many bound to the CYP 2B1/2B2. In certain cases, the MAbs yielded a positive precipitin reaction. Some of these MAbs also inhibited CYP 2B1/2B2-dependent AHH, although the latter is a minor substrate for these cytochrome P450s. Four of the MAbs, 2–66–3, 4–29–5, 4–7–1, and 2–8–1, completely inhibited both AHH and ECD activity of the PB-induced CYP 2B1/2B2. These MAbs did not cross-react with MC-induced CYP 1A1/1A2. There was a high degree of epitope specificity in the MAbs for CYP 2B1/2B2, because these MAbs showed no binding to BNF-induced CYP 1A1/1A2 or PCN-induced CYP 3A1. One of the MAbs, 2–66–3, inhibited the AHH and the ECD activity of microsomes from PB-treated rats by 20 to 40%, indicating that 20 to 40% of these enzyme activities in microsomes is due to CYP 2B1/2B2. Thus, the MAb can detect and measure the activity contribution of the epitope-containing cytochrome P450 induced in microsomes in PB-treated rats. Two additional MAbs, 4–7–1 and 4–29–5, completely inhibited AHH catalyzed by the purified PB-induced CYP 2B1/2B2. This series yielded four different MAbs capable of strongly inhibiting the PB-induced cytochrome P450s (Park et al., 1984). In a subsequent study, the specificity of these MAbs for different forms of PB-induced and related enzymes was determined (see below) (Park et al., 1984, 1986a,b).

In a different study MAbs were prepared against cytochrome P450 from PB-induced rat liver microsomes. It was suggested that only one form of cytochrome P450 was detected, although that is somewhat doubtful given the close homology of CYP 2B2 and 2B1. These MAbs were also incapable of inhibiting cytochrome P450 activity, making them somewhat less useful than inhibitory MAbs that have strong inhibitory activity specific for CYP 2B1 and 2B2.

Another study used MAbs to determine the relationship among various isozymes of cytochrome P450 from rabbit, rat, and human tissue. In this study, MAbs were made to CYP 2B1/2B2 and CYP 1A1 and rabbit liver cytochrome P450 form 4. Six of the MAbs obtained were studied. Each MAb reacted strongly with both the homologous antigen and fractions of microsomes that were selectively enriched with the antigen by pretreatment of the animal with compounds that induce particular cytochrome P450s. The inducers used to enrich the microsomes in this study were PB and MC, known inducers of CYP 2B1/2B2 and CYP 1A1/1A2, respectively. The MAbs were characterized as to immunoglobulin type. Several of the MAbs showed cross-reactivity between cytochrome P450s from rabbit, rat, and human. With the use of ELISA, Western blotting, and RIA, the study determined that each of the antibodies reacted with a different epitope. The antibodies were distinguished by proteolytic digestion of the cytochrome P450 antigen followed by Western blotting of the peptide fragments which permitted the localization of the epitope by its binding to the MAb. Although the MAbs were identical in their initial screening, the localization to different peptide fragments distinguished their uniqueness. This study is an example of epitope mapping that can localize the epitope within a region on the peptide that binds to a particular MAb. This general method can be used to determine whether an epitope is shared by more than one cytochrome P450. Furthermore, this approach may indicate whether the epitope is either structurally related

to the active site of the enzyme, is a site that may modify enzyme activity, or is unrelated to the active site. Epitope mapping is difficult and laborious. Methods that have been used with other proteins can be applied to the binding of MAbs to homologous cytochrome P450 proteins from different species. It is relatively easy to localize the epitope for an MAb in a large fragment of a target protein that has been cut in two or into relatively large fragments with proteolytic digestion. The localization of an epitope on a small peptide with a size ranging from five to ten amino acids is relatively difficult. Other methods, such as the construction of mutant proteins with site-directed mutagenesis, would be very useful for determining which mutations alter or eliminate either the binding or the inhibitory effect of the MAb (Reik et al., 1985).

The first MAb successfully constructed to a cytochrome P450 was to highly purified rabbit liver CYP 2B4 (LM2) which was used for the immunization (Park et al., 1980, 1981). MAbs to rabbit liver CYP 2B4 (LM2) were detected by RIA and by double-immunodiffusion analyses. In this first study, seven MAbs were positive by a binding assay to CYP 2B4. MAbs to CYP 2B4 showed a precipitin reaction and inhibition of CYP 2B4 enzyme activity. These MAbs did not inhibit the enzyme activity of other related isozymes, namely, cytochrome CYP 1A2 (LM4), cytochrome P450 fraction 1, and cytochrome P450 fraction 7, which are unpurified fractions of cytochrome P450. All of the MAbs formed against CYP 2B4 (LM2) were of the immunoglobulin subclass G. Certain MAbs of the panel strongly inhibited the CYP 2B4 (LM2) catalyzed formation of oxygenated metabolites of BP at various positions of the benzpyrene molecule, as well as inhibiting ECD. In addition to the hybridomas producing MAbs to CYP 2B4, four hybridomas were isolated that produced MAbs to CYP 1A2 (Park et al., 1981). Of these, one was of the IgM class and the other three of the IgG class. None of these MAbs precipitated the enzyme, and none of them cross-reacted with CYP 2B4 in a binding assay. The MAb to the CYP 1A2 (LM4) interacts with CYP 1A2. This complex displayed enzyme activity for BP hydroxylation which could be removed by complexing with protein A and centrifugation of the complex. Thus, the interaction between MAbs to CYP 1A2 did not inhibit the enzyme unless the enzyme MAb complex was precipitated with protein A. (Park et al., 1980, 1981).

One study utilized rabbit microsomal cytochrome P450 as antigen, and three MAbs, 1F11, 1G11 and 2F5 were developed to CYP 2C3 (Reubi et al., 1984a,b) and were used to immunoprecipitate several microsomal cytochrome P450 proteins. The antibodies bound to proteins from the solubilized microsomes, and all bound proteins exhibited the same electrophoretic mobility as CYP 2C5. One of the monoclonal antibodies, 2F5, recognized an additional protein with a different mobility, and the third MAb IGII reacted with several different microsomal proteins that included both CYP 1 and CYP 2C3. The results indicate that there were at least three electrophoretically distinct forms of cytochrome P450 that share one or more epitopes with CYP 2C5. These studies also indicated that the three antigenic determinants recognized by the different MAbs are spatially distinct and nonoverlapping. Reconstituted cytochrome P450 1 showed a 10-fold greater progesterone 21-hydroxylase activity than most preparations of rabbit liver microsomes. This was a relatively high rate compared to that of microsomes or that catalyzed by five electrophoretically distinct forms of cytochrome P450. Both the 1F11 and 2F5 antibodies inhibited the microsomal progesterone 21-hydroxylation very extensively. Thus, these MAbs to cytochrome P450 1 and rabbit liver microsomes can be used to reaction phenotype, i.e., to determine the contribution of the epitope-specific cytochrome P450s to the metabolism of specific substrates (Reubi et al., 1984a,b).

In one study, (Park et al., 1989) six hybridoma clones were obtained that produced MAbs of the IGM type, using as antigen the purified rat liver microsomal CYP 2C/RLM5, i.e., CYP 2C11 that was isolated from an untreated adult male. The six hybridoma clones each specifically immunoprecipitated the CYP 2C11 in the Ochterlony double-immunodiffusion test. Four of the MAbs bound, but did not precipitate, the CYP 2C11. The MAb 1–68–11 bound to the P340 2C11 did not bind to PB-induced CYP 2B1/2B2, CYP 1A2 of rabbit, or BNF-induced CYP 1A1 of rats. In contrast, polyclonal antibodies that were generated by the same antigen were far less specific and precipitated CYP 2C11, CYP 2B1/2B2, and cytochrome CYP B4. Thus, in a typical experiment, the polyclonal antibodies were far less specific than the MAbs. This is to be expected because the polyclonal is basically comprised of a large number of MAbs each derived from a different spleen cell. The MAbs obtained in this study were of extraordinary value because they completely inhibited both androstenedione 16α-hydroxylation and testosterone 16α-hydroxylation catalyzed by a reconstituted system with CYP 2C11. Androstenedione 6β-hydroxylation catalyzed by CYP 2C11 also was inhibited. Interestingly, the 7-hydroxylation was not inhibited by MAb 1–68–11, indicating that the 7-hydroxylation is catalyzed by a cytochrome P450 other than 2C11.

CYP 2C11 catalyzed 2α-, 16α-, and 6β-hydroxylation of progesterone in a reconstituted system, and these reactions were inhibited by MAb 1–68–11 by 60 to 80%. MAbs that immunoprecipitated the CYP 2C11 did not bind or precipitate CYP 2B1/2B2. In contrast, the mouse polyclonal antibodies produced by the immunization with the same preparation of purified CYP 2C11 strongly immunoprecipitated CYP 2C11 (RLM5) and, to a lesser extent, CYP 2B1 and rabbit CYP 2B4. Thus, the MAb

1–68–11 described in this study proved quite useful because it exhibits a positional specificity that defines the role of the individual cytochrome P450 in the metabolism of the steroid at different positions of the molecule. MAb 1–68–11 should prove very useful for reaction phenotyping or measuring the contribution of individual microsomal cytochrome P450s in the stereospecific oxidative metabolism of endogenous steroids and xenobiotic substrates (Park et al., 1989; Waxman et al., 1987).

In a related study, the cytochrome P450-dependent catalysis of BP metabolism, and its binding to DNA, was analyzed with MAb 1–68–11, (2C11) (Todorovic et al, 1991). In a previous study, MAb 1–7–1 bound to 1A1/1A2 inhibited BP metabolism by 70 to 80% in MC-induced microsomes of rat and mouse liver but had no effect on BP metabolism in the livers of untreated rats and mice. Thus, the constitutive cytochrome P450 responsible for BP metabolism is not CYP 1A1/1A2 which is inhibited by MAb 1–7–1. The MAb 1–68–11 (2C11/2C12), however, has strong inhibitory effects on BP metabolism in constitutive microsomes, thereby identifying 2C11/12 as a major cytochrome P450 responsible for BP metabolism in uninduced microsomes. In a follow-up study, the MAb 1–68–11 was found to have inhibitory activity that was regiospecific. Thus, the conversion of BP to the 9,10-diol and 7,8-diol was inhibited by MAb 1–68–11, and 4,5-diol formation was stimulated by MAb 1–68–11; BP-DNA adduct formation was completely inhibited by MAb 1–68–11 in microsomes of control rats and 70% inhibited in PB-treated microsomes. Thus, the use of MAb 1–68–11 identified the CYP 2C11/2C12 as the major cytochrome P450 catalyzing stereospecific BP metabolism and DNA binding in control rats (Todorovic et al., 1991).

The MAb 1–68–11 that precipitated CYP 2C11 also inhibited the androstanedione 16α-hydroxylase activity of untreated rat microsomes by more than 90%, but did not inhibit microsomal 6β- or 7α-hydroxylation. In addition, the MAb 1–68–11 completely inhibited both the androstenedione 16α-hydroxylation and testosterone 16α-hydroxylation of a reconstituted system using cytochrome CYP 2C11 as the cytochrome P450 antigen. Androstenedione 6β-hydroxylation, catalyzed by CYP 2C/RLM5 (2C11), was also inhibited, whereas the cytochrome P450-catalyzed 7α-hydroxylation was not inhibited by the same MAb. The CYP 2CII catalyzes 2α-, 16α-, and 6β-hydroxylation of progesterone in a reconstituted system. The formation of these metabolites was inhibited 60 to 80% by the MAb 1–68–11.

In a related study, five MAbs were prepared to cytochrome P450 isozymes: $CYP_{15\beta}$, $CYP_{15\alpha}$, and CYP_{DEa} (Beaune et al., 1985). These were all related to CYP 2C11 or 2C12 or other related cytochrome P450s. All the MAbs to $CYP_{15\beta}$ bound to the same epitope. These are immunochemically related by the finding of their cross-reactivity with polyclonal antibodies. In this study, MAbs to $CYP_{15\beta}$ and one antibody to $CYP_{16\alpha}$ showed very high selectivity with respect to antigen binding. Furthermore, there were high frequencies of cross-reactivities, indicating that there is a common epitope among the three antigens used. Furthermore, all of the $CYP_{15\beta}$ MAbs were found to bind the same epitope or closely grouped epitopes. The specificity of the MAbs was determined by ELISA and Western blotting. Two of the antibodies were apparently identical, and each was specific for $CYP_{15\beta}$ by the latter criteria. The specificity of two other MAbs for $CYP_{15\beta}$ and one for $CYP_{16\alpha}$ were highly dependent on the analytical method used to determine their cross-reactivity. All five of the MAbs inhibited microsomal $CYP_{15\beta}$ by a maximum of 70%. They also inhibited microsomal $CYP_{16\alpha}$. Another of the MAbs to the $P450_{16\alpha}$ was not found to be inhibitory. Thus, the study shows that MAbs can determine epitope relatedness between closely related cytochrome P450s and may be useful in defining positional specificity (Beaune et al., 1985).

Another extensive study determined the specificity of MAbs to different cytochrome P450 forms and especially to cytochrome P450s catalyzing steroid-dependent hydroxylations. MAbs that were inhibitory to different rat hepatic cytochrome P450s were examined. These consisted of nine different MAbs raised to four distinct rat liver cytochrome P450s. These were CYP 2C11, 3A1, 2B1, and 1A1. The study used a combination of ELISA, dot immunoblotting, Western blotting, immunodiffusion, and immunoinhibition. Four of the MAbs were fully inhibitory by >85% toward the corresponding immunoreactive cytochrome P450s when assayed with the purified reconstituted enzyme system. Two of the MAbs were partially inhibitory with a maximum of 50 to 80% inhibition in the presence of saturating levels of the MAb. Inhibitory MAbs that react with CYP 2C11, 3A1, and 2B1 demonstrated that the formation of various hydroxytestosterone metabolites by each of the cytochrome P450s examined is reflective of their inherent catalytic specificities and not due to the presence of the immunologically distinct cytochrome P450 enzyme contaminants.

The contributions of specific cytochrome P450 forms to rat hepatic microsomal steroid hormone hydroxylase activity were then assessed using inhibitory MAbs as probes. MAb-reactive CYP 2C11 was the major catalyst of testosterone and androstenedione 16α-hydroxylation in microsomes from either untreated or BNF-treated rats. This cytochrome P450 was responsible, however, for only 30% of the 16α-hydroxylase activity in PB-treated male rats and <10% in adult female rats in which the major part of 16-hydroxylase activity is catalyzed by an MAb-reactive CYP 2B1. Although the latter cytochrome P450 catalyzed >90% of microsomal androstanedione 16β-hydroxylation in PB-treated rats, the enzyme did not contribute to the low level of 16β-hydroxylase activity of control uninduced liver. MAb-sen-

sitive CYP 2C11 catalyzed >85% of microsomal androstanedione 17α-hydroxylation, and this inhibition was independent of the age, sex, or induction state of the animals. This series of experiments shows the extraordinary power of MAbs as probes for measuring the contribution of individual cytochrome P450 enzymes in the metabolism of steroid hormones. CYP 2E1 has received considerable attention because of its functioning in the metabolism of aniline and the carcinogenic nitrosamines. CYP 2E1 is also induced in rat liver by ethanol and is sensitive to fasting and diabetes (Ko et al., 1987; Park et al., 1986a,b). Thus, there are many pharmacological facets that make this CYP 2E1 of special importance (Ko et al., 1987).

Hybridomas were prepared from rats immunized with partially purified CYP 2E1 obtained from the livers of ethanol-induced rats. Thirty-one independent clones were produced that had a high affinity for the CYP 2E1. These clones produced IgG1, IgG2A, IGM, and IGA antibodies. Ten of the 31 MAbs also immunoprecipitated the CYP 2E1. One of the MAbs was tested for cross-reactivity with a large number of rabbit and rat liver cytochrome P450s and was found not to cross-react with any of these cytochrome P450s. Thus, CYP 2E1 did not cross-react with PB-induced CYP 2B1/2B2 or naphthoflavone-induced CYP 1A1/1A2 or CYP 3A1/2. One of the seven MAbs tested, MAb 1–91–3, inhibited aniline and nitrosamine metabolism in microsomes from acetone-, pyrazole-, and methylpyrazole-treated rats by 54, 47, and 48%, respectively (Ko et al., 1987). This indicates that at least 50% of the microsomal activity for aniline hydroxylation is a function of a CYP 2E1 that contains an epitope recognized by MAb 1–91–3. With both untreated and imidazole-induced microsomes, 32 and 21% inhibition of enzyme activity was observed. Thus, even control microsomes contain CYP 2E1 activity.

With the reconstituted system using purified CYP 2E1, the MAb 1–91–3 inhibited aniline hydroxylase by >90%. Nitrosodimethylamine demethylase activity of acetone-induced rat microsomes was inhibited 77% by the MAbs to CYP 2E1. Western blot analysis showed that the ethanol-inducible form of cytochrome P450 recognized by the MAbs was also detected in microsomes from rats treated with acetone, pyrazole, methylpyrazole, and imidazole. An interesting finding in this study was that MAb 1–91–3, which showed strong inhibitory activity toward the CYP 2E1, yielded no Western blot. On the other hand, MAb 1–98–1 gave a very strong Western blot but showed no inhibitory activity. The cytochrome P450 detected by MAb 1–98–1 is CYP 2E1 because it shows a single band on a Western blot. Thus, the epitope location on a cytochrome P450 can determine inhibitory activity as well as the binding activity on a Western blot, and these activities can be disassociated (Ko et al., 1987).

Two laboratories have succeeded in producing MAbs to PCN-induced rat CYP 3A1/2. In one study, 11 hybridoma clones were obtained after immunization with the purified CYP 3A1/2 that induced CYP 3A1/2 (Park et al., 1986a,b). Each of the MAbs were of the subclass IgG1, IgG2, or IgG2B. Each of the MAbs bound strongly to the CYP 3A1/2, as shown by RIA. Unexpectedly, of the 11 MAbs that bound strongly to the CYP 3A1/2, three also bound strongly to the PB-induced rat liver CYP 2B1/2B2. Thus, although CYP 3A1/3A2 have relatively little homology to CYP 2B1/2B2, three of the 11 MAbs recognized epitopes that were common to both the CYP 3A1/2 and the CYP 2B1/2B2.

Activities of one of the MAbs from each class, i.e., a class recognizing only CYP 3A1/2 and one recognizing both CYP 2B1/2B2 and CYP 3A1/2, were examined for their binding to eight other highly purified rat hepatic cytochrome P450s using solid phase (ELISA). The MAb 2–13–1 to CYP 3A1/2 was found to be specific for CYP 3A1/2 and did not cross-react with seven other cytochrome P450 forms. The MAb 2–13–1 was also an effective probe for monitoring, by Western blotting, the induction of microsomal CYP 3A1/2 induced by PCN. MAb 2–3–2 reacted with both CYP 3A1/2 and with the major PB-induced cytochrome P450 forms, i.e., CYP 2B1/2B2. None of the MAbs described in this study were inhibitory toward either the CYP 3A1/2 or the CYP 2B1/2B2 enzyme-dependent AHH, benzo(a)phetamine demethylase, ECD, or ethylmorphine N-demethylase activity, indicating that the epitopes recognized by these MAbs are not associated with these catalytic activities. The strong reactivities of MAb 2–3–2 to both the CYP 2B1/2B2 and CYP 3A1/2 classes of cytochrome P450 indicate that, although the MAb binds to cytochrome P450s that are structurally quite different, they, nevertheless, share at least one common epitope recognized by MAb 2–3–2. The binding of MAb 2–3–2 to both CYP 3A1/2 and CYP 2B1/2B2 is an example of how an MAb can recognize epitopes common to cytochrome P450s of different classes that may or may not be related to enzyme activity. It also exemplifies the possible use of MAbs for a taxonomic classification of cytochrome P450s based on their epitope content (Park et al., 1986a,b).

A study on the preparation and characterization of MAbs to sexually differentiated rat liver cytochrome P450 isozymes developed five MAbs to CYP 2C12 and one antibody to CYP 2C11; these MAbs showed selectivity for the respective antigens. There was, however, a high frequency of cross-reactivity which indicated a high degree of homology between, CYP 2C12, CYP 2C11, and CYP DE_a. All the CYP 2C12 specific MAbs bound to the same epitopes or closely grouped epitopes. The specificity of each MAb was characterized by ELISA assay, Western immunoblotting, and antibody Sepharose immunoabsorption of soluble rat liver microsomes. The identical antibodies, F22 and F23, were specific for CYP 2C12. All five MAbs that bound to CYP 2C12 inhibited the enzymatic activity of microsomal CYP 2C12 by up to 70%.

They also, however, inhibited microsomal CYP 2C11 indicating that they also have an affinity for a common epitope on CYP 2C11. In this study, a surprisingly large number of the MAbs obtained were partially inhibitory to enzyme activity, although none were completely inhibitory. The use of MAbs to study the role of each cytochrome P450 in metabolism leaves much to be desired unless a high level of inhibition, such as greater than 90%, is obtained with the MAb in a reconstitution system using a purified cytochrome P450. Inhibition of enzyme activity in microsomes may reflect inhibition of the target cytochrome P450, but if the inhibition is not >90% with the reconstituted cytochrome P450 system, the results with microsomes would be difficult to interpret and the microsomal activity may reflect enzyme activity of other cytochrome P450s (Morgan et al., 1987).

One study isolated 12 MAbs after immunization with PB-induced rat hepatic cytochrome P450. Ten of the MAbs bound to CYP 2B1, CYP 1A1, and CYP H. One MAb recognized CYP 2B1 and CYP 2B2 but not CYP 1A1. One MAb reacted strongly with CYP 2C7 and another with CYP 1A2, CYP 2C13, and CYP 2E1; but with a second MAb no cross-reactivity was observed with CYP 1A1, 1A2, and 2E1. The MAbs were directed to six distinct epitopes on CYP 2B1; two of the MAbs did not inhibit the metabolism of the two substrates, benzphetamine, and testosterone. The MAb to CYP 2B1 inhibited testosterone metabolism to the 16α- and 16β-hydroxytestosterone as well as androstenedione formation. The inhibition of these reactions in microsomes from PB-treated rats was between 67 and 94%. No other pathways of testosterone metabolism were inhibited by the MAbs to CYP 2B1. The degree of inhibition can be viewed as a measure of the contribution of the cytochrome CYP 2B1 toward the specific metabolism measured (Reik et al., 1985). The large variety of cytochrome P450s recognized by some of the MAbs is quite unusual given the diverse cytochrome P450 structures and may reflect the presence of an epitope of unusual commonality on cytochrome P450s to which the MAb is directed.

As discussed previously, in attempts to produce MAbs to PCN-induced CYP 3A1/3A2, a large number of hybridoma clones were obtained (Park et al., 1986a,b). In two studies, many MAbs were produced to the CYP 3A1/2 or 3A4. A few of the MAbs also recognized cytochrome P450s of a second class of cytochrome P450, primarily CYP 2B1/2B2. Neither of the studies produced MAbs that exhibited enzyme inhibitory activity toward the purified CYP 3A1/2 or 3A4. MAbs that bind to the CYP3A1/3A4 would be very useful for a variety of functions as stated above. However, the lack of inhibitory activity prevents the use of the MAb for determining the quantitative contribution of the targeted individual cytochrome P450 to the metabolism of a particular substrate. Because CYP 3A4 is a major human cytochrome P450 metabolizing a variety of drugs, many efforts are being expended to generate inhibitory MAbs to the CYP 3A4.

Human cytochrome P450s were used for immunization, and three similar hybridomas were constructed that recognized an epitope specific to a family of human cytochrome P450s. The epitope was also found in rat CYP 3A4. The isozyme binding to the MAb is different in molecular weight from rat 3A4. The MAbs were used to quantitate the epitope-related cytochrome P450s in the human liver (Morgan et al., 1987).

In a study of human CYP 3A4, six MAbs were obtained after immunization with human liver microsomes or partially purified microsomes (Barnes et al., 1987). All six of the MAbs recognized highly purified human liver cytochrome P450 protein of a molecular weight of 53 kDa. These MAbs gave a single band on a Western blot at 53 kDa with human liver microsomes from 11 individuals. These antibodies also recognized a 52- and 54-kDa protein in control and PCN-induced male rat liver microsomes which showed four different patterns of binding. Two of the antibodies recognized a 52-kDa protein. These were weakly expressed in untreated rats but strongly induced by PCN and are likely CYP 3A1/2. Two other MAbs recognized a constitutive 52-kDa protein which was expressed by treatment with MC and aroclor. MAbs HC4 and HP16 recognized a 52-kDa protein that was weakly expressed in untreated rats and strongly induced by PCN but not by PB, MC, isosafrole, clofibrate, or imidazole. MAb HP3 recognized a 54-kDa protein that was undetectable in control rats but strongly induced by PB, PCN, isosafrole, and aroclor. The constitutive proteins were male specific. The study also detected significant degrees of interindividual differences in the proteins from human liver detected by the different MAbs. Thus, the MAbs may be useful in defining differences in the expression of cytochrome P450s and interindividual differences among humans. In this study, unfractionated or partially fractionated microsomes were used as immunogens. This is a useful technique, but generally the antigen desired for specific MAb production is at a low concentration in this technique. Here the desired MAb would require considerably greater screening for its isolation. It is clear that the above study produced a large diversity of MAbs of different specificities, many of which were not characterized. Some of the MAbs isolated seem to have good potential value.

CYP aromatase P-19 is a cytochrome P450 of unusual character and of extraordinary interest because its inhibition may have chemotherapeutic potential for breast and prostate cancer. Two laboratories have isolated MAbs to CYP aromatase P-19 (Mendelson et al., 1985; Yoshida and Osawa, 1991). The enzyme was partially purified from human placenta microsomes by classical methods of affinity chromatography. There was an enrichment of two proteins with molecular weights of 50 and 55 kDa in the purified preparation. The protein

bands were removed from unstained polyacrylamide gels and injected separately or together into three rabbits. Serum fractions prepared from the rabbits injected with the 55-kDa band or with both the 50- and 55-kDa bands inhibited CYP P16 aromatase activity of human placenta by 80%, thus indicating the formation of polyclonal antibodies that were inhibitory to aromatase activity. The serum of the rabbit injected with the 50-kDa protein had little capacity to inhibit placental aromatase activity. Further immunoblot analysis showed that the IgG from serum of rabbit immunized with the 55-kDa protein specifically bound to a 55-kDa protein in human placental microsomes. MAbs were prepared and covalently linked to Sepharose and used for immunoaffinity chromatography of CYP aromatase P-19. The CYP aromatase P-19 was selectively retained by affinity column chromatography and, after reconstitution with cytochrome P450 reductase and phospholipid, exhibited aromatase activity. This was indicative that the 55-kDa protein is indeed CYP aromatase P-19. These findings also showed that both the MAb and polyclonal antibodies to CYP aromatase P-19 aromatase are specific for human CYP aromatase P-19 aromatase (Mendelson et al., 1985). The preparation and characterization of polyclonal and MAbs against human CYP aromatase P-19 and their use in its purification have also been described in parallel studies in another laboratory (Yoshida and Osawa, 1991).

A series of papers were published in which the construction and use of MAbs to fish cytochrome CYP E (1A1) of *Stenotomus chrysops* (scup) was described. Cytochrome CYP E 1A1 is present in scup and exhibits AHH activity. The MAb 1–12–3 was prepared from the purified cytochrome CYP E antigen and reacted only with cytochrome CYP E when tested by immunoblot analysis of five different cytochrome P450 fractions of scup liver. The MAb 1–12–3 and six other MAbs produced to the antigen cytochrome CYP E recognized the purified CYP E and only a single band that comigrated with authentic cytochrome CYP E. Both MAb 1–12–3 to cytochrome CYP E and polyclonal anti-cytochrome CYP E reacted only with cytochrome CYP E but not with other scup fractions. MAb 1–12–3 recognized single bands in scup liver from scup pretreated with polychlorinated biphenyls, BNF or MC, as well as in two trout species, killfish and winter flounder. MAb 1–12–3 also recognized rat CYP 1A1. The cytochrome CYP E is not detected in untreated fish. The AHH and EROD activities of induced scup and trout were also strongly inhibited by MAb 1–12–3. The MAb 1–12–3 recognition of purified rat CYP 1A1 indicates a common MAb 1–12–3 epitope shared by fish cytochrome CYP E and rat CYP 1A1. A second lower molecular weight band was detected on the gel that bound to MAb 1–12–3 and may be CYP 1A2 because MAb 1–7–1 binds to both CYP 1A1 and CYP 1A2.

The development of MAb 1–12–3, an MAb that recognizes an inducer-related cytochrome P450, suggests the possibility that the marine environment may be monitored for inducer-containing pollutants by their ability to detect induced cytochrome CYP E with MAbs (Park et al., 1986b; Stegeman et al., 1985). An interesting related finding was that the monoclonal 1–7–1 to rat CYP 1A1 had little effect on the AHH activity of scup cytochrome CYP E and did not recognize cytochrome CYP E by either RIA or Western blot; however, scup MAb 1–12–3 was recognized and inhibited strongly the AHH activity of rat CYP 1A1. The scup cytochrome CYP E and rat CYP 1A1 have at least one common epitope recognized by MAb 1–12–3, but the epitope recognized by MAb 1–7–1 in rat CYP 1A1 is absent in cytochrome CYP E. Thus, MAbs against cytochrome CYP E contain an epitope that is different from that in rat CYP 1A1. These MAbs may be useful for determining phylogenetic relationships of the class of CYP 1A1-inducible isozymes and their regulation by marine environmental factors (Kloepper-Sams et al., 1987; Park et al., 1986b; Stegeman et al., 1985).

Ten MAbs were prepared that were reactive with a high spin form of rat cytochrome P448 (P448–1). One of these MAbs bound to an epitope characteristic of P448. Five of the MAbs cross-reacted with a low spin form of cytochrome P448 but not with cytochrome P450. Some of these MAbs were inhibitory to cytochrome P450 enzymes in microsomes of rats pretreated with certain inducers (Hashimoto et al., 1985). Although this review is concerned primarily with MAbs to cytochrome P450, it is interesting that in one study MAbs have been successfully made to rat liver microsomal cytochrome b_5, an enzyme active in some cytochrome P450 catalyzed reactions. These were of the IgG1, IgG2B, and IgM types. All of the MAbs bound strongly to rat cytochrome b_5 when measured by RIA. Four clones of the MAbs were strongly sensitive to immunoprecipitation. Two of the MAbs partially inhibited cytochrome b_5 mediated NADH-cytochrome *c* reductase in liver microsomes. The MAb showed that the expression of the immunodetectable cytochrome b_5 was highest in liver, high in kidney, and quite low in all the other tissues examined by Western blotting. A unique feature of this study was the analysis of the specificity of binding of the MAbs to cDNA-expressed cytochrome b_5 which was derived from TK^- cells transfected with recombinant vaccinia virus encoding cDNA for human cytochrome b_5.

cDNA-expressed proteins may be very useful for examining the cross-reactivities of MAbs with human cDNA-expressed cytochrome P450 proteins. Thus, cytochrome P450s from humans and other sources can be examined for MAb specificity. This approach would yield a large number of cytochrome P450s that can be studied with the MAbs. The cytochrome b_5-directed MAb in this study may be useful for the identification, quantification, and immunopurification of cytochrome b_5 from animal

and human tissue and for understanding the role of cytochrome b_5 in drug metabolism and carcinogen activation (Park et al., 1992).

A. Antigen Preparation for Monoclonal Antibody Production

In the work cited above, the immunogens used for the preparation of MAbs were largely purified or semipurified preparations of cytochrome P450 from animal tissue. Classical purification of cytochrome P450s is cumbersome and difficult, and the amounts generally produced are quite small and sometimes inadequate for MAb production and screening. Generally, the amount of antigen needed to produce MAbs is in the range of 15 to 50 μg.

There are new approaches that seem promising for the preparation of antigens. One approach is by synthesizing peptide sequences that are specific to regions of particular cytochrome P450s. One study utilized two peptides with sequences unique in CYP 1A2 (Myers et al., 1990). The peptides were synthesized and used as antigens to generate the anti-peptide MAbs. The antisera to both peptides bound to rat CYP 1A2, but not the structurally similar CYP 1A1, as determined by ELISA assay. However, the antisera to both peptides detected both rat CYP 1A2 and CYP 1A1 on Western blots. This result contrasted to the MAb behavior with the ELISAs. In addition, when the MAbs were Western blotted with human liver microsomes, both antisera recognized 1A2 but not 1A1. This study demonstrates the potential utility of peptides of specific sequence as immunogens for MAb production.

Synthetic peptides have also been used to elicit polyclonal antibodies that specifically inhibit the activity of CYP 1A2 in rat hepatic microsomes. This was accomplished by using an antibody against the synthetic peptide (Ser-Glu-Asn-Tyr-Lys-Asp-Asn) which is a sequence that occurs in CYP 1A2 at positions 290 to 296. This sequence was selected based on various considerations, especially the relationship of the structure of CYP 1A2 relative to CYP 1A1. In this study polyclonal antibodies were produced. No MAb production was reported. The reason for the lack of MAb formation is not discussed but may be related to the known relatively poor binding of MAbs elicited from peptide antigens. It is also possible that attempts may not have been made to isolate MAbs. The polyclonal antibody strongly inhibited enzyme activity from MC-treated liver microsomes. The antibody specifically inhibited CYP 1A2-catalyzed phenacetin O-deethylase, but had no effect on AAF metabolism; nor did it have an effect on AHH, which is catalyzed by CYP 1A1. Furthermore, the antibody had no effect on aflatoxin B1 activation. This study accomplished the production of a highly specific polyclonal antibody to CYP 1A2 using a synthetic peptide derived from the structure of CYP 1A2. The reproductibility of producing this polyclonal antibody is not discussed, but each batch of polyclonal antibody varies to some degree. The approach presumably will also be useful for eliciting MAbs to specific cytochrome P450s (Edwards et al., 1988, 1990).

In a related study, a synthetic peptide was used to produce antibodies that recognized the two related CYP 1A1 and CYP 1A2. The peptide used for immunization has the same primary structure as that of a region of both the cytochrome P450 isozymes. There was no cross-reactivity of this antibody to liver microsomes induced with PB and isonicotinic acid hydrazide. The polyclonal antibodies obtained in this study, however, are not of any great advantage over the MAbs made in different laboratories because it recognizes both CYP 1A1 and CYP 1A2. This group also used a synthetic peptide in the region of the rat CYP 1A1 at residues 294 to 301. This is equivalent to a possible inhibitory region of CYP 1A2 and was identified by sequence alignment. Anti-peptide antibodies were raised to the peptide coupled through either the NH_2 or COOH terminus of a carrier protein. Both of the anti-peptide antibodies bound to CYP 1A1 inhibited AHH activity and the mutagenic activation of AFF. Each of these reactions is catalyzed by CYP 1A1. The antibodies did not affect high-affinity phenacetin O-deethylation, which is catalyzed by CYP 1A2. The studies suggested that the MAbs were directed to an epitope on the peptide opposite to the end that was coupled to the carrier protein. Both of the antibody preparations bound equally well to CYP 1A1, indicating that, in the native protein, the entire peptide region is exposed on the surface of CYP 1A1 and is available for binding to the antibody. The authors speculated that the role of this peptide region is similar in both 1A1 and 1A2 (Edwards et al., 1988, 1990).

The use of specific peptides for production of MAbs has great potential. One drawback has been that the MAbs made to small peptides are generally weakly bound to the cytochrome P450. Methods may be developed to increase the binding efficiency of these peptides by the use of different conjugates as antigen carriers. Their successful use as antigens may promote the development of a large library of MAbs.

Another approach for antigen production is the use of expression vectors for expressing cytochrome P450 cDNA into cytochrome P450 proteins. One expression system utilizes the vaccinia virus as a vector (Battula et al., 1987; Gonzalez, 1989). This vector has been used to express 15 individual human cytochrome P450s and numerous rodent cytochrome P450s (Gonzalez, 1989). The yield of cytochrome P450s is relatively small, and purification of the individual expressed cytochrome P450 may be required. Partial purification of the cytochrome P450 may yield insufficient amounts of cytochrome P450 for immunization. In any case, the expressed cytochrome

P450s could be used for determining the cross-reactivity of different MAbs.

The baculovirus expression system (Summers and Smith, 1987) yields very high levels of expressed cytochrome P450, and this system may be extraordinarily useful for obtaining sufficient antigens for both antibody production as immunogens and for screening for cross-reactivity. These methods could produce MAbs that would define immunologically and enzymatically closely related cytochrome P450s and could be of considerable usefulness for investigations of the role of individual cytochrome P450s in drug and carcinogen metabolism and activation.

III. Detection of Cytochrome P450s with Monoclonal Antibodies

A common detection method is the competitive RIA for MAb-specific cytochrome P450. Competitive binding assays are rapid and sensitive and can detect cytochrome P450 at a level of <100 ng of microsomal protein. Figure 2 shows a competitive RIA used to examine the distribution of MAb-specific cytochrome P450 in liver and various extrahepatic tissues of MC-treated rats (Cheng et al., 1984a,b). With this competitive assay, there was a 30- to 50-fold greater amount of MAb 1–7–1 (1A1/1A2) detectable cytochrome P450 in liver relative to lung and kidney. These values correspond well with the level of AHH activity in these tissues; AHH is a major enzyme activity of CYP 1A1. Thus, the MAbs can be used for the direct quantification of individual cytochrome P450s independently of their enzyme activity. The quantitation of MAb 1–7–1-detected CYP 1A1/1A2 and MAb 1–31–2-detected CYP 1A1 was examined in different tissues of several species induced by MC. The study compared the level of cytochrome P450 in liver microsomes from MC-induced rats, C57BL/6 mice, DBA/2 mice, hamsters, and guinea pigs. Rat liver microsomes were also compared to lung and kidney microsomes.

The results of the competitive assay determining the levels of epitope-specific cytochrome P450 in these animal tissues are shown in figure 2. MAb 1–7–1 (1A1/1A2) was used for figure 2A, and MAb 1–31–2 (1A1) was used for figure 2B. The microsomes from different species competed with wells coated with liver microsomes from MC-treated rats for binding to ^{35}S-MAb 1–7–1. As a positive control, the binding was reduced in the presence of competing microsomal proteins from MC-treated rats, with a 50% reduction of binding occurring with 0.042 μg of rat liver microsomal proteins. The greater competition gives a steeper slope, indicating a greater level of MAb-sensitive epitope. The microsomes from MC-treated C57BL/6 mice competed as effectively as rat liver microsomes for the binding of the ^{35}S-MAb 1–7–1, whereas liver microsomes from MC-treated DBA/2 mice, an AHH uninducible strain, competed only moderately for binding to the ^{35}S-MAb 1–7–1. MC-treated liver microsomes

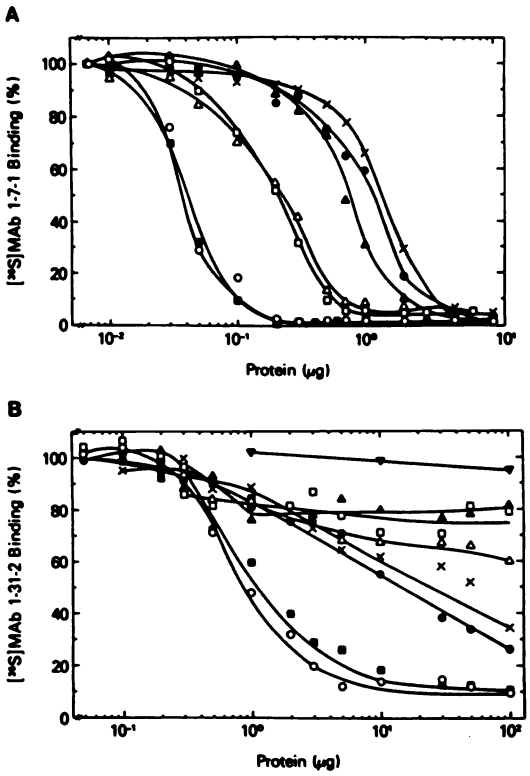

FIG. 2. RIA for cytochrome P450 in animal tissues using MAbs. A, Competitive RIA for MC-treated microsomes using [^{35}S]MAb 1–7–1 (1A1/1A2). Microtiter wells were precoated with 20 μg of MC-treated rat liver microsomes. [^{35}S]MAb 1–7–1 (15,000 cpm/well) was incubated overnight at 4°C with the following microsomal preparations: ○, rat liver; ■, C_{57}BL/6 mice liver; △, guinea pig liver; □, DBA/2 mice liver; ▲, hamster liver; ●, rat lung; and ×, rat kidney. The 100% binding levels (2400 cpm/well) were obtained in the absence of competing antigens. Each point represents the average of duplicate determinations. B, Competitive RIA for MC-treated microsomes using [^{35}S] MAb 1–32–2. Conditions are the same as in A. [^{35}S]MAb 1–31–2 (1A1) was incubated with the following preparations: ▼, bovine serum albumin; ○, rat liver; ■, C_{57}BL/6 mice liver; △, guinea pig liver; □, DBA/2 mice liver; ▲, MC-treated hamster liver microsomes; ●, rat lung; and ×, rat kidney. The 100% binding level was about 500 cpm/well.

from guinea pigs and hamsters competed very poorly for the ^{35}S-MAb 1–7–1.

The effectiveness of microsomes competing for binding increased in the amount of nanograms of protein required for a 50% reduction in binding as follows (μg): microsomes of MC-treated rats, 0.042; C57BL/6 mice, 0.050; guinea pigs, 0.21; DBA mice, 0.23; hamsters, 0.78; rat lung, 1.02; kidneys of rats, 1.04. Figure 2B shows the competitive binding curves for MAb 1–31–2 (1A1). This MAb recognizes 1A1 and not 1A2, and thus, the binding assay measures only CYP 1A1. Significant differences were seen between the competition curves with MAb 1–7–1 (fig. 2A) and MAb 1–31–2 (fig. 2B) that reflect the differences between recognition of both 1A1 and 1A2 with MAb 1–7–1 and only 1A1 with MAb 1–31–2.

Direct as well as competitive methods are also sensitive, efficient, and easily applicable for screening large numbers of tissue samples for MAb-recognized cytochrome P450s. These include sensitive radiometric tech-

niques (Rothwell et al., 1985). Other techniques common in immunological studies have been applied to cytochrome P450 detection. These include commonly used ELISAs (Paye et al., 1984).

The MAb-directed detection system for cytochrome P450 was also successfully used for detecting low levels of cytochrome P450 in human peripheral lymphocytes. The cytochrome P450-dependent AHH enzyme activity was increased after treatment of the mitogenized lymphocytes with the cytochrome P450 inducer benz[a]anthracene. Human placenta from women who smoked also showed by RIA higher levels of MAb 1–7–1-detected cytochrome P450. Thus, the MAbs offer a new potential for the detection of extremely low levels of epitope specific cytochrome P450s such as those contained in human placenta and lymphocytes (section VII).

Another important factor supporting the use of the MAb-based immunoassay is that the stability of the epitope available for MAb binding may be far greater than that of the cytochrome P450-dependent enzyme activity which is often quite labile (see section VII).

A. Competitive Radioimmunoassay to Determine Epitope Relatedness

The cytochrome P450 content of liver microsomes from MC-treated rats was examined by RIA with a series of MAbs to the major form of MC-induced rat liver cytochrome P450 (Song et al., 1985a). The relatedness of the epitopes to which the MAbs bind was examined by competitive RIA (fig. 3). Binding of a reference radiolabeled MAb to wells coated with microsomes was measured in the presence of each of a series of MAbs. The degree of inhibition of binding of the radiolabeled MAb by the individual MAb indicates whether the two competing MAbs bind to entirely independent epitopes or to epitopes that are identical or are overlapping. The binding inhibition curve obtained with a radiolabeled MAb in the presence of the identical unlabeled MAb is a useful standard curve, because both labeled and unlabeled MAbs are directed toward the same epitope. The extent to which each different MAb inhibits binding of the MAb relative to this standard curve depends on the relatedness of the epitopic sites which are specific for each of the pair of MAbs.

A competitive inhibition curve, using wells coated with MC-treated rat liver microsomes and added [^{3}H]MAb 1–7–1 as the reference MAb is shown in fig. 3a. With unlabeled MAb 1–7–1 as competitor, significant inhibition of binding is observed, with 20% of maximal binding occurring at 1 μg of MAb. MAb 1–8–1 also decreases binding but to a lesser extent; with 1 μg only 54% of maximal binding is observed. None of the MAbs 1–2–10, 1–25–3, 1–31–2, 1–36–1, and 1–56–2, or anti-lysozyme MAb HyHEL-9, inhibit [^{3}H]MAb 1–7–1 binding. These results indicate that of the seven MAbs examined, only MAb 1–8–1 recognizes an epitope overlapping or inter-

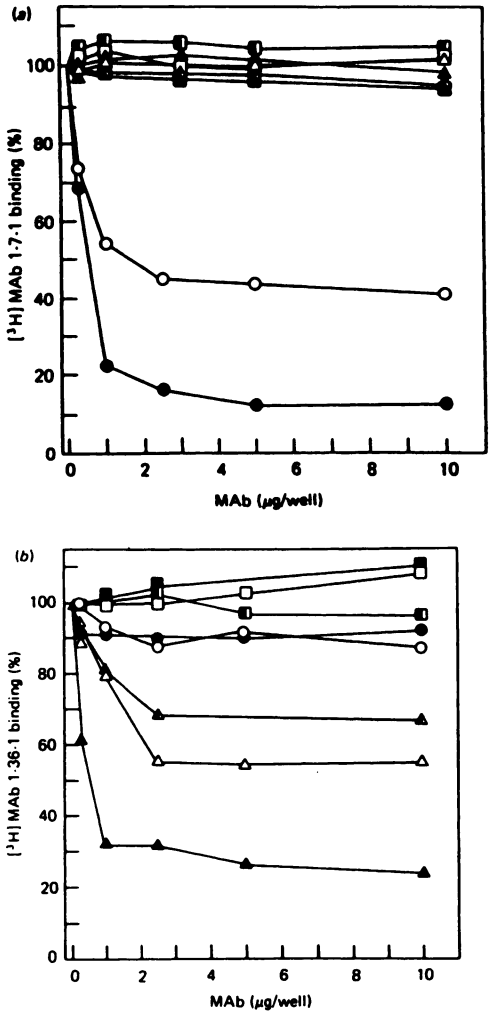

FIG. 3. Competitive binding of [^{3}H]MAb 1–7–1 and [^{3}H]MAb 1–36–1 to microsomes from liver of MC-treated rats by unlabeled MAbs. □, anti-lysozyme MAb HyHEL-9; ◧, MAb 1–2–10; ●, MAb 1–7–1; ○, MAb 1–8–1; ■, MAb 1–25–3; △, MAb 1–31–2; ▲, MAb 1–36–1; and ▲, MAb 1–56–2. The 100% binding level (1000 cpm/well) was obtained in the absence of competing antigens. Each point represents the average of duplicate determinations.

active with the epitope recognized by MAb 1–7–1. However, because slightly different curves were obtained with MAbs 1–7–1 and 1–8–1, the epitopes on cytochrome P450 to which these MAbs bind are not identical. The remaining five MAbs to CYP 1A1/1A2 are entirely noncompetitive with MAb 1–7–1, indicating that they are directed toward target epitopes that are nonoverlapping and noninteracting with the MAb 1–7–1 specific epitope.

The epitope specificity of different MAbs relative to MAb 1–36–1 was also measured by competitive RIA using [^{3}H]MAb 1–36–1 as the reference MAb (fig. 3b). The unlabeled MAb 1–36–1, as expected, effectively inhibited the binding of the identical labeled MAb 1–36–1. The MAbs 1–56–2 and 1–31–2 also significantly inhibited the binding of [^{3}H]MAb 1–36–1, indicating that these two MAbs target epitopes that overlap or interact with the MAb 1–36–1-specific epitope. The MAbs 1–2–10, 1–7–1, 1–8–1, 1–25–3, and anti-lysozyme MAb HyHEL-9

do not inhibit the binding of [^{3}H]MAb 1–36–1, demonstrating that they are directed toward epitopes that are different from, and noninteracting with, the epitope recognized by MAb 1–36–1.

Thus, the two reference radiolabeled MAbs, 1–7–1 and 1–36–1, have been successfully used to identify at least three distinct categories of epitopes on rat liver cytochrome P450: one recognized by MAbs 1–7–1 and 1–8–1; a second by MAbs 1–36–1, 1–56–2, and 1–32–1; and a third by MAbs 1–2–10 and 1–25–3, which bind neither of the epitopes on cytochrome P450 that are recognized by the MAbs of the first two groups (Song et al., 1985a).

The MAbs have also been successfully used as antibodies using the Western blotting technique. The ability to bind cytochrome P450 in this method, however, does not always parallel the inhibitory activity of the MAb. Thus, MAb 1–98–1 to CYP 2E1 gives a strong positive Western blot but is not inhibitory to CYP 2E1 enzyme activity, whereas MAb 1–91–3 is inhibitory to CYP 2E1 but does not give a positive Western blot (Ko et al., 1987). Inhibitory MAbs and immunoblotting (Western blots) were both used to study developmental aspects of cytochrome P450 in chickens (Lorr et al., 1989). Four MAbs, 1–7–1 (1A1/1A2), 2–66–3 (2B1/2B2), 1–91–3 (2E1), and 2–13–2 (3A4), were used. Of the four classes of MAbs, only MAb 2–13–2 against CYP 3A1 detected a cytochrome P450 in chicken liver microsomes on a Western blot. The protein detected by this MAb exhibited identical electrophoretic mobility as authentic CYP 3A1 that was isolated and purified from dexamethasone-treated rats.

Developmental studies found that the most predominant presence of the CYP 3A1 occurred 1 day posthatching. Lower levels of the CYP 3A1 were observed in the embryo at much later times after hatching. Both PB and dexamethasone induced the CYP 3A1 protein. The study also determined that chicken liver microsomal erythromycin demethylase, an enzymatic function of P450 3A1, was similar in its developmental and induction profile as the CYP 3A1 protein detected by Western blots with MAb 2–13–2 (3A1). Other enzyme activities of different cytochrome P450s, e.g., CYP 2B1/2B2, were poorly correlated with the immunodetected CYP 3A4 protein. These activities included aldrin epoxidase, benzphetamine demethylase, ethylmorphine demethylase, and aminopyrine demethylase. The latter enzyme activities were functions of CYP 2B1/2B2 and were identified by inhibition by MAb 2–66–3 (2B1/2B2). The MAbs were crucial to this study and very useful in determining the developmental course of the specific CYP 3A1 during the embryonic posthatching and later developmental stages. Thus, the MAb detection technique can be most usefully applied to a variety of developmental and other studies in which there is a changing pattern of individual cytochrome P450s caused by different ages, sex, tissues, organs, and changing hormonal, nutritional, or environmental conditions.

MAbs were used to distinguish possible differences among the cytochrome P450s induced by pyrazole, ethanol, and acetone. All of these compounds induce rat CYP 2E1 and rabbit CYP LM3$_a$. This induction has been examined in inbred strains of mice including DBA/2N, AKR/J, and BALB/c mice. Pyrazole strongly induced coumarin 7-hydroxylase in DBA/2N but was considerably less active in the other strains. The effect of pyrazole on aniline *p*-hydroxylase and ethanol oxidase was strain dependent. Pyrazole only increased these enzymes in DBA/2N mice. Neither acetone or ethanol induced coumarin 7-hydroxylase, but both increased aniline hydroxylase and ethanol oxidase from 1.4- to 3.3-fold in all strains of mice examined. The MAb 1–91–3 (CYP 2E1), which was raised against acetone-induced CYP 2E1, did not inhibit the coumarin 7-hydroxylase, whereas aniline hydroxylase was inhibited by 46 to 76% and ethanol oxidase by 25 to 70%. In immunoblots of these microsomes, anti-CYP COH recognized only its own antigen but not CYP ac (2E1), whereas the MAb 1–98–1 (CYP 2E1) detected CYP 2E1 and another form of the enzyme from the D2 mouse, which corresponded to CYP 2E1. It did not, however, detect the CYP COH.

Purified CYP 2E1 and CYP Coh have molecular weights of 52 and 50 kb as determined by gel electrophoresis. These cytochrome P450 antigens were expressed differentially in response to pyrazole, ethanol, and acetone. The CYP Coh was increased only after pyrazole treatment, but MAb 1–98–1-detectable protein was elevated in D2 mouse liver only by ethanol and acetone treatment but not by pyrazole. Thus, the use of the MAbs led to the conclusion that mouse CYP Coh and rat CYP 2E1 (ac) are different forms of cytochrome P450 and that the CYP 2E1-like protein, which is a catalyst for aniline hydroxylation and ethanol oxidation, is present in the D2 mouse liver. These two cytochrome P450 isozymes are also expressed differently in the mouse liver in response to inducer administration. Thus, the MAbs were instrumental in discriminating between cytochrome P450 induced by pyrazole, acetone, and ethanol (Honkakoski et al., 1988).

MAb 1–36–1 (1A1) was used to determine that congenitally jaundiced (*jj*) Gunn rats had a greater hepatic microsomal content of a CYP 1A1 than did the nonjaundiced (*Jj*) rats. No differences in content of CYP 2B1/2B2, CYP 1A1, and PCN-induced CYP 3A1 were found between *jj* and *Jj* rats. This demonstrates a constitutive increase in a specific cytochrome P450 isoenzyme in association with a genetic defect (Kapitulnik et al., 1987b). These studies demonstrated the value of MAbs in measuring a constitutive increase in specific cytochrome P450 enzymes, i.e., 1A1/1A2, 2B1/2B2, and 3A4. Thus, the MAbs detect and localize changes in

specific cytochrome P450 content under different physiological or pathological conditions.

MC increases the CYP 1A1/1A2 content of various rat tissues, as measured by the levels of AHH. In one study, two MAbs, 1–7–1 specific for 1A1/1A2 and 1–31–2 specific for 1A1, were used to determine by Western blots the presence of CYP 1A2 in various tissues. With the MAb-based method, CYP 1A1 was found in rat nasopharynx and pancreas in addition to its previously reported presence in liver, lung, and kidney (Ueng et al., 1987). In contrast to CYP 1A1 induction, the immunorelated CYP 1A2 was found only in the liver. The specific content of immunodetected CYP 1A1 in the tissue homogenates decreased in the order: liver, nasopharynx, pancreas, lung, and kidney. The corresponding AHH activity decreased in the order: liver, kidney, lung, nasopharynx, pancreas. The ratio of AHH activity to cytochrome P450 content was widely different among tissues. Thus, the ratios varied from 37:1.7:47:04:02 for kidney, liver, lung, nasopharynx, and pancreas, respectively. The finding that the ratios of AHH to CYP 1A1 are different in different tissues suggests that extrahepatic AHH activity may result from both CYP 1A1 and other cytochrome P450 forms with different specific activities (Wilson et al., 1990). Western blot analysis has been used extensively in conjunction with MAb-based detection. MAb-based detection of individual cytochrome P450s can be used to make an atlas of cytochrome P450 distribution of the entire organism.

IV. Monoclonal Antibody-directed Immunohistochemistry

MAbs specific for a single epitope are unique tools for identifying epitope-specific cytochrome P450s in organs, tissues, cells, and subcellular organelles. MAb-based immunohistochemical methods can be applied to localize and examine the distribution of individual cytochrome P450s after different inducer administration, during various physiological states related to nutrition, age, and sex, and in different species and tissues. Furthermore, the intracellular distribution of the cytochrome P450 can be clearly determined in a way not possible by standard biochemical methods which generally cannot identify the presence of specific forms of cytochrome P450 proteins in isolated tissues and organelles.

The first application of MAbs to the analysis of cytochrome P450 in liver cells showed that the MAb 1–7–1 (CYP 1A1/1A2) detects the epitope containing cytochrome P450 in MC-induced rat liver and is localized in both the rough and smooth ERs as well as in the nuclear envelope of hepatocytes (Brands et al., 1985). Previous biochemical studies left uncertain the presence of cytochrome P450 in the nuclei or the nuclear envelope of rat liver hepatocytes. This was due to the inadequacy of the procedures used for isolation of the nuclei, because cytoplasmic contamination of the nuclei by the ER could not be excluded. The MAb-based study was the first to directly demonstrate the presence of CYP 1A1/1A2 in hepatocyte nuclear membranes in situ. This study presents the most compelling evidence for the presence of cytochrome P450 in the nuclear membrane to date. This elegant study utilized the MAbs with a protein A-gold conjugate and both light and electron microscopy. Because the MAbs do not bind protein A-gold conjugates, the investigators applied rabbit anti-mouse IgG as an intermediate antibody to localize cytochrome P450 isozymes. The MAbs clearly detected CYP 1A1/1A2 in both smooth and rough ERs and in the nuclear envelope of hepatocytes. These cytochrome P450s were not found in Golgi stacks, indicating a lack of processing of cytochrome P450s through Golgi bodies. The conclusion of the study was that the ER membrane proteins are retained during a highly selective export process. These include the ASN-linked oligosaccharide chains of two well-characterized ER membrane proteins, glucosidases and hexose-6 phosphate dehydrogenase. Thus, the MAb 1–7–1 was used as a marker for cytochrome CYP 1A1/1A2, which is contained in the ER and which is not processed through the Golgi apparatus. This information led to the conclusion that the ER efficiently retains its specific proteins (Brands et al., 1985).

Subsequently, a panel of MAbs was produced against PB-induced CYP (2B1/2B2); MAb-5/23/64 was used to study the induction process in situ by immunoelectron microscopy and in vitro by ELISA techniques. The study utilized protein A-colloidal gold for labeling and found that the rough ER was the primary site of cytochrome P450s induced by PB (Marti et al., 1990). These are likely CYP 2B1/2B2 and perhaps several other related PB-induced cytochrome P450s. Twelve hours after PB was administered, the rough ER showed the highest density of MAb-detected cytochrome P450 and reached the highest level of cytochrome P450 in the smooth ER in 5 days. Thus, there seems to be a shift in cytochrome P450 content from the rough ER to the smooth ER during a 5-day period. The maximum increase of the PB-induced cytochrome P450s was 21-fold as measured by morphometric analysis and 15-fold as measured by ELISA. Furthermore, this study is similar to one (Brands et al., 1985) reporting no evidence of recycling of the PB-induced cytochrome P450s in the Golgi apparatus; nor was there integration of the cytochrome P450 into the lysosomes after maximal induction. These two studies show the efficacy of MAbs for determining the intracellular localization of different cytochrome P450s in situ. (Brands et al., 1985; Marti et al., 1990).

Extensive studies (Anderson et al., 1987; Forkert et al., 1986, 1988, 1989) using MAbs for cytochrome P450 localization have localized individual cytochrome P450s in different tissues of different strains of mice. One study utilized MAb 1–7–1 (1A1/1A2) and MAb 2–66–3 (2B1/2B2) and studied the distribution of cytochrome P450s

in frozen sections of mouse lung with MAb-directed immunohistochemical analyses. In MC-induced mice the MAb 1–7–1 reacted intensely with the parenchymal cells, but not with the epithelial cells, of the pulmonary bronchioles. MAb 1–7–1 also did not react with pulmonary cells of either untreated or PB-treated mice. The MAb 2–66–3 (2B1/2B2) reacted with parenchymal and bronchiolar epithelial cells in the lungs of PB-treated, MC-treated, and untreated mice. Thus, this study successfully localized different constitutive and induced forms of cytochrome P450 in different cells of the lungs of MC-treated, PB-treated, and control mice. The PB-induced cytochrome P450s, which were reactive with MAb 2–66–3, were also present in MC-treated and constitutive mice. In contrast, the MC-induced MAb 1–7–1-sensitive 1A1/1A2 was not present in either the PB-induced or control mice. Studies such as these may provide useful information concerning the nature of cell-specific damage related to unique forms of cytochrome P450 and the enzymatic activity of cytochrome P450 (Forkert et al., 1989).

In other comprehensive studies by the same group, MAb-directed immunofluorescence was used to study the distribution of cytochrome P450s induced by MC and PB in the lungs of inducer-responsive C57BL/6 and nonresponsive DBA/2 mice (Forkert et al., 1989). In this study MAbs of different specificities were used to more clearly localize the cytochrome P450. The MAb 1–7–1 (1A1/1A2) detected cytochrome P450 in cells of the alveolar septa, including type II cells and epithelial cells lining the blood vessels. MAb 1–31–2 (1A1) was also used. In contrast to MAb 1–7–1, the latter MAbs reacted only with 1A1 and not 1A2. These MAbs detected cytochrome P450s in type II cells of the alveolar septa but were not found in the epithelial cells. Thus, epithelial cells contain CYP 1A2 but not the CYP 1A1. These studies indicated that the alveolar septa cells contain both the CYP 1A1 and CYP 1A2, whereas the epithelial cells of the vasculature contain CYP 1A2 but not CYP 1A1. Surprisingly, immunolocalization of these cytochrome P450s was observed in both MC-treated C57BL/6 and DBA/2 mice. MAb 2–66–3 (2B1/2B2) was immunoreactive only in type II alveolar and bronchiolar epithelial cells, including nonciliated Clara cells. The epitope for MAb 2–66–3 is present constitutively within the lungs and did not increase with PB treatment. In contrast, the epitopes for MAb 1–7–1 were not expressed constitutively but were only detected after induction by MC. The regional and cellular localization of different cytochrome P450s in lung tissue for the major MC- and PB-inducible forms of cytochrome P450 indicate that individual tissues exhibit quite specific expressions of different cytochrome P450s (Forkert et al., 1986).

In other histochemical studies (Forkert et al., 1986, 1988), MAb 1–7–1 and MAb 2–66–3 were used with an unlabeled peroxidase-antiperoxidase immunohistochemical procedure to study the intralobular induction and intralobular distribution of cytochrome P450s in the livers of CD-1, C57BL/6, and DBA/2 mice. CYP 1A1/1A2 were localized predominantly in the centrilobular hepatocytes of mice from all strains. The amount of this cytochrome P450, however, was much lower in DBA/2 mice. Pretreatment with MC or BNF caused large increases in the MAb 1–7–1 (1A1/1A2) sensitive cytochrome P450s in hepatocytes from all regions of the hepatic lobule in CD-1 and C57BL/6 mice but had no effect in DBA/2 mice. The PB-specific cytochrome P450s binding to MAb 2–66–3 (2B1/2B2) were localized in hepatocytes in all segments of the lobule of the control mice with somewhat higher cytochrome P450 content in the centrilobular hepatocytes. PB pretreatment caused an increase in these cytochrome P450s in all regions of the lobule. Strain-related differences were not observed with the PB-induced CYP 2B1/2B2. The results demonstrated that in control mice the cytochrome P450s are localized predominantly in the central lobular hepatocytes of murine livers, and MC or PB induction of the cytochrome P450 is observed to the greatest extent in the periportal hepatocytes, resulting in a more uniform distribution of the cytochrome P450s in the hepatic lobule. CYP 1A1 is not believed to be a constitutive cytochrome P450 in mouse liver. Thus, these results suggest that the MAb 1–7–1-detected enzyme may be CYP 1A2 (Forkert et al., 1988).

In a related study, MAb 1–7–1 (1A1/1A2) was used for immunohistochemical staining of formalin-fixed tissues. The stained tissue was examined for MAb 1–7–1 epitope-containing cytochrome P450s in control and MC-treated C57BL/6, DBA/2, and [(C57BL/6 × DBA/2)F_1 × DBA/2]F_2 mice. MAb 1–7–1 stained the cytoplasm of the centrilobular hepatocytes of C57BL/6 mice that were pretreated with MC. No staining was observed in constitutive C57BL/6 or with MC-treated DBA/2 mice. In the F_2 mice, 50% of the cytochrome P450 enzyme was expected to be MC inducible. Inducibility phenotype was measured by the conversion of C14-MC to oxidized and conjugated products in the liver homogenates. In freshly fixed tissue from MC-treated mice, the liver phenotype was determined by the metabolism of MC. The inducible phenotypes also stained with MAb 1–7–1, whereas the mice showing a negative histochemistry had the noninducible phenotype. There was a significantly positive correlation between the amount of MAb 1–7–1-directed staining and the amount of MC metabolism.

The immunohistochemical procedure was also reproducible for the determination of inducibility phenotype of livers that had been stored in paraffin blocks for up to 2 years. This finding is extraordinary and indicates that the MAb 1–7–1-detected cytochrome CYP 1A1/1A2 epitope can be stabilized for 2 years. In lung the MAb 1–7–1 stained only the alveolar walls and endothelial blood vessels in the MC-induced C57BL/6 mice. Control MAbs

and other strains of mice were negative. In the kidney of MC-induced C57BL/6 mice, MAb 1–7–1 stains only interstitial cells and glomeruli; the endothelium of the blood vessels in the colon of the mice also was strained. The MAb 1–7–1 epitope-specific CYP 1A1/1A2 was found in the endothelium cells, a finding consistent with another study that demonstrated the presence of cytochrome cytochrome P450 in vascular endothelium (Anderson et al., 1987). Thus, a number of studies utilizing immunohistochemical techniques directed by MAbs indicate large promise for the highly specific localization of different forms of cytochrome P450 containing MAb-specific epitopes (Anderson et al., 1987).

In a study in which a variety of tissues was examined(Foster et al., 1986), MAb 3/4/2 (CYP 1A1) was used to examine the cellular distribution of cytochrome CYP 1A1 in the liver and the extrahepatic tissues of the rat by immunohistochemical methods. The epitope-containing cytochrome CYP 1A1 was found in the liver, proximal tubules of the kidney, and Clara cells of the lung, olfactory epithelium, and Bowman's glands of olfactory tissue. The immunoreactive cytochrome P450 was not found in small intestine, testes, or the adrenal gland, although these tissues are known to contain some form of cytochrome P450. This study also examined the effects of PB, BNF, and clofibrate on the distribution of CYP 1A1. Surprisingly, PB pretreatment induced CYP 1A1 in the centrilobular cells of the liver but had no inductive effect on the other tissues containing constitutive CYP 1A1. Clofibrate had no effect on the level of CYP 1A1 in any of the tissues studied. BNF induced CYP 1A1 in the periportal region of the liver, Clara cells of the lung, enterocytes of the small intestine, and the proximal tubules of the kidney. The olfactory epithelium was unique in that it did not respond to any of the enzyme inducers. Other MAb-directed studies (Forkert et al., 1986) showed that mouse lung contained a PB-induced type of cytochrome P450, whereas the MC-induced form of CYP 1A1/1A2 was detectable only after MC pretreatment.

Brain nerve fibers were examined for CYP 1A1 immunoreactivity and was observed in the globus pallidus. Immunoreactive fibers were also observed in the caudate putamen, amygdala, septum, ventromedial nucleus of the hypothalamus, medial forebrain bundle, ansa lenticularis, and ventromedial portion of the internal capsule and crus cerebri. Cell bodies with cytochrome P450 immunoreactivity were observed in the caudate putamen and in the perifornical area of the hypothalamus. These studies were performed with polyclonal antibodies to CYP 1A1/1A2. The MAbs were not immunoreactive (Kapitulnik et al., 1987a). A library of MAbs should prove useful when used in conjunction with immunohistochemical methods, for constructing a topographic distribution of individual cytochrome P450s in both human and rodent tissues.

V. Immunopurification

One of the major tasks facing cytochrome P450 research is the development of methods for the rapid and simple purification of the cytochrome P450 enzymes and especially those forms that are difficult to obtain or are present in low concentration. Generally, cytochrome P450 isolation has been accomplished by classical methods of enzyme purification, which most often are laborious and require numerous steps and considerable time. MAbs have properties that make them unique reagents for immunopurification of cytochrome P450. The MAbs introduce an entirely new approach to cytochrome P450 isolation. Unlike polyclonal antibodies, the MAbs are chemically defined, homogeneous reagents that are highly reproducible and specific probes for single epitopes on a cytochrome P450.

The basic procedure for MAb-based immunoaffinity purification requires only one or two steps and can yield highly purified milligram quantities of individual cytochrome P450s. In the immunopurification procedure, the MAb is covalently bound to Sepharose, which is made into the form of either a column or a slurry for batch purification. The epitope containing cytochrome P450 is then passed through the column and binds to the MAb-linked Sepharose. The nonbound material containing unrelated proteins and cytochrome P450s lacking the MAb-sensitive epitope is thoroughly eluted leaving the epitope-specific cytochrome P450 as the only protein bound to the MAb-Sepharose column. The MAb-recognized cytochrome P450 can then be eluted and used for a variety of chemical and physical studies. This technique was used to isolate individual cytochrome P450s from liver microsomes of MC-induced rats, C57BL/6 mice, DBA/2 mice, guinea pigs, hamster, and lung. The isolated cytochrome P450s from each preparation were of sufficiently high purity so that NH_2-terminal sequencing of each of the cytochrome P450s was accomplished (Cheng et al., 1984a,b).

The experiment shown in table 3 demonstrates that MAb 1–7–1 absorbed two species of cytochrome P450 of 56,000 and 57,000 kDa from MC-induced rat liver microsomes. These two species were identified as CYP 1A1 and CYP 1A2. Similar results were obtained with liver

TABLE 3

Antigenically related cytochrome P450 purified with Sepharose-bound MAbs (Cheng et al., 1984a,b)

Species, tissue	Molecular weight of cytochrome P450 purified with MAb-Sepharose	
	MAb 1-7-1	MAb 1-31-2
Rat liver	56,000, 57,000	57,000
Rat lung	57,000	57,000
C57BL/6 mouse liver	56,000, 57,000	57,000
DBA/2 mouse liver	56,000	ND*
Guinea pig liver	53,000	ND
Hamster liver	57,000	ND

* ND, not detectable.

microsomes from MC-induced C57BL/6 mice yielding a 56,000-kDa CYP (1A2) and a 57,000-kDa CYP (1A1) Kd protein. MAb 1–7–1 also bound the 57,000-kDa (1A1) from rat lung; the CYP 1A2 was not detected. Similar results were obtained with hamster liver. MAb 1–31–2, which is specific for CYP 1A1 and not CYP 1A2, absorbs only the 57,000-kDa (1A1) polypeptide from MC-induced rat liver, rat lung, and C57 mouse liver. These results reveal that livers from both MC-induced rats and C57BL/6 mice contain cytochrome P450s of 56,000 and 57,000 kDa which were absorbed by MAb 1–7–1. Cytochrome P450 1A1 (57,000 kDa) is present in rat lung and contains the epitope recognized by MAb 1–31–2. The MAb 1–31–2 does not bind to the 56,000-kDa CYP 1A2 which is bound by MAb 1–7–1. Thus, 1–7–1 recognizes two cytochrome P450s, which subsequent studies showed are CYP 1A1 and CYP 1A2, whereas the MAb 1–31–2 recognizes only 1A1. The tandem use of these two MAbs permits the clean isolation of both the CYP 1A1 and 1A2. Other MAb-based immunopurification studies shown in table 3 demonstrate that cytochrome P450s from different tissues and species can be directly immunopurified. These were cytochrome P450 of 56,000 kDa from DBA/2 mice, a 53,000-kDa cytochrome P450 from guinea pig, and a 57,000-kDa cytochrome P450 from hamster.

The one-step immunoaffinity purification procedure easily purified milligram quantities of single cytochrome P450 from the microsomes of MC-induced animals as shown in table 3. The protein isolated from guinea pig is unique in that it has a lower molecular weight than CYP 1A1 and CYP 1A2 but is epitopically related to 1A1 and 1A2. The amino acid composition of the six immunopurified cytochrome P450s were determined. The compositions of these polypeptides were similar; all contained 40 to 50% hydrophobic amino acids. The 57-kDa rat polypeptide had an amino acid composition most similar to that of the 57-kDa polypeptide of C_{57}BL/6 mice, except for slight differences in the histidine and valine content. The 56-kDa polypeptides of rats and both mice strains had similar amino acid compostions. Comparison of the amino acid composition of the 57-, 56-, and 53-kDa polypeptides reveals significant differences in their amino acid content, especially for histidine, isoleucine, leucine, valine, and serine. The isolated cytochrome P450s were of sufficiently high purity to permit NH_2-terminal sequencing (Cheng et al., 1984a,b).

NH_2-terminal sequence data were obtained for each of the immunopurified proteins. Six proteins exhibited an unblocked and unique NH_2-terminal amino acid. The initial yields for coupling and cleavage of the NH_2-terminal residue varied from 10 to 40%, and the repetitive yield of coupling and cleavage usually reached 95%. The first 25 cycles yielded positively identifiable amino acids. Sequence homology among the immunopurified proteins is illustrated in figure 4.

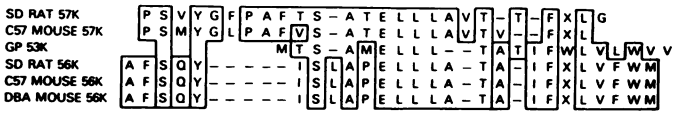

FIG. 4. Sequence homology among the immunopurified proteins.

Cytochrome P450 from microsomes of PB-treated rats was also purified by a single immunopurification step using an MAb. The amino-terminal sequence of the isolated cytochrome P450 displayed a microheterogeneity related to the different forms of PB-induced cytochrome P450. Thus, the use of MAbs allows the classification of different, but related, cytochrome P450 isozymes (Friedman et al., 1985a,b,c,d,e). It is also possible that a taxonomic system may be developed in which cytochrome P450s can be defined by their content of epitopes recognized by different MAbs.

In one study (Reubi et al., 1984a,b) a rabbit cytochrome P450 was isolated using an MAb and in other studies MAbs were utilized for the purification of several different cytochrome P450s (Davies et al., 1982; Thomas et al., 1984a,b). One study (Lewis et al., 1982) reported success in the immunopurification of cytochrome P450 from rabbit liver with an MAb called MBS 105. The MBS 105 had no effect on cytochrome P450 enzyme activity. This antibody was termed a null antibody. The null antibody was covalently bound through activated Sepharose, and the complex was incubated with cholate-solubilized microsomes. The Sepharose-null antibody selectively absorbed a cytochrome P450 of 55,000 kDa. The null antibody-bound-cytochrome P450 exhibited enzyme activity. The cytochrome P450-antibody complex could not be dissociated, however, without losing active enzyme activity. In this (Lewis et al., 1982) and a similiar study (Friedman et al., 1985b,c), it was not possible to elute cytochrome P450 from its antibody-Sepharose-bound form and retain enzyme activity. However, in a subsequent study a low level of active enzyme activity could be eluted from the Sepharose-antibody complex under certain conditions, using an antigen exchange technique (Friedman et al., 1985e). The use of Sepharose for binding an MAb-bound cytochrome P450 described above can be very useful for a simple purification of a single cytochrome P450, which can then be examined for its structural properties, sequence, and, under certain conditions, enzyme activity.

Another study (Robinson et al., 1989) (table 4) utilized the MAb 1–91–3 to CYP 2E1 for identification and isolation of the cytochrome 2E1 from both rat and human

TABLE 4

NH_2-terminal sequence of rat and human CRP 2E1 (Robinson et al. 1989)

	1 5 10 15
Rat	A V L G I T I A L L V W V A T L L V I
Human	* A L G V T V A L L V W A A F L L L V

* Several amino acids were observed, including P, C and W.

tissues. The generality of the cross-species reactivity depends on epitope identity. Table 4 shows the NH_2-terminal sequence of the rat and human CYP 2E1. The immunopurified rat and human proteins migrate with apparent molecular weights of 53 and 56 kDa, respectively. Peptide mapping of the two proteins showed them to be relatively resistant to proteolytic digestion; prolonged incubation with a high ratio of proteolytic enzyme to cytochrome P450 protein was required. This indicated that the target cleavage sequences are partly inaccessible, possibly due to conformational limitations or aggregation of the polypeptide. The human CYP 2E1 protein yielded a significantly different peptide pattern than did the rat CYP 2E1. The sequences for the first 19 amino acids of each cytochrome P450 are shown in table 4.

These sequences were identical with those that were deduced from human and rat cDNAs and were isolated with MAb 1–91–3 or 1–98–1 to rat CYP 2E1, except for the internal residue of the human cytochrome P450 protein, which was undetermined owing to several amino acids in the first cleavage cycle. The two proteins differed in their peptide maps but displayed similar amino-terminal sequences in the first 19 residues. This study is an example of the use of MAbs made to rodent cytochrome P450s that can be useful for the isolation of human cytochrome P450s (Robinson et al., 1989).

Another example of the use of MAbs for the study of the interrelationship of cytochrome P450 in different tissues is that in which a pulmonary cytochrome P450 was purified from lung microsomes of MC-treated rats by immunoaffinity chromatography which utilized an MAb to MC-induced rat liver CYP 1A1. The isolated pulmonary cytochrome P450 was identified by its molecular weight of 57 kDa determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as well as by its NH_2-terminal sequence of the first nine amino acids. The cytochrome P450 from lung was identical with that of the epitopically related rat liver cytochrome CYP 1A1. In addition, partial proteolysis of both cytochrome P450s yielded indistinguishable peptide patterns on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Thus, treatment of rats with MC induces a pulmonary cytochrome P450 structurally identical with the MC-induced hepatic enzyme by several criteria. This study also showed that there were two epitopes common to these cytochrome P450s that were recognized by MAbs 1–31–2 and 1–7–1. The amino-terminal sequence of the immunopurified polypeptides was Pro-Ser-Val-Tyr-Gly-Phe-Pro-Ala-Phe (Robinson et al., 1986).

One of the major limitations in the use of MAb-Sepharose-directed immunopurification is the significant loss in enzyme activity of the bound cytochrome P450 when eluted from the antibody-Sepharose column. To overcome this problem, an antigen exchange technique was used to elute the cytochrome P450 in active form (Friedman et al., 1985c,e). This technique used inactive cytochrome P450 as contained in the elution fluid. Also, immunoaffinity-purified cytochrome P450 that required denaturants for efficient desorption from the column matrix showed a significant lack in catalytic activity. These cytochrome P450s, however, were found to retain epitopic structural integrity as determined by RIA using the appropriate MAbs. In these experiments the eluted cytochrome P450s retained low levels of AHH and ECD activities. Thus, epitope-specific exchange of denatured antigen for native antigen on a solid phase matrix can be generally applicable to the preparation of proteins with the retention of some activity. Although this procedure was somewhat successful, the yields were disappointingly low, and the technique, although of theoretical potential for immunopurification, may require much work to make it a practical useful technique (Friedman et al., 1985c,e).

The purification of several other human cytochrome P450s has been accomplished with the use of MAbs (Beaune et al., 1985). MAbs were reacted with human liver microsomal cytochrome P450. Three similar hybridomas produced antibodies that recognized an epitope-specific family of human cytochrome P450 isozymes. The epitope was also present in CYP 3A1 of rat liver microsomes but differed from human cytochrome P450s by its molecular weight. The MAbs were also used to quantify cytochrome P450s in human liver microsomes. Their distribution suggested a polymorphism in the human liver cytochrome P450. Quantitation of the human cytochrome P450s was determined with the MAbs to seven different human liver samples, and there was a wide distribution of cytochrome P450 ranging from 0.13 to 0.53 nmol/mg protein among the samples. The MAbs did not cross-react with isozymes 1 and 8 which represent another class of cytochrome P450 enzymes in human liver. The cytochrome P450s bound to MAb showed a common epitope with rat liver PCNE-induced CYP 3A1. Both MAb 13–1–13- and 13–7–8-based analyses suggested a significant polymorphism in the CYP 3A1 (Beaune et al., 1985).

Immunopurification techniques were used for the purification of aromatase (P-16) (see section II). Also, cytochrome P450s involved in μ-2 prostaglandin hydroxylation utilized immunopurification methods to identify the cytochrome P450 catalyzing hydroxylation (see section VI).

VI. Reaction Phenotyping of the Metabolic Contribution of Individual Cytochrome P450s

The high specificity of MAbs, their interaction with individual epitopes on proteins, homogeneity as defined chemical reagents, immortality, and ease of handling all contribute to their extraordinary utility. MAbs that are highly inhibitory to a specific form of cytochrome P450 make them especially and uniquely useful for the quantitative determination of the contribution of an

epitope-specific single or class of cytochrome P450 to the metabolism of a substrate when the cytochrome P450 is contained within a mixture of cytochrome P450s. Thus, within a mixture of cytochrome P450s, as is present in common tissue preparations such as microsomes or cell homogenates, the inhibitory MAbs can measure the contribution of a single cytochrome P450 to the metabolism of a specific substrate.

The amount of conversion of a substrate to a product catalyzed by a specific cytochrome P450 can be quantitatively measured by the amount of inhibition of activity caused by the inhibitory MAb. This analysis, utilizing an inhibitory MAb, has been termed reaction phenotyping because it identifies the phenotype of the responsible cytochrome P450. Reaction phenotyping can be generally and usefully applied to the analysis of any metabolic reaction catalyzed by a single or epitope-defined class of cytochrome P450 for which an inhibitory MAb exists. The types of reaction that can be analyzed in this manner include substrate utilization, product formation, and positional and stereospecific metabolism. Reaction phenotyping with inhibitory MAbs can also be applied to cytochrome P450 dependent secondary biological effects, such as mutagen and carcinogen activation, toxicity, or metabolite binding to DNA (table 1).

The inhibitory MAb-based analysis is applicable to both xenobiotic and endobiotic metabolism. Inhibitory MAbs specific for a single cytochrome P450 can be used to determine the contribution of that single cytochrome P450 to a specific metabolic reaction. MAbs that recognize two or more cytochrome P450s and are inhibitory to each of them designate the epitope-specific class of cytochrome P450 responsible for the reaction.

Furthermore, MAb reaction phenotyping in combination with the cDNA expression offers a unique analytic advantage. cDNA expression can be used to define the specificity of individual cytochrome P450s and can often help identify the responsible cytochrome P450 when more than one cytochrome P450 share a common epitope and are both inhibited by an MAb. cDNA expression (Gonzalez, 1989) can determine when one or both of the epitope-sharing cytochrome P450s are responsible for the studied reaction. However, cDNA expression of cytochrome P450 does not quantitate the activity or metabolic contribution of a cytochrome P450 in a tissue in situ, such as occurs in microsomes, cell homogenates, or in vivo. Thus, two epitope common cytochrome P450s recognized by the same MAb can be distinguished as to their metabolic specificity by their cDNA expression if they exhibit nonoverlapping catalytic activity. Where there is uncertainty concerning the specificity of an MAb for one of a pair or more cytochrome P450s, the use of cDNA-expressed cytochrome P450 designates the specificity of each cytochrome P450, and the MAb inhibition measures the quantitative contribution of the individual cytochrome P450 to metabolism. Thus, the two techniques are complementary, cDNA expression defining individual cytochrome P450 specificity and the inhibitory MAb determining the quantitative contribution of the specific cytochrome P450 to the enzyme reaction.

The results of a reaction phenotyping study in which various mammalian tissues were examined with inhibitory MAbs to quantitatively measure the contributions of specific cytochrome P450s to cytochrome P450-dependent reactions are shown in tables 5 and 6 (Fujino et al., 1984b). Table 5 shows the inhibitory effects of MAb 1–7–1 (1A1/1A2) on two enzyme activities, AHH and ECD, in MC- and PB-induced and control rat liver, lung, and kidney microsomes. In this experiment the degree of inhibition by the MAb 1–7–1 quantitatively determines the contribution to AHH and ECD of the epitope-containing cytochrome P450s in rat liver. Table 6 shows a similar study of AHH and ECD of liver, lung, and kidney microsomes from C57BL and DBA/2 mice, guinea pigs, and hamsters.

The MAb 1–7–1 (1A1/1A2) inhibited the AHH-induced CYP 1A1 purified from MC-treated rats by 100%, establishing the MAb 1–7–1 as a powerful inhibitor of CYP 1A1. The MAb 1–7–1 inhibited the MC-induced rat liver microsomal AHH by 80% and the ECD by 65%, indicating that the AHH is catalyzed primarily by 1A1 and the ECD by 1A1 and a second cytochrome P450 not inhibited by MAb 1–7–1. Another possibility is that the MAb interaction with the cytochrome P450 has differential effects on the binding of each substrate to the active site. The contribution of 1A2 to AHH was relatively little because cDNA-expressed CYP 1A2 showed only negligible AHH activity compared to the cDNA-expressed CYP 1A1 (Battula et al., 1987). Thus, the MAb 1–7–1 inhibition of AHH is largely due to inhibition of 1A1.

CYP 1A1 contributes at least 80% of MC-induced microsomal AHH, as shown by its 80% inhibition by MAb 1–7–1. In contrast, MAb 1–7–1 inhibits ECD in liver microsomes from MC-treated rats by only 65%, indicating that 1A1 is responsible for 65% of ECD activity, which is considerably less than the AHH inhibition. The AHH and ECD of microsomes from control and PB-treated rats were not inhibited by the MAb 1–7–1. This seemingly negative result is of large importance because it demonstrates that the AHH and ECD activities in both the control and PB-treated microsomes are not a function of 1A1 or 1A2, because they are completely insensitive to MAb 1–7–1 (1A1/1A2). Thus, the AHH and ECD in the control and PB-treated microsomes are functions of cytochrome P450s other than 1A1 and 1A2, and the cytochrome P450 responsible for AHH and ECD lacks the epitope sensitive to MAb 1–7–1. Thus, the MAb 1–7–1 characterizes the epitopic character of the cytochrome P450s responsible for AHH and ECD in MC-treated rodents as entirely different from those cyto-

TABLE 5

*AHH and ECD of rat tissues and inhibition by MAb 1-7-1**

Treatment	Enzyme inhibition					
	Liver		Lung		Kidney	
	Control	+1-7-1	Control	+1-7-1	Control	+1-7-1
AHH (pmol/min/mg)						
Control	229	239 (0)	7.7	4.5 (42)	1.8	1.6 (11)
PB	141	128 (5)	3.2	2.4 (24)	2.3	1.4 (39)
MC	2099	409 (81)	53.2	11.6 (78)	151.3	19.2 (87)
ECD (pmol/min/mg)						
Control	1.06	1.08 (0)	0.16	0.16 (0)	0.01	0.10 (0)
PB	2.73	2.86 (0)	0.10	0.10 (0)	0.02	0.02 (0)
MC	8.37	2.96 (65)	0.24	0.15 (38)	0.27	0.08 (70)

* Values in parentheses indicate percentages of inhibition by MAb 1-7-1. From Fujino et al. (1984b).

TABLE 6

AHH and ECD of different mouse and guinea pig tissues and inhibition by MAb 1-7-1 (Fujino et al., 1984b)*

Strain and treatment	Enzyme inhibition					
	Liver		Lung		Kidney	
	Control	+1-7-1	Control	+1-7-1	Control	+1-7-1
AHH (pmol/min/mg)						
C57BL/6						
Control	186.1	186.1 (0)	14.3	7.2 (50)	1.2	1.2 (0)
PB	854.8	820.3 (4)	18.0	10.3 (43)	1.7	1.6 (6)
MC	4882.7	714.6 (85)	141.5	24.3 (83)	57.5	7.8 (86)
DBA/2						
Control	327.6	292.8 (11)	22.2	18.4 (17)	2.2	1.6 (27)
PB	610.0	504.0 (17)	18.1	19.9 (0)	2.9	2.7 (7)
MC	283.0	276.2 (2)	44.4	21.7 (51)	1.5	1.1 (27)
ECD (pmol/min/mg)						
C57BL/6						
Control	2.46	2.10 (15)	0.18	0.17 (6)	0.12	0.12 (0)
PB	10.88	10.06 (8)	0.22	0.21 (5)	0.14	0.13 (7)
MC	13.98	6.26 (55)	0.22	0.12 (45)	0.12	0.12 (0)
DBA/2						
Control	4.81	4.41 (8)	0.67	0.63 (6)	0.34	0.35 (0)
PB	16.17	15.73 (3)	0.45	0.50 (0)	0.65	0.59 (9)
MC	4.02	3.35 (17)	0.48	0.48 (0)	0.22	0.21 (5)
AHH (pmol/min/mg)						
Guinea Pigs						
Control	281.7	208.5 (26)	68.3	56.3 (18)	4.9	1.7 (65)
PB	703.4	922.1 (0)	69.9	68.3 (2)	6.0	3.3 (45)
MC	1642.3	804.1 (51)	302.1	233.9 (23)	168.8	16.3 (90)

* Values in parentheses indicate percentages of inhibition by MAb 1-7-1. From Fujino et al. (1984b).

chrome P450s responsible for AHH and ECD in control and PB-treated rats.

In the lung and kidney, as in the liver of MC-treated rats, AHH was inhibited by 78 and 87%, respectively. In contrast to liver, the AHH and ECD in both the control and PB-treated rat lung microsomes were inhibited by 42 and 24%, suggesting that a significant part, but not all, of the activity in lung microsomes is the result of CYP 1A1, whereas a second cytochrome P450 not containing the MAb 1–7–1 epitope is responsible for the noninhibited activity (Fujino et al., 1984b). In kidney, (table 5) both AHH and ECD enzyme activities were very low in both the control and PB-treated microsomes, and, therefore, the inhibitory effects could not be adequately measured. However, in kidney microsomes from MC-treated rats there was a large induction of both AHH and an 87% inhibition by MAb 1–7–1, indicating the presence of a high level of the MAb 1–7–1 epitope-specific CYP 1A1 that is responsible for MC-induced kidney AHH. Thus, these results indicate that extrahepatic tissues, such as lung and kidney, contain cytochrome CYP 1A1 epitopically identical with that found in MC-treated liver, sensitive to inhibition by MAb 1–7–1, and responsible for AHH.

The microsomes from PB-treated and control rats also exhibit AHH activity, but it is not the result of CYP 1A1 but rather other cytochrome P450s that are insensitive to MAb 1–7–1. With ECD activity, similar results were obtained as with rat liver AHH. With ECD moderate inhibition was observed in the MC-treated microsomes, and no inhibition was observed in either the control or PB-treated microsomes. Similar results were observed

for lung, i.e., the lung showed very low ECD activity in control and PB-induced microsomes; this is in contrast to the high levels of AHH observed in lung.

In a recent study, MAb 1–68–11 (2C11/12) was found to significantly inhibit AHH and BP metabolism in microsomes from control and PB-induced rat liver, thereby implicating CYP 2C11/12 in BP metabolism in these tissues. Enzymatic insensitivity to MAb 1–7–1 (1A1/1A2) indicated that the cytochrome P450 lacked the epitope for MAb 1–7–1. In this case, the MAb 1–68–11 inhibited BP metabolism and, for the first time, identified CYP 2C11/2C12 or primary agents for BP metabolism in control and PB-induced microsomes (Todorovic et al., 1991). Table 6 shows a similar study examining AHH and ECD activity in different strains of mice, inducible C57BL/6 and noninducible DBA/2, and in guinea pigs. In each case, the MAb 1–7–1 and MAb 1–68–11 were able to define the contribution of the epitope-specific CYP 1A1 to AHH and ECD activity of liver, lung, and kidney of the control and the MC- and PB-induced rodents.

The results with the MC inducible C57BL/6 mice (table 6) were very similar to the results obtained with the MC-induced rat liver, lung, and kidney as shown in table 5. The AHH was highly inhibited in all three tissues, and the ECD was moderately inhibited (45 to 55%) in rat liver and lung by MAb 1–7–1 and not inhibited in the low activity kidney. With noninducible DBA/2, MAb 1–7–1 was only slightly inhibitory for AHH in both induced and in noninduced mice and in the other tissues except for the MC-induced lung where the inhibition was 50%. ECD activity was only negligibly inhibited in each of the tissues of DBA/2 mice by MAb 1–7–1. The liver and kidney AHH in MC-induced guinea pig was inhibited by 51 and 90%, respectively. Thus, the amount of metabolic activity can be quantitatively assigned to individual cytochrome P450s with the use of inhibitory MAbs.

Cytochrome P450-dependent AHH and ECD activities of a cloned line of human lymphoblastoid AHH-1 cells are inhibited by MAb 1–7–1 (1A1). The MAb inhibition determined that a single MAb 1–7–1-sensitive type of cytochrome P450 is responsible for all AHH expression in both the basal and benz[a]anthracene-induced cells. Partial inhibition by the MAb 1–7–1, however, indicates that at least two forms of cytochrome P450 catalyze ECD in both the basal and the induced cells. Thus, a single cloned cell line is capable of expressing two classes of cytochrome P450s, and the observed multiplicity of cytochrome P450 in animal tissues is not a result of cell heterogeneity in which each cell type expresses a different cytochrome P450. A sensitive MAb 1–7–1-based RIA also directly demonstrated the presence in these cells of an MAb 1–7–1-specific type of cytochrome P450, as well as its elevation in the induced cells (Friedman et al., 1985d).

The inhibitory MAb 1–7–1 has been productively used for reaction phenotyping of cytochrome P450 enzyme alterations observed with nutritional changes (Hietanen et al., 1987). Intestinal AHH and ECD activities in MC- and PB-induced rats fed cholesterol-free diets were examined. Cholesterol-free diets considerably decrease the cytochrome P450-catalyzed AHH and ECD activities. However, both AHH and ECD in intestine are markedly increased in rats treated with MC. In this study MAb 1–7–1 was found to inhibit both the constitutive and the MC-induced intestinal cytochrome P450-catalyzed AHH and ECD activity. This indicates that the constitutive intestinal cytochrome P450 catalyzing the inhibitable AHH and ECD contains an MAb 1–7–1-sensitive epitope identical with that present in the cytochrome P450 induced by MC in both intestine and liver.

CYP 1A1 is inhibited by MAb 1–7–1 and appears to be under both cholesterol and MC regulation in the intestine. The data indicate that CYP 1A1 is a constitutive cytochrome P450 in rat intestine responsible for both AHH and ECD and is induced by MC in intestine and liver. In contrast to the observations with intestinal AHH activities, the liver AHH in control rats and PB-treated rats was insensitive to MAb 1–7–1. Only the AHH and ECD of MC-treated rat liver microsomes were sensitive to the inhibition by MAb 1–7–1, and thus, the constitutive cytochrome P450 for catalyzing AHH and ECD is different in the liver than in the intestine.

Of interest is the finding that the feeding of cholesterol-free diets markedly decreased both AHH and ECD activities in the liver as well as in the small intestine. The highest level of enzyme activity was observed after feeding a high cholesterol diet for 1 month. A moderate change, dependent on diet, was observed for total AHH and ECD in liver. In the same animals, however, no change was observed in AHH or ECD of lung or kidney. Thus, cholesterol may modulate to a certain degree total monooxygenases activity, with the largest effect being in the intestine. Part of this modulation is reflected by a cholesterol-induced change in cytochrome P450 isozyme pattern in liver and intestine, as reflected by altered enzyme sensitivity to MAb 1–7–1 (Hietanen et al., 1987).

The AHH and ECD in the livers of woodchucks were examined with MAbs. Woodchucks infected by hepatitis virus, a virus related to hepatitis B, elevated the cytochrome P450s at the AHH locus. Thus, monooxygenases related to 1A1 were highly elevated, whereas the monooxygenases related to other cytochrome P450s were either unchanged or decreased. MAb 1–7–1 strongly inhibited AHH in the infected woodchucks. The results suggest that the hepatitis virus infection activates the AHH locus related to CYP 1A1. The MAb 1–7–1 inhibition of the AHH confirmed the identification of CYP 1A1 as the hepatitis-induced cytochrome P450 (Hietanen et al., 1989).

MAbs were also used for developmental studies of

cytochrome P450-catalyzed ECD and EROD activities. The levels of these enzymes were studied in newborn rats and rats 1 week, 2 weeks, 1 month, and 2 months of age. The rate of O-deethylation of both substrates was lowest in the newborn rats. At 1 week the ECD reached its highest value and thereafter decreased to a minimum value. The rate of EROD increase, however, was different, reaching its highest value in 1-month-old rats. The monoclonal MAb 1–7–1 (1A1/1A2) inhibited the EROD in the livers by 30%, and the MAb 2–66–3 (2B1/2B2) had no effect on activity. Surprisingly, no effect was observed of the MAb 1–7–1 on ECD, although it inhibited the metabolism of EROD (Pacifici et al., 1988a).

MAbs have been utilized to characterize the cytochrome P450 responsible for several cytochrome P450 enzyme activities expressed in a large series of different rat hepatoma cells. The contribution of each of the different cytochrome P450s to enzyme activity was determined in cell lysates or microsomes. In this study (Wiebel, 1984) nine differentiated or dedifferentiated cell lines derived from rat hepatomas were used. The hepatoma cells were examined for the expression of aldrin epoxidase, ECD, and AHH. The cells were all induced by MC or PB, and the amount of expression was measured as enzyme activity with and without the added inhibitory MAbs, 1–7–1 (1A1/1A2) and 2–66–3 (2B1/2B2). Six differentiated cell lines examined expressed CYP 2B1/2B2. MAb 2–66–3 (2B1/2B2) inhibited aldrin epoxidase by 30 to 75%, indicating the contribution of 2B1/2B2-sensitive cytochrome P450s to be 30 to 75% for aldrin epoxidase activity. Thus, the aldrin epoxidase activity was due to a PB-induced cytochrome P450 enzyme variably sensitive among the cells to the inhibition of MAb 2–66–3.

Most of the cell lines induced by PB showed marked ECD inhibition by MAb 2–66–3. The MAb 2–66–3, however, had a far weaker affect on AHH of these cells. In contrast MAb 1–7–1 inhibited the MC-induced AHH and ECD in two dedifferentiated cell lines by 50 to 80%, indicating that 50 to 80% of the AHH in these lines were catalyzed by CYP 1A1/1A2. The MAb 1–7–1 also inhibited AHH in poorly differentiated lines by 40 to 65%. These results show that hepatoma cells that express various amounts of either PB-inducible enzyme activities or MC-inducible cytochrome P450s can be analyzed for the nature of the cytochrome P450 responsible for a specific amount of enzyme activity by the addition of inhibitory epitope-specific MAbs. Thus, the MAbs are easily applied to the examination of xenobiotic and endobiotic metabolism in a variety of cell types and under different regulational conditions (Wiebel et al., 1984).

Four MAbs to PB-induced CYP (2B1/2B2) show different patterns of inhibition of PB-cytochrome P450-catalyzed AHH, ECD, benzphetamine demethylase, and ethylmorphine demethylase. The inhibition constants vary depending on the individual MAb and the individual substrate. Two of the four MAbs completely inhibit the reduction of cytochrome P450 by NADPH cytochrome *c* (P450) reductase. The same cytochrome P450 bound to carbon monoxide, however, can be reduced chemically by sodium dithionite in the presence of the MAb. These data indicate that the two MAbs examined completely prevent electron transfer by NADPH cytochrome *c* (P450) reductase. Substrate binding is partially inhibited by the MAb. The type I substrate-binding spectrum of benzphetamine is inhibited more than the type II-binding spectrum on aniline. The degree of inhibition of the substrate binding, as indicated by the spectrum, is less than that observed for the inhibition of catalytic enzyme activity by the MAbs. The data indicate that each of the MAbs is directed toward different epitopes on the cytochrome P450s with different relationships to the active catalytic site (Fujino et al., 1990).

Two studies utilized MAbs to examine the role of specific cytochrome P450s in the metabolism of AAF. In one of these studies, a series of MAbs was used to examine the effect on AAF metabolite formation at specific positions, i.e., 7-hydroxy, 9-hydroxy, 5-hydroxy, 3-hydroxy, 1-hydroxy, and N-hydroxy AAF. In addition, AHH was used as a positive control for the CYP 1A1 enzyme activity. Two of the 10 MAbs, 1–7–1 (1A1/1A2) and 1–32–2 (1A1), inhibited AAF hydroxylation. The effect of the MAbs on MC-induced rat microsomal AAF metabolism was also examined. The formation of 7-hydroxy, 5-hydroxy, 3-hydroxy, 1-hydroxy, and N-hydroxy AAF was inhibited by the inhibitory MAbs 1–7–1 and 1–31–2, and the metabolism at each position was catalyzed by the same epitope-specific cytochrome P450. Thus, positional specificity of individual cytochrome P450-catalyzed metabolism can be determined with inhibitory MAbs (Thorgeirsson et al., 1983). The MAb 1–31–2 was inhibitory at the time of this study. Since then, however, through many passages the MAb 1–31–2 has lost inhibitory activity (see table 1).

AAF was added to the diet, and MAbs were used to determine the content of epitope-specific cytochrome P450-dependent liver microsomal and nuclear metabolism of AAF. This study utilized both MAb and polyclonal antibodies to CYP 1A1 and CYP 1A2. CYP 1A2 was immunodetected in liver microsomes, the nuclear envelope, and nuclei from untreated rats; CYP 1A1 was not detected in any of the same preparations. The levels of 1A1 and 1A2, however, were elevated after a diet of AAF for a period of 1 or 3 weeks. Supplementation of the AAF diet with butylated hydroxytoluene protects against AAF hepatocarcinogenesis in high fat-fed rats. Butylated hydroxytoluene enhanced AAF-dependent induction of CYP 1A1 but not of CYP 1A2. The study provided additional evidence that cytochrome P450 regulation in the nuclear envelope is independent of that in microsomes and suggests that regulation of cytochrome

P450 may play a crucial role in the nutritional modulation of AAF carcinogenesis (Friedman et al., 1989).

Induction by MC pretreatment results in a large increase of AHH in the livers of rats and C57 mice. MAb 1–7–1 (1A1/1A2) inhibits 70 to 80% of AHH activity in both the MC-induced C57 mouse and rat. MAb 1–7–1, however, has no effect on AHH in control or PB-treated rats or mice. Thus, the induced AHH is sensitive to MAb 1–7–1 and a function of 1A1/1A2. Thus, the AHH activity in the microsomes of control and PB-treated must be a function of a cytochrome P450 other than 1A1 because it is not inhibited by MAb 1–7–1 (see above).

Another MAb, 1–68–11 (2C11/2C12), was prepared to constitutive rat CYP 2C11 (2c/RLM5) from control male rat livers (Park et al., 1989). This MAb shows strong inhibitory effects on BP hydroxylation in both control and PB-treated rats. It exhibits only negligible inhibitory activity toward the AHH induced in rat liver by MC treatment. Thus, BP metabolism and AHH activity are a function of 2C11/12 rather than of 1A1 in control and PB-treated rat liver.

A study (Todorovic et al., 1991) in which various metabolites of BP were isolated after incubation with rat liver microsomes yielded three BP dihydrodiols, three BP phenols, and BP quinones. MAb 1–68–11 (2C11/2C12) was found to exhibit positional specificity in its inhibitory effects. MAb 1–68–11 inhibited BP 9,10-dihydrodiol formation by 80% in liver from untreated male rats. In livers from untreated female rats, the inhibition was 100%. BP 7,8-dihydrodiol was inhibited from 38 to 77% in males and 50% in the females. On the other hand, the formation of 4,5-dihydrodiol, located on the opposite side of the BP molecule from the 7,8 and 9,10 positions, was enhanced by 80% in microsomes from MC-treated rats. No inhibition by MAb 1–68–11 was observed in MC-treated rats for either BP metabolism or BP binding to DNA. In contrast, the binding of BP to DNA was completely abolished by MAb 1–68–11 in microsomes from control rats and inhibited 70% in PB-induced liver microsomes. Thus, MAb 1–68–11 (2C11/2C12) inhibits regiospecifically the metabolism of BP at different positions. This MAb, 1–68–11, was made to rat CYP 2C11, and it is likely that it may cross-react with other 2C family cytochrome P450s, e.g., the female-specific 2C12.

The metabolism of commonly used drugs has also been reaction phenotyped with the use of inhibitory MAbs. The metabolism of aminopyrine, antipyrine, and theophylline were studied with MAb 1–7–1 (1A1/1A2) and MAb 2–66–3 (2B1/2B2) (Slusher et al., 1987). The addition of either of the latter MAbs to liver microsomes from untreated rats had only a negligible effect on the metabolism of aminopyrine, antipyrine, and theophylline, suggesting that the metabolism of these drugs in the untreated animal are not due to 1A1/1A2 or 2B1/2B2. However, in microsomes from MC- and PB-treated rats the two MAbs differentially inhibited individual pathways of metabolism. For example, at 20 mM 4-aminopyrine, 55% of aminopyrine metabolism resulted from the action of cytochrome P450 isozymes that were not inhibited at 4 mM aminopyrine substrate concentration and 4-methylaminopyrine formation at both low and high substrate concentrations. The results of this study indicated that there were several pathways of metabolism of the drug, some of which were inhibited by both MAb 1–7–1 (1A1/1A2) and MAb 2–66–3 (2B1/2B2). Other pathways were inhibited by the MAb 2–66–3 only, and still others were inhibited by MAb 1–7–1 only. Some metabolic pathways were unaffected by the addition of either class of MAb. These results indicate that these drugs are metabolized by a variety of different cytochrome P450s, some of which are 1A1/1A2 and 2B1/2B2. An important finding was that the cytochrome P450s that function at 20 mM substrate concentration are different from those that function at 4 mM substrate concentration. This study indicates a greater heterogeneity of cytochrome P450s responsible for the metabolism of these drugs than previously believed (Slusher et al., 1987).

The MAbs have been useful in examining the role of cytochrome P450s in the metabolism of environmental chemicals. Various inducers of cytochrome P450 including acetone, PB, and MC were given to rats, and the pharmacokinetics of styrene metabolism were examined (Elovaara et al., 1991). Styrene is converted to phenylglyoxylic acid, the enantiomers of mandelic acid, and thioethers. The amount of these metabolites excreted in the urine was measured. After MC was administered, styrene metabolism in liver measured in vitro was increased by 140%; pretreatment with acetone plus styrene increased metabolism by 190%, MC pretreatment plus styrene increased metabolism by 180%, and PB plus styrene increased metabolism by 250%. MAb 1–7–1 (1A1/1A2), MAb 2–66–3 (2B1/2B2), MAb 1–91–3 (2E1) were used in conjunction with Western blots to identify the various cytochrome P450s induced by the above treatments. Use of these MAbs and the high inhibitory activity of MAb 1–91–3 (2E1) indicated clearly that styrene inhalation primarily induced CYP 2E1. Styrene, given by gavage at a high narcotic dosage, induced both CYP 2E1 and CYP 2B1/2B2. The study showed that styrene metabolism was autoinduced by styrene and also induced by other foreign compounds, all of which induced primarily CYP 2E1, as measured by N-nitrosomethylamine N-demethylase activity, and to a lesser extent CYP 2B1/2B2, which was measured by its PROD activity (Elovaara et al., 1991).

In a related study MAbs were used to characterize the cytochrome P450s responsible for toluene metabolism in rat liver. Six inhibitory MAbs, 1–7–1 (1A1/1A2), 2–66–3 (2B1/2B2), 4–7–1 (2B1/2B2), 4–29–5 (2B1/2B2), 1–68–11 (2C11/2C12), and 1–91–3 (2E1), were used to measure the contribution of each of the various cytochrome P450s to toluene metabolism. Toluene is metabolized at the

methyl group to BA as well as by ring hydroxylation to *o*- and *p*-cresol. MAb 1–7–1 inhibited *o*-cresol formation in MC-treated rats only. MAbs 2–66–3 (2B1/2B2), 4–7–1 (2B1/2B2), and 4–29–5 (2B1/2B2) all strongly inhibited BA, *o*-cresol, and *p*-cresol formation in PB-induced microsomes only. MAb 1–68–11 (2C11/2C12) inhibited BA formation in PB-induced microsomes and only at a high toluene concentration. MAb 1–91–3 (2E1) inhibited BA formation at a low, but not high, toluene concentration. Different pretreatments of the animals modified the inhibitory response. These treatments included MC, ethanol, and PB with and without fasting. The results indicated that the CYP 2E1 and CYP 2C11 are constitutive isozymes that are primarily responsible for the formation of BA, *p*-cresol, and *o*-cresol. Furthermore, PB-induced CYP 2B1/2B2 contributes to the formation of all three metabolites, whereas MC-induced 1A1/1A2 forms primarily *o*-cresol from toluene. The MAb-based study was also able to distinguish the sensitivity to MAbs of the high and low K_m enzymes (Nakajima et al., 1991).

Pyrazole was found to be different from acetone or ethanol as an inducer of the cytochrome P450 system in mice. Evidence was presented that pyrazole-induced cytochrome P450 Coh is different from acetone-inducible CYP 2E1. MAb 1–98–1 (2E1) detected 2E1 on a Western blot but did not detect cytochrome P450 Coh. Furthermore, ethanol and acetone did not induce cytochrome P450 Coh-catalyzed coumarin 7-hydroxylase, whereas pyrazole strongly increased the enzyme in DBA mice (Honkakoski et al., 1988).

Benzene is another environmental agent metabolized by cytochrome P450. The MAbs were used to study the contribution of different cytochrome P450 isozymes to the metabolism of benzene in liver microsomes from fed, fasted, pyrazole-treated, PB-treated, and ethanol-treated rats. Controls were isocaloric, and the inhibitory effects on metabolism in microsomes were studied with MAb 1–7–1 (1A1/1A2), MAb 2–66–3 (2B1/2B2), and MAb 1–91–3 (2E1). MAb 1–7–1 failed to inhibit benzene metabolism in any of the rats, whereas MAb 2–66–3 inhibited benzene metabolism only in PB-induced microsomes at a high concentration of benzene. MAb 1–91–3 (2E1) inhibited benzene metabolism in liver microsomes from fed isocaloric controls and PB-, ethanol-, and pyrazole-treated rats. The degree of inhibition by 1–91–3 (2E1) of the variously treated rats was as follows: fed = isocaloric control = PB < fasted < pyrazole = ethanol. The pattern of inhibition by MAb 1–91–3 was examined at a low benzene concentration of 0.23 mM and a high benzene concentration except in the PB-induced microsomes. Western blot analysis indicated that MAb 1–7–1 did not detect any protein in the region of cytochrome P450s, whereas MAb 2–66–3 detected a band only in liver microsomes from PB-treated rats. MAb 1–98–1 (2E1) detected a band in microsomes from all treated groups. The thickness of the bands was in the order of PB = isocaloric control < fed < fasted < pyrazole < ethanol. Thus, the study indicates that CYP 2B1/2B2 contributes to benzene metabolism in PB-treated rats and CYP 2E1 metabolizes benzene in the variously treated rats described above. The PB-induced 2B1/2B2 has a low affinity for benzene. CYP 2E1 has a high affinity for benzene and is induced by 1-day fasting, pyrazole, and ethanol treatment but decreased by PB treatment. This study clearly identified CYP 2E1 as the major cytochrome P450 responsible for benzene metabolism. Benzene is a compound commonly used in industry and is present in the environment, thereby having the potential for large-scale population exposure (Nakajima et al., 1989, 1990).

In a related study, MAbs were used to measure the contribution of 1A1/1A2, 2B1/2B2, 2C11/2C12, and CYP 2E1 to the metabolism of benzene, EROD and PROD in liver microsomes from fed, 1-day fasted, and PB-, MC-, and ethanol-treated rats. Enzyme activity varied with the pretreatment and the induction of the various cytochrome P450s. MAb 1–91–3 (2E1) had no influence on EROD dealkylation but did inhibit benzene hydroxylase in a manner similar to that described above (Nakajima et al., 1990); the amount of inhibition increased with the inducibility of the enzyme activity. The degree of induction was in the following order: MC, PB < fed < fasted < ethanol. MAb 2–66–3 (2B1/2B2), MAb 4–7–1 (2B1/2B2), and MAb 4–29–5 (2B1/2B2) had no affect on EROD but inhibited the activities of high-K_m benzene hydroxylase and PROD. MAb 1–7–1 (1A1/1A2) inhibited benzene hydroxylase formation by 58%, EROD by 79%, and PROD by 50% and the high-K_m benzene hydroxylase by 42% in microsomes form MC-treated rats. The MAb 1–68–11, which inhibits 2C11/2C12 and possibly other related 2C family cytochrome P450s, inhibited EROD, PROD, and high-K_m benzene hydroxylase activity. Thus, the CYP 2E1 is active for benzene metabolism at a low K_m but not active in EROD metabolism. 2B1/2B2 was responsible for a major part of PROD metabolism and also, selectively, for benzene hydroxylation at high benzene concentrations. 1A1/1A2 contributed primarily to EROD metabolism and only slightly to PROD and high-K_m benzene hydroxylase activity. CYP 2C11/2C12 contributed slightly to high-K_m benzene hydroxylase and to alkoxyresorufin metabolism. This study used MAbs to five different cytochrome P450s to demonstrate the contribution of each of the cytochrome P450s, to benzene hydroxylation, under different conditions, EROD, and PROD activity (Nakajima et al., 1990).

The cytochrome P450 pattern induced by 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene and pyrazole were compared to the pattern induced by PB with the use of MAbs. Earlier studies showed that 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene was 650 times more potent than PB as an inducer of the 2B1/2B2 and other cytochrome P450s, although the inducers elicited the same profile of cytochrome P450s. Pyrazole was also a potent and selec-

tive inducer of coumarin 7-hydroxylase in the DBA/2 mice. Different strains of mice were used in which different cytochrome P450 enzymes were induced. This study determined the effect of MAb 2–66–3 (2B1/2B2) on coumarin 7-hydroxlase and PROD. MAb 2–66–3 did not affect coumarin hydroxylase but inhibited 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene-induced activity. The MAbs 1–7–1 and 2–66–3 were able to distinguish between the different types of inducers with respect to a CYP 1A2 and CYP 2B1/2B2 enzyme activity. With Western blots MAb 2–66–3 detected protein that migrated with CYP 2B1/2B2 obtained from PB-treated rat liver. This cytochrome P450 protein was greatly increased in 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene-treated mice (Raunio et al., 1988).

Four monoclonal antibodies to individual P450s were tested and only the MAb 2-13-2 against PCN-inducible P450 3A1 was immunodetected in chicken liver microsomes. This protein migrated identically with the PCN-inducible P450 detected in microsomes from dexamethasone-treated rats. It was most predominant in liver microsomes from chickens 1 day posthatching, whereas much lower levels were observed in the embryo and at 36 days posthatching. Phenobarbital and dexamethasone were both effective inducers of 3A1. Chicken liver microsomal erythromycin demethylase, a characteristic activity of rat PCN-inducible P450 3A1, was similar in developmental profile and induction to the MAb 2-13-2-sensitive protein. Aldrin epoxidase, benzphetamine demethylase, ethylmorphine demethylase, and aminopyrine demethylase were more similar to each other in development and induction and less correlated with the 3A1. This evidence for PCN-inducible P450 3A1 in chickens agrees with sequence information that suggests the early evolution of this form and demonstrates the suitability of the chicken for studies of P450 evolution (Lorr et al., 1989).

MAbs have also been used to examine the cytochrome P450-based metabolism of fusarin C, a potent mutagen present on *Fusarium moniliforme*-contaminated corn. The compound requires metabolic activation, and microsomes from PB-induced rats are most effective for the mutagen activation. Inhibition of the simultaneously induced esterase of the microsomes yields a less mutagenic metabolite. As a positive control, MAb 2–66–3 (2B1/2B2) inhibited the O-demethylation of *p*-nitroanisole and aflatoxin B_1 mutagenicity. MAb 2–66–3, however, had no affect on fusarin C mutagenicity. Thus, enzymes involved in the fusarin C mutagenicity differ from those involved in the demethylation of *p*-nitroanisole and aflatoxin B_1 mutagenic activation. and, hence, fusarin C mutagenicity is not due to activation by CYP 2B1/2B2 (Lu et al., 1989).

Nitrofluoranthenes are common environmental contaminants that are carcinogenic. Both fluoranthene and nitrated nitrofluoranthene, as well as a mixture of nitrofluoranthenes, induced hepatic and pulmonary cytochrome P450s. These compounds all induce AHH and EROD. The isozymes induced by these agents were characterized by Western blot analysis with MAb 1–7–1 (1A1/1A2) and MAb 2–66–3 (2B1/2B2). The hepatic microsomes from 3-nitrofluoranthene- and nitrofluoranthene-treated rats showed two distinct immunoprecipitin bands with MAb 1–7–1, whereas microsomes from fluoranthene-treated rats showed a sharp single band with MAb 2–66–3. MAb 1–7–1 strongly inhibited AHH activity (80%) induced by 3-nitrofluoranthene and nitrofluoranthenes. On the other hand, fluoranthene-induced activity was only moderately inhibited (30%) by MAb 1–7–1. High-performance liquid chromatography analysis indicated that phenol formation in the 3-nitrofluoranthene- and nitrofluoranthene-induced rats was inhibited by 60 to 75% and BP 7,8-diol formation by 52 to 60% by MAb 1–7–1. These studies suggested that fluoranthene treatment results in an increase in an MAb 2–66–3-sensitive cytochrome P450, i.e., CYP 2B1/2B2 and the nitrated compound nitrofluoranthene and a mixture of nitrofluoranthenes induce the MAb 1–7–1-sensitive 1A1/1A2. Thus, subtle differences in inducer structure yield induction of different cytochrome P450s that can be identified with the MAbs (Khan et al., 1987).

A series of studies on cytochrome P450-catalyzed metabolism in rat skin used MAb 1–7–1 (1A1/1A2) and MAb 2–66–3 (2B1/2B2) to investigate the metabolism of various compounds in skin and to identify individual cytochrome P450s responsible for specific substrate metabolism. MC, topically applied to rat skin, resulted in a large induction of AHH, ECD, and EROD in the microsomes of the epidermis. An RIA of epidermal microsomes from untreated control rats showed a significant binding of $S^{35}32S$?-MAb 2–66–3 to skin protein. Relatively little binding of $S^{35}32S$?-MAb 1–7–1 was observed in the skin of uninduced rats. On the other hand, high binding was observed with $S^{35}32S$?-MAb 1–7–1 with epidermal microsomes prepared from MC-treated rats. However, histochemical staining of the epidermis from control rats showed no immunoreactivity with MAb 2–66–3 or MAb 1–7–1. The lack of immunohistochemical reactivity with MAb 2–66–3 is inconsistent with its reactivity in the RIA. MAb 1–7–1 showed immunohistochemical binding to epidermis from MC-treated animals but MAb 2–66–3 did not. Western blot analysis of control epidermal microsomes failed to yield any band with either MAb 1–7–1 or MAb 2–66–2, whereas preparations from MC-treated rats showed a distinct immunoreactive band with MAb 1–7–1. MAb 2–66–3 inhibited AHH and ECD activity 40 to 50% in microsomes from control animals. This is a somewhat surprising result because 2B1/2B2 is generally thought not to be present in epidermis. MAb 1–7–1 inhibited AHH, EROD, and ECD from MC-treated rat epidermis by 70 to 80%. The same enzyme activities in control rats were inhibited to a considerably lesser degree

by MAb 1–7–1. The results demonstrated that the MAb 1–7–1-detected 1A1/1A2 is clearly present at lower levels in control rats and is highly inducible by MC in rat epidermal tissue (Khan et al., 1989b).

Crude coal tar is often applied to skin for therapeutic purposes. A single application of crude coal tar was applied to the skins of neonatal rats and was found to induce both epidermal and hepatic cytochrome P450-dependent enzyme activities. MAb 1–7–1 (1A1/1A2), MAb 2–66–3 (2B1/2B2), and MAb 1–98–1 (2E1) were used to characterize the nature of the cytochrome P450s induced by crude coal tar treatment. The crude coal tar-treated rats showed a significant increase in the cytochrome P450 region in gel electrophoresis. Western blot analysis of epidermal and hepatic microsomes with MAb 1–7–1 showed a heavily stained band in both liver and epidermal tissue. The MAb 2–66–3, however, showed immunoreactivity only with hepatic microsomes. Furthermore, crude coal tar treatment resulted in a suppression of immunoreactivity of MAb 1–98–1 with hepatic microsomes, suggesting that the crude coal tar treatment suppresses the formation of CYP 2E1. Epidermal and hepatic microsomes from the crude coal tar-treated rats both showed increases in BP metabolism. The increase in BP metabolism was inhibited by both MAb 1–7–1 and MAb 2–66–3 in both the hepatic and epidermal tissues; MAb 1–7–1 was more inhibitory than the MAb 2–66–3. This result is as expected because the MAb 2–66–3 binds to CYP 2B1/2B2 which has far less BP-metabolizing activity. This result suggests, however, that there is a low level of 2B1/2B2 in epidermis. The topical application of therapeutic doses of crude coal tar results in the induction of specific CYP 1A1/1A2 in the epidermis and both CYP 2B1/2B2 and 1A1/1A2 in liver. This induction by crude coal tar was paralleled by a suppression of the level of CYP 2E1 (Khan et al., 1989c). These findings may be relevant to crude coal tar treatment for therapeutic purposes.

Another commonly used drug in the treatment of certain skin diseases is 8-methoxypsoralen which is metabolized by liver microsomes of CD-1 mice. The metabolites formed are covalently bound to microsomal protein in control mice and in mice pretreated with PB or BNF. In BNF-pretreated mice, MAb 1–7–1 (1A1/1A2) inhibited the metabolism of 8-methoxypsoralen by 57% and 8-methoxypsoralen covalent binding to microsomal protein by 40%. No effect of MAb 1–7–1 was observed on the metabolism of 8-methoxypsoralen in microsomes from PB-pretreated mice or control mice. MAb 2–66–3 (2B1/B2) enhanced the covalent binding of 8-methoxypsoralen metabolites to microsomal protein in control mice by 64% and in PB-pretreated mice by 44% without affecting the disappearance of 8-methoxypsoralen. Preincubation of liver microsomes from BNF-pretreated mice with 8-methoxypsoralen decreased the activity of ECD. Preincubation with 8-methoxypsoralen for 10 minutes decreased the V_{max} from 3.4 to 1.2 nmol/min/mg protein and increased the K_m from 46 to 90 mM. Cysteine trapped three-fourths of the reactive intermediates of 8-methoxypsoralen but was ineffective in preventing the irreversible inhibition of ECD or the 45% loss of cytochrome P450 as measured spectrally. There was no spectral evidence that 8-methoxypsoralen converted cytochrome P450 to cytochrome P420 or to metabolite-intermediate complexes with cytochrome P450. These studies support the hypothesis that irreversible inactivation of cytochrome P450 by 8-methoxypsoralen is caused by a modification of the apoprotein by reactive metabolites. The MAb studies suggest that CYP 1A1/1A2 is responsible for about 50% of this inactivation. The stimulating effect of MAb 2–66–3 may be due to inhibition of a cytochrome P450 metabolizing the reactive intermediate to an inactive form (Mays et al., 1990).

Another drug that is used in the treatment of dermatological disease is clotrimazole, a nitrogen-substituted imidazole that is used as an antifungal agent. It both inhibits and induces cytochrome P450. In this study the isozyme spectrum induced by clotrimzole was examined with the use of MAbs. Clotrimazole administered for 4 days increased the overall level of cytochrome P450 in the liver by 86%. The microsomes were assayed for ECD, aminopyrine demethylase, benzphetamine demethylase, *p*-nitrophenol hydroxylase, and EROD, which were all significantly induced by clotrimazole; AHH surprisingly remained unchanged. Western blot analysis of microsomes from clotrimazole-treated animals with MAb 2–66–3 (2B1/2B2) and MAb 1–98–1 (2E1) revealed strong bands, whereas a moderate band was observed with MAb 2–13–1 (3A1). These results are consistent with the results obtained with the inhibitory effects of the MAbs on the various enzyme activities. MAb 2–66–3 (2B1/2B2) significantly inhibited ECD by 45%, whereas MAb 1–7–1 inhibited EROD by 30%. The MAb-based studies suggest that clotrimazole is an inducer of several different cytochrome P450 enzymes in rat liver which are similar to those cytochrome P450s induced by PB, PCN, and ethanol (Khan et al., 1989a).

The role of various forms of cytochrome P450 in the activation of compounds to their mutagenic forms was studied with two MAbs, 1–7–1 (1A1/1A2) and 2–66–3 2B1/2B2. These MAbs inhibited the metabolism of various carcinogens and xenobiotics as well as some endogenous compounds in two strains of mice, the C57BL/6 (B6) and the DBA/2 (D2). The effect of the MAbs on the S9-mediated mutagenicity of aflatoxin, BP 7,8-diol, AAF, and N-nitrosomorpholine in the Ames test (Ames et al., 1973) was examined. In parallel studies, the effects of the same MAbs were examined for AHH, ECD, EROD, aminopyrine N-demethylase, and testosterone 6β-, 7α- and 16β- hydroxylases. With the S9 preparations, which contain microsomes as the activating agent, the MAb 1–7–1 (1A1/1A2) inhibited only the enzyme activities of

the MC-inducible cytochrome P450s, those that are predominantly responsible for AHH and ECD, and the mutagen activation of AAF and BP 7,8-dihydrodiol. In contrast, the MAb 2–66–3 inhibited the PB-inducible enzymes aminopyrene N-demethylase and testosterone 6β-, 7α-, and 16β-hydroxylase, and aflatoxin B mutagenicity. Thus, the cytochrome P450s largely responsible for mutagen activation of AAF, BP 7,8-dihydrodiol, and aflatoxin B1 were identified by their inhibition by the two MAbs. The MAb 2–66–3 also inhibited testosterone 7α-hydroxylase of PCN-treated B6 mice. MAb 1–7–1 did not inhibit N-nitrosomorpholine mutagenicity, but the latter was increased by MAb 2–66–3 by 2- to 6-fold. Thus, when these two MAbs were used, it was possible to measure the quantitative contribution of epitope-specific single cytochrome P450 or classes of cytochrome P450 responsible for both metabolic reactions and mutagen activation (Hietanan et al., 1986).

In a related study from the same laboratory, the mutagenic activation of N-nitroso, N-benzyl-methylamine, N-benzyl-N-nitrosourea, and N-methyl-N-nitrosourea was investigated with MAbs and the Ames test. MAb 1–91–3 (2E1) did not inhibit the N-demethylation of N-benzyl-methylamine at either low or high substrate concentration, thus ruling out an activation role for CYP 2E1. MAb 1–7–1 (1A1/1A2) causes only a slight inhibitory effect on this activation to mutagenic metabolites, indicating that CYP 1A1/1A2 contributed only a small amount to the activation. Debenzylation was catalyzed primarily by CYP 1A1/1A2 at high N-benzyl-methylamine concentrations, and MAb 2–66–3 enhanced the mutagenicity of N-benzyl-methylamine up to 17-fold at a low substrate concentration. This antibody, however, was without effect at high substrate concentrations. An interesting observation was a 100% glutathione-dependent reduction of N-benzyl-methylamine mutagenicity with liver S9 from untreated Wistar rats. The mechanism of stimulation by MAb 2–66–3 is not known but may be due to the inhibition of a form of cytochrome P450 that is very active in detoxification, thereby increasing the amount of substrate available for mutagen activation (Lin et al., 1990).

The MAbs have also been successfully used to characterize the rat hepatic cytochrome P450s that catalyze ω-1 and ω-2 hydroxylation of prostaglandin. In earlier studies, MC induction caused a 10-fold increase in ω-2 hydroxylation of PGE_2. In the MAb-directed study, a major portion of the mechanism of the ω-2 hydroxylation was determined by identifying the responsible cytochrome P450 form. Another related question was whether the same cytochrome P450 enzyme catalyzed the reaction in control microsomes. The three MAbs, 1–7–1 (1A1/1A2), 1–31–2 (1A1), and 1–36–1 (1A1), all raised against the major liver cytochrome P450 (1A1) from MC-treated rats, were used. MAb 1–7–1 (1A1/1A2) inhibited ω-2 PGE_2 hydroxylation and ω-1 PGE_2 hydroxylation in liver microsomes from MC-treated rats by 70 and 45%, respectively. Furthermore, the MAbs 1–31–2 (1A1) and 1–31–1 (1A1), known not to be inhibitory, were also not inhibitory in this system. MAb 1–7–1, however, did not inhibit the ω-2 PGE_2 hydroxylation in microsomes from control or PB-treated rats, indicating that the CYP 1A1/1A2 catalyzing the hydroxylation of ω-2 PGE_2 is different in control and PB-treated rats than the major CYP 1A1/1A2 in MC-induced animals. MAb 1–7–1 binds and inhibits both 1A1 and 1A2.

The MAbs 1–31–1 and 1–36–1 bind to 1A1 on a Western blot but do not inhibit the enzyme. The question of whether 1A1 or 1A2 was responsible for μ-2 PGE_2 metabolism was resolved by immunoaffinity-isolation of CYP 1A1 using immunopurification with MAb 1–31–2. The isolated MAb 1–31–2-bound purified enzyme was supplemented with NADPH-cytochrome P450 reductase and shown to catalyze ω-2 hydroxylation to a large extent and ω-1 hydroxylation to a lesser extent. No activity was observed in the absence of reductase. Thus, immunopurification directed by MAb 1–31–2 yields a purified CYP 1A1 complex that can be reconstituted with reductase and exhibits hydroxylase activity. The reconstituted cytochrome P450 composed of CYP 1A1 and reductase purified by conventional means, hydroxylated PGE_2 at the ω-2 sites at a ratio of 2.8, similar to that obtained with the CYP 1A1 MAb complex. These studies clearly demonstrate that a major portion of the ω-2 hydroxylation of prostaglandins present in MC microsomes are catalyzed by CYP 1A1. Other purified cytochrome P450s, such as 2C11, from untreated control rat liver also catalyze the ω-2 hydroxylation of PGE_2. The study shows that all the ω-2 hydroxylation by cytochrome P450s in control microsomes and 25% of the activity in MC microsomes are not inhibited by a cytochrome P450 sensitive to MAb 1–7–1 and thus are not catalyzed by 1A1 or 1A2 (Holm et al., 1989).

MAbs were used for a mechanism-based study of the effect of various 4-alkyl analogs of DDC which inactivated cytochrome P450 by destroying the heme prosthetic group. This study used MAbs to identify the individual cytochrome P450 that is sensitive to this inactivation. The study was done with Western blots and by utilizing two MAbs to CYP 1A1, MAb 1–31–2 and MAb 1–36–1, and to CYP 3A1 MAb 2–13–1 (3A1). The MAb 1–31–2 and MAb 1–36–1 reacted with cytochrome P450s from β-nitrofluoranthene-treated rats following DDC analog treatment. In microsomes from dexamethasone-treated rats, DDC analogs caused the formation of higher molecular mass protein (80, 94, and 115 kDa), showing an immunoreactivity with MAb 2–13–1, directed against CYP 3A1, a major dexamethasone-inducible isozyme. These immunochemical findings were supported by the fact that DDC analogs also inhibited the activity of CYP 1A1-catalyzed EROD activity and erythromycin N-demethylase of CYP 3A1 but not any of the the major

PB-inducible cytochrome P450 enzymes such as PROD. The study demonstrates the usefulness of combining MAbs for mechanism studies. Thus, CYP 1A1 and CYP 3A4 are targets for mechanism-based inactivation by DDC analogs, and the MAbs were successfully used to determine the nature of the form of cytochrome P450s inhibited by DDC (Riddick et al., 1989).

VII. Monoclonal Antibodies for Analyses of Human Tissues

A. cDNA-expressed Human and Rodent Cytochrome P450s for Analysis of Monoclonal Antibody Specificity

The determination of cross-reactivity of MAbs with human cytochrome P450s has been a difficult task, given the relative unavailability of the human enzymes obtained by classical purification. The cDNA expression of human and rodent cytochrome P450s is easily accomplished and the presence in individual cytochrome P450s of MAb-specific epitopes can be determined immunologically. Table 7 shows the cross-reactivity of 13 MAbs, 12 to rat cytochrome P450s and one to a scup (fish) cytochrome P450 with 12 vaccinia cDNA-expressed human, mouse, and rat cytochrome P450s (Goldfarb et al., 1993). The cross-reactivity was considered positive if either the ELISA or Western blot was positive. The rat MAbs 1–36–1 (CRP 1A1), 1–31–2 (CRP 1A1), and 1–7–1 (CRP1A1/2) and the fish scup 1–12–3 (E 1A1) all cross-reacted with cDNA expressed CRP 1A1. The MAb 1–7–1 also cross-reacted with mouse CRP 1A2. The MAb 1–12–3 to CRP E(1A1) scup also reacted with human CRP 2E1 which was an unexpected result. The two MAbs to rat CRP 2B1/2B2, MAb 4–7–1 and MAb 2–66–3, cross-reacted with rat CRP 2A1 and CRP 2B1. Two other MAbs that cross-reacted with CRP 2B1/2B2, MAb 4–29–5 and MAb 2–8–1, made to rat CRP 2B1/2B2 reacted less strongly to rat CRP 2B1. MAb 1–68–11 made to rat CRP 2C11 cross-reacted with human CRP 2C9. Also, MAbs 1–91–3 and 1–98–1 made to rat CRP 2E1 cross-reacted with human CRP 2E1. The MAbs 2–3–2 and 2–13–1 made to rat 3A1 gave strong Western blots with cDNA-expressed human CRP 3A4. The use of cDNA-expressed cytochrome P450s can be applied to the analysis of cross-reactivity of MAbs made to different cytochrome P450s of different species and thus clarify the usefulness of each MAb for cross-species studies. The results with the human cDNA-expressed cytochrome P450s is especially interesting because it represents the first major method for examining the immunological reactivity of human cytochrome P450s with MAbs.

B. Monoclonal Antibody Analyses of Cytochrome P450s in Human Tissue

MAbs are uniquely suited for the analyses and characterization of individual cytochrome P450s in human tissues with detection, immunohistochemical, or immunoinhibition methods. The MAbs can be used for the measurement of the metabolic contribution of epitope-specific single or classes of cytochrome P450 in human tissues. The success of these MAb- directed analytical approaches has been amply demonstrated with animal

TABLE 7

*Immunocross-reactivity of 10 vaccinia cDNA-expressed human, rat, and mouse P450s with 13 MAbs raised to individual P450s (Goldfarb et al., 1993)**

Vaccinia cDNA Expressed P450	Immuno Assay	Cytochrome P450												
		Rat 1A1	Rat 1A1	Fish 1A1	Rat 1A1/2	Rat 2B1/2	Rat 2B1/2	Rat 2B1/2	Rat 2B1/2	Rat 2C11	Rat 2E1	Rat 2E1	Rat 3A1	Rat 3A1
		Monoclonal Antibodies												
		1-36-1	1-31-2	1-12-3	1-7-1	4-7-1	2-66-3	4-29-5	2-8-1	1-68-11	1-91-3	1-98-1	2-3-2	2-13-1
M1A1	ELISA	+++	+	+	+++	–	–	–	–	–	–	–	–	–
	WB	++	+	+++	+++	–	–	–	–	–	–	–	–	–
M1A2	ELISA	–	–	–	++	–	–	–	–	–	–	–	–	–
	WB	–	–	–	+	–	–	–	–	–	–	–	–	–
H1A2	ELISA	–	–	–	–	–	–	–	–	–	–	–	–	–
	WB	–	–	–	–	–	–	–	–	–	–	–	–	–
R2A1	ELISA	–	–	–	–	+	++	–	–	–	–	–	–	–
	WB	–	–	–	–	–	–	–	–	–	–	–	–	–
R2B1	ELISA	–	–	–	–	+++	++	+	+	–	–	–	–	–
	WB	–	–	–		–	+++	+	+	–	–	–	–	–
H2B6	ELISA	–	–	–	–	–	–	–	–	–	–	–	–	–
	WB	–	–	–	–	–	–	–	–	–	–	–	–	–
H2C8	ELISA	–	–	–	–	–	–	–	–	–	–	–	–	–
	WB	–	–	–	–	–	–	–	–	–	–	–	–	–
H2C9	ELISA	–	–	–	–	–	–	–	–	++	–	–	–	–
	WB	–	–	–	–	–	–	–	–	–	–	–	–	–
H2E1	ELISA	–	–	–	–	–	–	–	–	–	+	+++	–	–
	WB	–	–	+	–	–	–	–	–	–	–	–	–	–
H3A4	ELISA	–	–	–	–	–	–	–	–	–	–	–	–	–
	WB	–	–	–	–	–	–	–	–	–	–	–	+++	+++

* M, mouse; H, human; R, rat; WB, Western blot.

tissue as well as with human tissue. In studies of human tissue (Fujino et al., 1982; Song et al., 1985b), MAb 1–7–1 (1A1/1A2) was used to measure the amount of 1A1-dependent AHH in human placenta and lymphocytes (fig. 6). Other studies indicated the absence of 1A2 in placenta and lymphocytes, and thus, the AHH activity inhibited by MAb 1–7–1 would be a function of 1A1.

Human placental AHH is highly induced in women who smoke cigarettes (Nebert et al., 1969). Immunoassays of the RIA or ELISA type utilize MAbs for the analyses of human tissue cytochrome P450s that are present at low levels and that cannot be easily detected or quantitated by direct enzyme assay. MAb-directed competitive immunoassay was used to assay human placenta and lymphocytes for CYP 1A1. Figure 5 shows that all placentas from women who smoked cigarettes showed a relatively high content of CYP 1A1/1A2, as detected by MAb 1–7–1. The placentas from women who did not smoke cigarettes showed virtually no increase over control levels which were only slightly above background. Thus, the competitive immunoassay showed a clear-cut demonstration of a relatively large increase in levels of cytochrome P450 in women who smoke cigarettes compared to nonsmokers. The use of the MAb was of significant utility from the standpoint of sensitivity and the greater stability of the CYP 1A1 epitope recognized by MAb 1–7–1 than the 1A1 enzymatic activity as AHH. CYP 1A1 has been commonly measured by its AHH activity, one of the CYP 1A1 catalytic functions. The AHH activity of 1A1 has limited stability and is lost over a relatively short period. Highly induced AHH of placental tissue incubated for 48 h at 4°C results in a 22% loss of AHH activity. When incubated at 21°C for 24 h, there is a 50% loss of AHH activity. However, using the same incubation conditions with a RIA detection system, there is essentially no loss in the level of CYP 1A1 detected by RIA using MAb 1–7–1. Thus, the MAb-based RIA detection system is superior to AHH with respect to 1A1 stability and would be extremely useful under conditions in which the tissue enzyme activity could not be rapidly analyzed. The RIA system also may be more advantageous for the determination of other cytochrome P450s compared to the assay for their enzyme activities. Under conditions that require transport and freezing or, in certain epidemiological field studies that lack optimal laboratory facilities (Song et al., 1985b), the immunological measurement may be more useful than enzyme analyses. Thus, the immunological approach may be useful for the study of polymorphisms in populations.

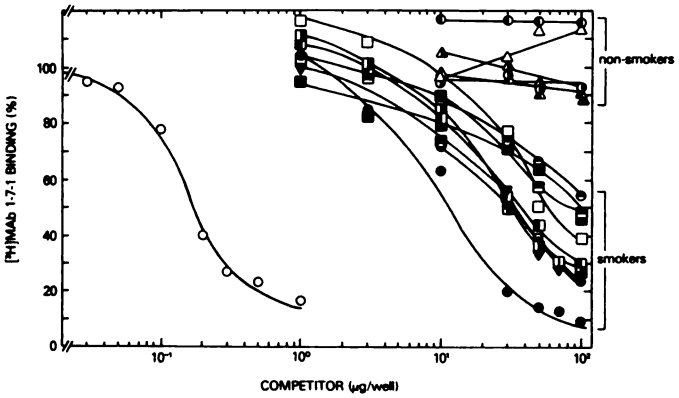

FIG. 5. Competitive RIA for human placental CYP 1A1. The 96-well microtiter plate was coated with rat liver microsomes induced with MC (2 μg/well) and stored overnight at 4°C. ^{3}H-labeled MAb 1–7–1 3691 (cpm/well) diluted in 5 mM phosphate-buffered saline (pH 7.4), 0.2% bovine serum albumin, and 0.6% 3-(3 cholamidopropyl)dimethylammonio-1-propane sulfonate was incubated overnight at 4°C with different concentrations of rat liver microsomes from rats treated with MC (O) and placental microsomes from smokers and nonsmokers (each symbol represents an individual placenta). The 100% binding level (1103 cpm/well) was obtained in the absence of competing antigens. The data reported were the means of duplicate determinations. The SE values for the individual points averaged ±5.6%.

Lymphocytes were reported to contain measurable levels of AHH activity after they were treated with mitogens and induced with benz[a]anthracene (Whitlock et al., 1972). This work subsequently became the subject of a great deal of research attempting to clarify possible relationships between the inducibility of AHH in lymphocytes and individual susceptibility to lung cancer (Gelboin, 1977, 1983; Kellerman et al., 1973; Paigen et al., 1977). The data concerning this relationship are conflicting, and the issue is still largely unresolved (Gelboin, 1977, 1983). Part of the problem may be due to the relative instability of AHH in lymphocytes which may be the cause of the lack of reproducibility of results from different laboratories. Because the enzymatic methods of analysis may in some cases be unstable or insufficiently sensitive, the use of detection methods based on MAb binding may increase the potential for accurate and reproducible determinations of the level of MAb 1–7–1 detected 1A1/1A2 in lymphocytes.

The current belief is that CYP 1A2 is liver specific, and therefore, the MAb 1–7–1-based measurement of cytochrome P450 in lymphocytes and placenta is specific for cytochrome CYP 1A1. The addition of mitogens to lymphocyte cultures, followed by the addition of the inducer benzanthracene, significantly increased the level of MAb 1–7–1 detected cytochrome P450. In noninduced lymphocytes, the basal levels were similar to control levels.

MAb 1–7–1 (Friedman et al., 1985d) was used to determine the amount of cytochrome P450-dependent AHH and the ECD in a cloned line of lymphoblastoid cells termed AHH-1 cells. The two enzyme activities in these human cells were inhibited by MAb 1–7–1. The AHH was almost entirely inhibited by the MAb 1–7–1, suggesting that a single form of the cytochrome P450 is responsible for all AHH activity in the AHH-1 cells. With respect to ECD activity, however, the MAb 1–7–1 only partially inhibited the enzyme, suggesting that there are at least two forms of cytochrome P450 responsible for ECD in the lymphoblastoid cell, one cytochrome P450

being inhibited by MAb 1–7–1 and the second cytochrome P450 being an ECD active form that is not inhibited by MAb 1–7–1. Similar results were obtained in both basal and induced cells, suggesting that both basal and induced cells contain qualitatively different cytochrome P450s responsible for AHH and ECD. This study demonstrates that a single cloned cell line is capable of expressing two different forms of cytochrome P450, and leads to the important conclusion that the multiplicity of cytochrome P450s found in animal tissues is not a result of a heterogenicty of cells each containing a different single cytochrome P450 but rather that different forms of cytochrome P450 are present and expressed in a single cloned cell line. Thus, the MAb-based procedures can determine the contribution of epitope-specific cytochrome P450s to the metabolism of a variety of compounds metabolized by cytochrome P450 enzymes, including xenobiotics and endobiotics.

MAbs to rat liver cytochrome P450s have previously been used for successful immunopurification of cytochrome P450s from animal tissues. This MAb-based immunopurification technique was applied to the human lymphoblastoid AHH-1 cell line. Immunopurification carried out with three different MAbs each yielded a 45-kDa polypeptide. The purified protein contains an MAb-specific epitope present on cytochrome P450s and may, therefore, be a human cytochrome P450. The AHH-1 cells are currently used extensively for cDNA expression of single or multiple cytochrome P450s. These AHH-1 cells can be transfected to express single or several cytochrome P450s and thus can be used to analyze individual cytochrome P450 for their specificity for substrate and product, their ability to activate mutagens, and their sensitivity to individual MAbs (Crespi et al., 1989).

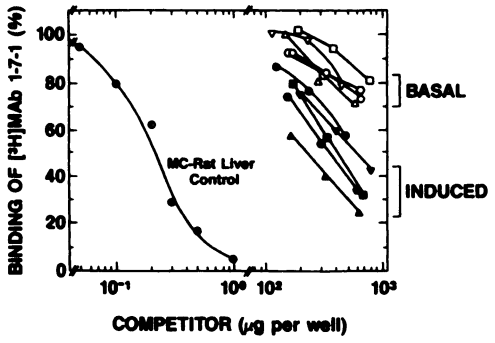

FIG. 6. Competitive RIA for human lymphocytes with MAb 1–7–1. Lymphocytes from normal human donors were treated with 1% phytohemagglutinin and 1% pokeweed mitogen for 48 h. For induction, lymphocytes were induced with benz[a]anthracene for 16 h (2 μg/ml) in 0.1% dimethyl sulfoxide. Untreated and induced lymphocytes were harvested and assayed using ^{3}H-labeled MAb 1–7–1 (6804 cpm/well) incubated overnight with different concentrations of rat liver MC microsomes. Homogenates of noninduced lymphocytes (open symbols) and lymphocytes induced with benz[a]anthracene (closed symbols) represent lymphocytes from a different individual. The 100% binding level (1993 cpm/well) was obtained in the absence of competing antigens. The data are reported as means of duplicate determinations. The *se* values for the individual points averaged ±1.4%.

An MAb prepared to purified rat liver cytochrome CYP 2E1 was found to cross-react with the human 2E1 enzyme. The MAb 1–98–1 detected CYP 2E1 in both rat and human tissue by Western blot analysis and by competitive RIA. The human CYP 2E1 and the rat CYP 2E1 were immunopurified by MAb bound to Sepharose (Robinson et al., 1989). The immunopurification resulted in cytochrome P450s of such high purity and quality that it was possible to analyze the proteins for the terminal 20-amino acid sequences which were determined for both the rat and human 2E1 (see section V). Thus, MAbs are proven instruments for simple purification of individual epitope-specific cytochrome P450s that can be isolated from the large multiplicity of cytochrome P450s present in microsomes. Immunopurification directed by MAbs is quite rapid and is less likely to result in proteolysis of the cytochrome P450 that often occurs during a slow multistep purification (Robinson et al., 1989). This technique is especially superior for human tissues and cytochrome P450s of limited availability. The enzyme activity of the immunopurified cytochrome P450, however, is reduced considerably when the cytochrome P450 is separated from the MAb-bound Sepharose (see section V).

The first use of inhibitory MAbs for measuring the metabolic contribution of induced cytochrome P450, i.e., phenotyping of cytochrome P450 in human tissues (Fujino et al., 1982), utilized a series of MAbs made to MC-induced cytochrome P450 purified from rat liver. The MAbs isolated were MAb 1–7–1 (1A1/1A2), MAb 1–8–1 (1A1), and MAb 1–31–2 (1A1). The MAbs inhibited AHH and ECD activity of the purified rat CYP 1A1 and were all of the IGg1 type. The MAb 1–31–2, after many passages, lost its inhibitory activity, perhaps through a mutational event, but still maintained its CYP 1A1-binding activity. A number of different human tissues were examined for the inhibition of AHH and ECD by the three MAbs. MAb 1–7–1, MAb 1–8–1, and MAb 1–31–2 were used to analyze human placenta, lymphocytes, monocytes, liver, and several human cell lines. The AHH of placentas of women who smoked cigarettes was highly inhibited by the three MAbs, the inhibition ranging from 83 to 95%. Thus, >83% of placental AHH was due to CYP 1A1.

The same three MAbs inhibited ECD from 21 to 50%. This indicates the presence in placenta of a second cytochrome P450 other than 1A1 that is responsible for a significant part (50 to 80%) of the ECD activity in placentas of women who smoke cigarettes. MAb 1–7–1 inhibited AHH from 65 to 78% in both BA-induced and noninduced human lymphocytes. The ECD activity of the lymphocytes was inhibited approximately 30 to 89%. It is noteworthy that the amount of inhibition of the BA-induced AHH and ECD in lymphocytes was the same as that observed in noninduced lymphocytes. This suggests that the cytochrome P450s responsible for AHH and ECD in noninduced basal lymphocytes are identical

with AHH and ECD active cytochrome P450s induced by BA. BA-induced monocyte AHH and ECD were not inhibited by the MAb 1–7–1, which suggests that the monocytes express cytochrome P450s for AHH and ECD that are different from MAb 1–7–1-sensitive cytochrome P450s expressed in lymphocytes and placenta (Fujino et al., 1982).

Human liver obtained from surgical specimens showed essentially no inhibition of AHH or ECD activity by MAb 1–7–1. This is consistent with the view that CYP 1A1 is not present in normal noninduced human liver. Various human cells grown in culture were also examined. The AHH of HeLa and A49 were inhibited variably by the MAb 1–7–1, signifying the presence of the cytochrome CYP 1A1 sensitive to MAb 1–7–1 and catalyzing AHH activity. This study, also examined CYP 1A1-catalyzed BP metabolite formation, i.e., three dihydrodiols, four phenols, and quinones were examined for the specificity of inhibition by MAb 1–7–1 in placenta and lymphocytes. There was a variation in the amount of MAb 1–7–1 inhibition of metabolite formation. The 7,8-diol formation was inhibited by 80%, whereas the 4,5-diol was only inhibited by 28%. The 9-hydroxy formation was inhibited by 85% and the 3-hydroxy by 65%. Thus, there is positional specificity in the inhibitory action of MAb 1–7–1 on CYP 1A1-catalyzed BP metabolism (Fujino et al., 1984b; Gelboin, 1980). This may be due to an MAb active site interaction that preferentially directs metabolism at specific positions in the substrate. MAbs may be useful in elucidating the nature of the active site-substrate complex that determines the flow of substrate to specific products into activation or detoxification pathways.

MAb 1–7–1 (1A1/1A2), as shown above, antigenically defines and inhibits a form of cytochrome P450 responsible for AHH and ECD activity in human placenta. In a unique study (Fujino et al., 1984a,b) the placentas from single and twin births from mothers who smoked cigarettes and nonsmokers were analyzed. The placentas from both dizygotic and dichorionic monozygotic twins showed extraordinarily high intrapair concordance for both the absolute amounts of AHH and ECD and their inhibition by MAb 1–7–1 compared with unrelated individuals; this indicates that interindividual differences in these parameters are not due to random variation or experimental error and that the activity of antigenically unique types of cytochrome P450 responsible for different drug and carcinogen reactions can be measured in different individuals by the amount of their inhibition by highly specific MAbs (figs. 7 and 8).

In figure 7, each point represents either a placenta from a single birth or the average of values of the two placentas from a twin, dichorionic birth. Figure 7 shows the percentage of inhibition by MAb 1–7–1 of AHH and of ECD in the same sample of placental microsomes from smokers. There was no parallel between the percentage

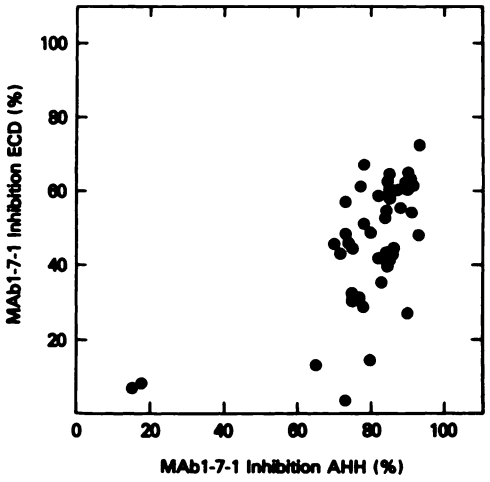

FIG. 7. Relationship between AHH and ECD inhibition determined by MAb 1–7–1 (Fujino et al., 1984a).

of inhibition of AHH and that of ECD activity. AHH activity was inhibited >80% except in two placentas. These two placentas showed extremely low absolute values of AHH of <1.0 unit/mg of protein. In most placentas, AHH was inhibited by >80%, with the median being 79%. Those few placentas exhibiting low inhibition of the AHH also exhibited low inhibition of the ECD. There were three samples, however, in which the AHH was highly inhibited (>60%), and the ECD activity was inhibited by <20%.

Thus, the ECD activity of individual placentas is a function of two types of cytochrome P450, a type lacking the epitope for MAb 1–7–1, and, thus, entirely insensitive to the MAb 1–7–1, and a cytochrome P450 that is sensitive to MAb 1–7–1 inhibition. The ratio of these two

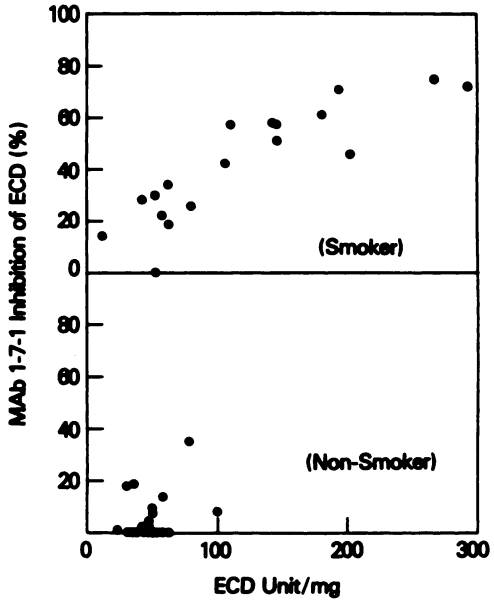

FIG. 8. Relationship between ECD activity and percentage of inhibition by MAb 1–7–1 in placentas. The average of the twin sets of placentas was taken as a single value. The coefficient of correlation between ECD activity and the percentage of inhibition by MAb 1–7–1 in smokers was 0.87 (Fujino et al., 1984a).

cytochrome P450s can be determined with the MAb 1–7–1. In certain individuals, mostly nonsmokers, all of the ECD is a function of the insensitive cytochrome P450. Thus, smoking largely induces the 1A1, which is MAb 1–7–1 sensitive and responsible for a proportion of ECD in smokers. This cytochrome P450 form appears to be absent in nonsmokers. There is a high correlation (r = 0.87, $P < 0.01$) between absolute levels of ECD and the degree of MAb 1–7–1 inhibition in the smoker group (fig. 8). The two lowest values of ECD inhibition were from individuals who had stopped smoking 1 to 2 weeks prior to delivery. The ECD activity of nonsmokers, with few exceptions, showed very little inhibition by the MAb 1–7–1. These findings may have general application to studies of the relationship of cytochrome P450 phenotype to population differences in drug and carcinogen biotransformation.

In a different study, the various levels of AHH, ECD, and EROD were examined in human placental microsomes of six nonsmokers and five smokers. In parallel, an immunohistochemical study was conducted of the placenta of each of the subjects. In the nonsmokers only one placenta gave a marginal positive result with MAb 1–7–1, whereas in the smokers the immunohistochemical analyses with MAb 1–7–1 showed highly positive staining, indicating the presence of CYP 1A1/1A2. This study also found that MAb 1–7–1 had a large inhibitory effect on AHH, EROD, and ECD in the placenta from smokers but had no effect on these enzymes in the nonsmokers who had much lower levels of enzymes. Furthermore, MAb 2–66–3 (2B1/2B2) failed to inhibit any of the enzyme activities in placenta of either the smokers or the nonsmokers. This MAb also failed to give positive staining in the placenta. Relatively little cytoplasmic staining was observed in large villi in a few trophoblastic cells. The primary finding was that in women smoking induces an Mab 1–7–1 detected cytochrome P450 that results in heavy staining of the placenta in the trophoblastic layer (Pasanen et al., 1988).

In a study utilizing human tissues, two cytochrome P450 enzyme activities, EROD and AHH, in liver microsomes from seven human fetuses were examined. MAb 1–7–1 (1A1/1A2) and MAb 2–66–3 (2B1/2B2) were used to determine the contribution of the epitope-specific single or class of cytochrome P450 to the total reaction. MAb 1–7–1 inhibited fetal hepatic EROD with a large variation from 0 to 100% but had only negligible effects on AHH activity, i.e., only an average of 9% inhibition. When tissues were obtained from women who smoked cigarettes, extensive variability was observed in the EROD inhibition by MAb 1–7–1; MAb 2–66–3 inhibited AHH activity by only 12% and EROD activity by 18%. When compared to the results with placental, smoking-induced AHH, which is highly inhibited by MAbs 1–7–1, these studies suggest that there may be large individual and tissue-specific differences in EROD and AHH activity. This may be due to differences between adult and fetal tissues in cytochrome P450 expression. ECD and EROD activities were examined in microsomal fractions from five human adult livers, three fetal livers, and five human fetal adrenals by Pacifici et al., 1988b. The enzyme activity of EROD, expressed as pmol/min/mg microsomal protein, was higher in all specimens examined with average values (±SEM) of 74 ± 27, 13 ± 3, and 12 ± 1 in adult, fetal livers, and fetal adrenals, respectively. MAb 1–7–1 (1A1/1A2) and MAb 2–66–3 (2B1/2B2) were examined for their inhibitory effect on O-deethylation of both ECD and EROD in human adult liver. The MAb 1–7–1 inhibited O-deethylation of EROD by 64 to 79%, and the MAb 2–66–3 had no effect on either the ECD or EROD activity. Thus, EROD metabolism is partially catalyzed in human adult liver by a cytochrome P450 that contains an epitope recognized by MAb 1–7–1. With fetal liver, MAb inhibition of enzyme activity was too low to measure. In a different study (Pasanen et al., 1987) of human tissues, six MAbs, 1–7–1 (1A1/1A2), 1–36–1 (1A1), 2–66–3 (2B1/2B1), 4–29–5 (2B1/2B2), 2–13–1 (3A4), and 2–3–2 (3A4), were used to detect epitope-specific cytochrome P450s in human aborted fetuses that were 14 to 24 weeks of age. These studies utilized Western blot analysis in parallel with ECOD determinations on the same tissue specimen. Of the six different MAbs used, MAb 2–13–1 (3A4) was the only one that consistently immunodetected a protein in all fetal liver preparations, showing a band whose intensity varied among samples. No immunodetectable bands were detected in preparations from adrenal and renal tissues obtained from the same fetuses. Human adult liver also contained an MAb 2–13–2-detected CYP 3A4 band. EROD was detected in all but one of the human fetal livers and varied between 0.22 and 47.5 pmol/min/mg protein as compared to 113 to 489 pmol/min/mg protein in human adult livers. Surprisingly, in all of the fetuses except one, the adrenal ECD activity was greater than that in the liver. The renal ECD activity, however, was low. Although EROD is a function of different cytochrome P450s, there was, nevertheless, a correlation (r = 0.78) between the EROD activity and the MAb-immunodetected protein band intensity in Western blots of human fetal liver microsomes. The MAb 2–13–1-identified CYP 3A4 band in fetal liver may be related to steroid effects in the fetus. In earlier studies, MAb 2–13–1 (3A4) was found not to be inhibitory to CYP 3A4 enzyme activity. In this study, a number of the MAbs that were used for Western blots were also inhibitory to enzyme activity. The MAb 4–29–5 (2B1/2B2), MAb 2–66–3 (2B1/2B2), MAb 1–7–1 (1A1/1A2), and MAb 1–36–1 (1A1) were not immunoreactive with any of the fetal or adult livers examined (Ladona et al., 1988).

The formation of BP diol epoxide-DNA adducts formed in vitro by rat and human liver and human placenta microsomes have been studied with the use of

MAb 1–7–1 (1A1/1A2) and MAb 2–66–3 (2B1/2B2). Microsomes from human placenta and liver, as well as rat liver, were incubated with BP and DNA, and BP diol epoxide-DNA adducts were measured by fluorescence spectrophotometry. The only fluorescence peak on the DNA that gave the same fluorescence peak as the chemically modified BP diol epoxide-DNA was the one with BP-7,8-dihydrodiol as a precursor. Of the 29 human placentas, five placentas were from women who smoked cigarettes, and five human livers capable of activating BP to the BP diol epoxide-DNA adduct were studied. The MAb 1–7–1 completely inhibited the formation of the BP diol epoxide-DNA adduct in placental microsomes. The inhibition by MAb 1–7–1 using rat and human liver, however, was less, being 50 to 60% for rat microsomes and 90% for human liver microsomes. 1A1 is not normally found in human liver, and metabolism in this organ may be due to 1A2. The MAb 2–66–3 (2B1/2B2) had no affect on any of the tissue enzyme activities or adduct formation. In fact, adduct formation was inhibited to a greater degree by MAb 1–7–1 than was the inhibition of AHH activity. Apparently, CYP 1A1 in human placenta, and possibly CYP 1A2 in human liver, may play a major role in BP activation; 1A1 is not present in normal human liver. Other cytochrome P450s may also be involved in the activation in liver, and thus, BP activation to the diol epoxide may be the result of the activity of different cytochrome P450 (Vahakangas et al., 1989).

In a population study, lung tissue from 25 patients with lung cancer was examined by immunohistochemistry with MAbs. The pulmonary activity of CYP 1A1-dependent AHH was also measured. CYP 1A1 was localized primarily in the peripheral airways in type I and II alveolar epithelium and in ciliated columnar and cuboidal bronchiolar epithelium. The amount of CYP 1A1 in the bronchial wall was minimal and was localized mainly in the endothelium and epithelium of the bronchial glands. The most important single factor that related to the presence of CYP 1A1 and AHH in lung tissue was smoking. None of the 10 ex-smokers, but all except one of the current smokers, had MAb 1–7–1-detectable levels of CYP 1A1. The cancer tissues contained detectable levels of CYP 1A1 and the localization of the cytochrome P450 in cancer tissues was also correlated with the presence of CYP 1A1. Thus, peripheral lung tissue stained positively in all patients with a peripheral adenocarcinoma who were currently smoking but in less than half of those with a bronchial cancer who were smokers. The data suggest that the smokers who have an inducible CYP 1A1 are especially at increased risk of developing lung cancer of the peripheral adenocarcinomatous type (Anttila et al., 1991).

Pelkonen et al. (1986) used MAbs as analytic reagents to measure four cytochrome P450 activities, EROD, coumarin hydroxylase, ECD, and AHH. These were measured in human liver tissues from needle biopsy samples from smokers and nonsmokers. The effect of MAb 1–7–1 and MAb 2–66–3 on the above activities was measured. EROD in the livers of cigarette smokers was inhibited, whereas AHH, coumarin hydroxylase, or ECD were not affected by cigarette smoking. No correlation was observed between plasma cotinine concentration and EROD activity. The MAb 1–7–1 inhibited hepatic EROD activity to a variable extent (from 0 to 65%) but had little effect on AHH, coumarin hydroxylase, or ECD. Thus, the latter enzyme activities are not due to CYP 1A1/1A2. The MAb 1–7–1 inhibition of EROD was >50% in smokers, whereas MAb 2–66–3 had no inhibitory effect on any of the enzymes measured. In contrast to the results with EROD, the MAb 1–7–1 produced considerable inhibition of placental microsomes from cigarette smokers. The MAb 2–66–3 did not have an effect on placental AHH or EROD. Thus, cigarette smoking induces a form of cytochrome P450 in human liver that is responsible for EROD that contains an epitope recognized by MAb 1–7–1. This form of cytochrome P450 is insensitive to MAb 2–66–3 and does not contribute to AHH, coumarin hydroxylase, or ECD activity in human liver. The MAb 1–7–1 can distinguish tissue enzyme activities that are related to a particular epitope-containing cytochrome P450.

In earlier studies, the AHH of human placenta was shown to be considerably increased by cigarette smoking (Nebert et al., 1969). Compared with other human tissues, the placenta was considerably more sensitive to cigarette smoking than were the other tissues examined. There is evidence for multiple forms of cytochrome P450 in human placenta, but not much is known about the inducibility and substrate specificity of the cytochrome P450 isozymes other than 1A1. Only placental CYP 1A1 responsible for AHH has been studied extensively with MAbs and immunoinhibition. These studies included immunohistochemical analysis utilizing MAb 1–7–1, which showed a very positive stain reaction in the trophoblast cells of the microvilli, as well as in large villous structures, of all placentas from women who smoked. Evenly distributed immunoreactivity was observed in the cytoplasm of trophoblasts in microvilli as well as in large villous structures (Pasanen et al., 1988).

VIII. Epilogue

MAbs produced by the hybridoma technology are one of the great achievements of modern biology. MAbs are pure chemical reagents of the highest cognitive specificity, and their generative capacity and potential immortality make them reagents *par excellence* for studies of the highly multienzyme families of cytochrome P450 isozymes. The MAbs that recognize epitope-specific single or multiple forms of cytochrome P450 have been successfully used for the qualitative and quantitative detection, immunohistochemical localization, and im-

munopurification of cytochrome P450s. Furthermore, they are reagents useful for the analyses of cytochrome P450 enzyme mechanisms and topographical studies of cytochrome P450.

The MAbs that inhibit cytochrome P450 enzyme activity are uniquely suited for reaction phenotyping, i.e., determining the contribution of epitope-specific cytochrome P450s to metabolic reactions involving substrate disappearance and product formation as well as positional, regio-, and stereospecificity. In addition, the MAbs can measure the contribution of specific cytochrome P450s to cytochrome P450-based reactions that lead to DNA binding, mutagen activation, carcinogen activation, and formation of toxic products. The use of MAbs for cytochrome P450 research is arising from its infancy and with its certain maturation will become a most powerful tool for the study of cytochrome P450 structure and function. The knowledge gained from these studies will be of the utmost usefulness in understanding the role of cytochrome P450 in xenobiotic and endobiotic metabolism.

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