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RAPID COMMUNICATIONS

CROSS-REACTIVITY OF THIRTEEN MONOCLONAL ANTIBODIES WITH TEN VACCINIA cDNA EXPRESSED RAT, MOUSE AND HUMAN CYTOCHROME P450s

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ABSTRACT - Twelve monoclonal antibodies (MAbs) to rat cytochrome P450s and one MAb to a scup (fish) P450 have been isolated, characterized, and are currently in common use. Expression of cDNAs for different P450s from a vaccinia vector offers a rapid and simple way toward the production of individual P450s. The thirteen MAbs were examined for their cross-reactivity with ten cDNA expressed human, rat, and mouse P450s. Three MAbs to rat 1A1 and fish 1A1 cross-reacted with cDNA expressed mouse 1A1. One of the latter MAbs, 1-7-1 but none of the others cross-reacted with mouse 1A2. Surprisingly, the fish MAb to 1A1 also cross-reacted with human 2E1. Two MAbs to rat 2B1/2B2 cross-reacted with rat 2A1. An MAb to rat 2C11 cross-reacted with human 2C9. Two MAbs to rat 2E1 cross-reacted with human 2E1. Finally, two MAbs to rat 3A1 cross-reacted strongly with human 3A4. These studies open the door to constructing a library of MAbs with defined binding activity to the P450s of human and other species.

INTRODUCTION

The cytochrome P450s are the metabolic interface between xenobiotics and their metabolism in human and other species as well as for the metabolism of endobiotics. The large array of drugs, mutagens, carcinogens and environmental chemicals as well as steroids are metabolized by individual forms of cytochrome P450 which may direct substrates into detoxication or activation pathways. The cytochrome P450s are a paradigm for multi-isozyme systems which may govern metabolic pathway choice. More than 200 P450 genes in 27 gene families have been identified [1]. P450 structural homology varies greatly and large differences in substrate and product specificity among P450s make difficult the determination of the precise role of each P450 in the metabolism of individual substrates. The often subtle diversity of cytochrome P450 structure and function requires unusually specific and sensitive methods for identification and quantification of individual P450s and for the measurement of their contribution to the metabolism of xenobiotics and endobiotics. The reagents that fulfill these requirements are the monoclonal antibodies (MAbs) [2]. MAbs are pure, chemically defined reagents that recognize a single determinant or epitope on the antigen. The specificity remains for the lifetime of the MAb-producing cell and its progeny. In respect to chemical purity, specificity, and limitless generation of cells, the MAbs are a superior class of reagents. MAbs can measure individual or epitope related P450s with radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA) or western blots or by immunohistochemistry. Inhibitory MAbs can measure the contribution of an individual P450 to any P450 catalyzed reaction [2].

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A large number of rodent and human P450s have been cloned and expressed into cytochrome P450 protein with different vectors [1,3,4]. We have used the vaccinia virus vector to express rodent and human cDNAs into P450s. The specificity of cross-reactivity of each P450 with the MAb can be precisely determined. The P450 activities of human and rodent P450s can differ greatly and thus it is important to utilize human P450s to study human drug metabolism, carcinogenesis and risk assessment. This study examines the cross-reactivity of individual MAbs with cDNA expressed mouse, rat and human P450s and can be a model study for defining the cross-reactivity of a library of MAbs.

MATERIALS AND METHODS

cDNAs coding for the following cytochrome P450s were constructed into vaccinia virus vectors: human 1A2, 2B6, 2C8, 2C9, 2E1 and 3A4; mouse 1A1 and 1A2; and rat 2A1 and 2B1 [4]. The enzymes were expressed by infecting TK^- cells for periods of 24-48 hr [4]. Cell lysates were used as a source of the cytochrome P450 and the content of P450 was determined by spectral analysis [5]. P450 content was dependent on the P450 expressed and varied between 10 and 40 pmol/mg protein. The lysate from cells infected with wild-type vaccinia virus served as a negative control and the liver microsomes of induced rats were used as a positive control.

Monoclonal antibodies. MAbs were raised to rat liver P450 1A1 [6], 1A2 [6], 1A1 (fish) [7], 2B1/2B2 [8], 2C11 [9], 2E1 [10], and 3A1 [11].

Enzyme-linked immunosorbent assay. The assay was performed by the generally accepted method [12] using alkaline phosphatase-conjugated goat F(ab')2 fragment to mouse IgG or IgM. The wells of microtiter plates were precoated with a solution containing 5-10 pmol P450/100μL phosphate-buffered saline.

Western immunoblot analysis. The assay was performed as previously described [10] using 4-7 pmol P450/lane and alkaline phosphatase conjugated goat F(ab')2 fragment to mouse IgG or IgM.

RESULTS AND DISCUSSION

Thirteen individual MAbs were prepared over a period of years to twelve rat and one fish (scup) cytochrome P450s. All of these MAbs were shown previously to bind to the classically purified cytochrome P450 used for immunization as determined by RIA. MAb 1-36-1, MAb 1-31-2, and MAb 1-7-1 bind to rat P450 1A1; MAb 1-7-1 also binds to P450 1A2. MAb 1-12-3 binds to fish scup 1A1, rat 1A1 and 1A2. Four MAbs prepared to rat 2B1/2B2, MAb 4-7-1, 2-66-3, 4-29-5 and 2-8-1, bind to rat 2B1/2B2. Mab 1-68-11 binds to rat P450 2C11 and two MAbs, 1-91-3 and 1-98-1, bind to rat P450 2E1. MAbs 2-3-2 and 2-13-1 bind to rat P450 3A1. MAb 1-7-1 [6] and MAb 1-12-3 [7] inhibit rat and fish P450 1A1, respectively. MAb 1-7-1 also inhibits rat 1A2. MAb 1-7-1 has extensive species cross-reactivity and inhibits 1A1-dependent aryl hydrocarbon hydroxylase (AHH) activity in rat, mouse, hamster and guinea pig [13] and human placenta and lymphocytes [14]. The four MAbs to rat 2B1/2B2, 2-66-3, 4-29-5, 2-8-1 and 4-7-1, inhibit the enzyme activities of rat expressed 2B1/2B2 [8]. MAb 1-68-11 inhibits rat 2C11 [9] and MAb 1-91-3 inhibits rat 2E1 [10]. MAb 2-3-2 and 2-13-1 to rat 3A1 bind strongly to 3A1 but are not inhibitory [11]. Table 1 shows the cross-reactivity of the above MAbs with cDNA expressed P450s. The cross-reactivity was considered positive when either the ELISA or western blot was positive. Rat MAbs 1-36-1, 1-31-2 and 1-7-1 and fish scup 1-12-3 all cross-reacted with cDNA expressed mouse 1A1. Of the latter MAbs, only 1-7-1 also cross-reacted with mouse 1A2. The MAb 1-12-3 to scup 1A1 surprisingly also reacted with human 2E1. Two of the four MAbs made to rat 2B1/2B2, MAb 2-66-3 and 4-7-1, cross-reacted with rat 2A1 and 2B1 and the 4-29-5 and 2-8-1

reacted less strongly and only to rat 2B1. MAb 1-68-11 to rat 2C11 cross-reacted with human 2C9. MAbs 1-91-3 and 1-98-1 to rat 2E1 cross-reacted with human 2E1. The MAbs 2-13-1 and 2-3-2 gave very strong western blot with human 3A4. These studies exhibit an atlas of cross-reactivity of MAbs with cDNA expressed P450 from different species including human. Knowledge of MAb cross-reactivity will be very useful for utilizing MAbs as reagents for the detection and quantitation of P450s and the determination of the contribution of individual P450 forms to total cellular metabolism by cytochromes P450.

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Table 1. Immuno-cross-reactivity of ten vaccinia cDNA expressed human, rat and mouse P450s with thirteen monoclonal antibodies raised to individual P450s

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