OROSOMUCOID PHENOTYPING USING MONOCLONAL ANTIBODIES

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INTORODUCTION

Orosomucoid(ORM) is a major glycoprotein in human serum and shows extensive variation. About 30 alleles heve been described at ORM1 and ORM2 locus, respectively (Yuasa et al. 1993). The ORM1 and the ORM2 alloproteins are encoded by two closely linked genes on the long arm of the chromosome 9 (Dente et al. 1987) and are distinguishable in band intensity from each other (Yuasa et al. 1986). In the course of ORM phenotyping, we have occasionally encountered some bands which are difficult to determine whether they belong to ORM1 or ORM2 product. In this study to overcome such confusions we attempted to prepare locus-specific monoclonal antibodies.

MATERIALS AND METHODS

Monoclonal antibodies – Female BALB/c mouse was immunized twice at 2-weeks interval by intraperitoneal injection of the purified desialylated ORM (about 100 μg protein, Cosmo Bio, Tokyo, Japan) emulsified in 100 μl Hunter's TiterMax adjuvant (CytRx, Norcross, GA). Three monoclonal antibodies, OR35 (IgG1), OR40 (IgM) and OR48 (IgG1), were selected. The ascitic fluid was used for immunological detection of ORM bands without further purification.

SDS-PAGE and immunoblot analysis – Sera reduced with 2-mercaptoethanol were separated on SDS polyacrylamide gel (8-16%), and blotted onto an Immobilon-P membrane (Millipore, Bedford, USA) by electroblotting. Then the filter was reacted to monoclonal or polyclonal antibodies for 40 min. Immunostaining was performed using Alp-labeled goat anti-mouse Ig (Dako, Glostrup, Denmark) or Alp-labeled porcine anti-rabbit Ig (Dako).

Subjects tested – Plasma samples from 658 Aboriginal Taiwanese were collected from the following a subpopulations: 100 Atayal, 64 Saisiat, 87 Bunun, 80 Tsou, 72 Ami, 63 Puyuma, 54 Rukai, 60 Paiwan and 78 Yami. Preliminary genetic studies on them were reported by Umetsu et al. (in press).

ORM phenotyping – Polyacrylamide gel IEF was carried out as described elsewhere (Yuasa et al. 1987), except that the concentration of Triton X-100 was decreased from 0.2% to 0.05%. The ORM patterns were developed by immunoprinting with polyclonal antibody, and also the ORM phenotyping was performed by immunoblotting with monoclonal antibodies. The symbols of these alleles were based on the nomenclature proposed by Yuasa et al. (1993).

Table 1. Distribution of ORM phenotypes and allele frequencies in nine subpopulations of Aboriginal Taiwanese.

Phenotypes		Atayal	Saisiat	Bunun	Tsou	Ami	Puyuma	Rukai	Paiwan	Yami
ORM1	F1	49	49	37	56	48	38	47	42	75
	F1-S	23	11	34	19	13	18	4	14	3
	S	0	0	6	3	0	1	0	1	0
	F1-dF1S	0	2	4	0	0	2	0	1	0
	F1-B9	0	0	0	0	3	0	0	0	0
	S-B9	0	0	0	0	0	1	0	0	0
	F1-Q0	25	1	4	2	8	3	3	2	0
	S-Q0	2	1	1	0	0	0	0	0	0
	Q0	1	0	1	0	0	0	0	0	0
Total		100	64	87	80	72	63	54	60	78
Allele frequencies										
*ORM1*F1*		0.730	0.875	0.667	0.831	0.833	0.786	0.935	0.842	0.981
*ORM1*S*		0.125	0.094	0.270	0.156	0.090	0.167	0.037	0.133	0.019
*ORM1*dF1S*		-	0.016	0.023	-	-	0.016	-	0.008	-
*ORM1*B9*		-	-	-	-	0.021	0.008	-	-	-
*ORM1*Q0*		0.145	0.016	0.040	0.013	0.056	0.024	0.028	0.017	-
ORM2	M	99	64	84	79	62	56	53	54	78
	M-H19	1	0	2	1	10	7	1	6	0
	M-H2	0	0	1	0	0	0	0	0	0
Total		100	64	87	80	72	63	54	60	78
Allele frequencies										
*ORM2*M*		0.995	1.000	0.983	0.994	0.931	0.944	0.991	0.950	1.000
*ORM2*H19*		0.005	-	0.012	0.006	0.069	0.056	0.009	0.050	-
*ORM2*H2*		-	-	0.006	-	-	-	-	-	-

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RESULTS AND DISCUSSION

Figure 1 shows the bond patterns of three different types analyzed by SDS-PAGE under native and desialylated conditions using polyclonal and monoclonal antibodies. The OR35 and OR48 antibodies recognized ORM1 and ORM2 bands, respectively. On the other hand, the OR40 antibody reacted to both ORM1 and ORM2 bands and provided essentially similar patterns obtained with the polyclonal antibodies. The three monoclonal antibodies reacted to native and desialylated ORM.

ORM phenotyping was performed on 658 individuals from nine subpopulations of Aboriginal Taiwanese by IEF and immunoprinting using polyclonal antibody. Some samples were difficult to determine the phenotype, as shown in lanes 2–4 of figure 2A. The monoclonal antibodies were used to find the correct ORM phenotyps. The OR35 monoclonal antibody reacted only to ORM1 alloproteins and OR48 reacted only to ORM2 bands. On the other hand, OR40 reacted strongly to ORM1 bands and faintly to ORM2 bands (figure 2B–D). The results revealed the bands corresponding to ORM2 M to originate only from ORM2 M band, and the samples in the lanes 2–4 of figure 2 were identified as ORM1 F1–Q0/ORM2 M, Q0/M and S–Q0/M, respectively. The distributions of ORM phenotypes and allele frequencies are given in Table 1. In the present study, five ORM1 alleles (*ORM1*F1*, *ORM1*S*, *ORM1*dF1S*, *ORM1*B9* and *ORM1*Q0*) and three ORM2 alleles (*ORM2*M*, *ORM2*H19* and *ORM2*H2*) were recongnized by IEF method in combination with immunodetection using monoclonal and polyclonal antibodies.

In conclusion, we have found the technique using monoclonal and polyclonal antibodies to be of great value in ORM phenotyping of *ORM1*Q0* carrier. Futher study will be necessary to confirmation by genetical analysis using nucleic acids.

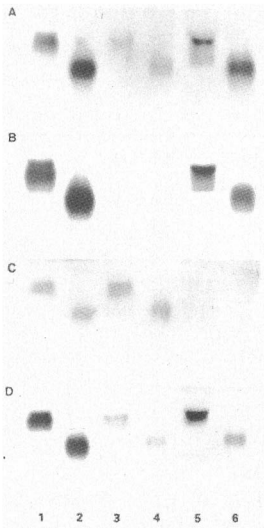

Fig.1. SDS-PAGE and immunoblot analysis of ORM before (1,3,5) and after (2,4,6) treatment with sialidase using polyclonal (A;DAKO) and monoclonal (B;OR35, C;OR48, D;OR40) antibodies. 1,2: ORM1 F1/ORM2 M, 3,4: Q0/M, 5,6: S/Q0.

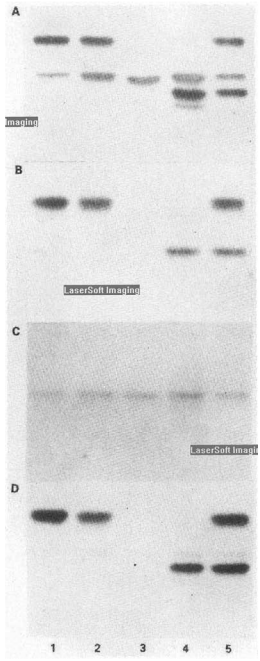

Fig.2. Immunoprint (A) using polyclonal antibody to ORM (Dako) and immunoblot using monoclonal antibodies to ORM with: (B) OR35, (C) OR48 and (D) OR40. 1: ORM1 F1/ORM2 M, 2: F1–Q0/M, 3: Q0/M, 4: S–Q0/M, 5: F1–S/M.