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Human Antigen and Enzyme Markers in Man–Chinese Hamster Somatic Cell Hybrids: Evidence for Synteny Between the *HL-A*, PGM_3, ME_1, and *IPO-B* Loci

(human gene linkage/species-specific antigens)

H. VAN SOMEREN*†‡, A. WESTERVELD†, A. HAGEMEIJER§, J. R. MEES†, P. MEERA KHAN¶, O. B. ZAALBERG†

†Medical Biological Laboratory TNO, P.O.B. 45, Rijswijk 2100, The Netherlands; ‡Laboratory for Molecular Genetics, State University, Leiden, The Netherlands; §Department of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands; and ¶Department of Human Genetics, State University, Leiden, The Netherlands

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ABSTRACT **In man–Chinese hamster somatic cell hybrids the segregation of the loci for 27 human enzyme markers and the species-specific surface antigens, including the HL-A histocompatibility antigens, was studied. The results show a synteny of the human loci for phosphoglucomutase 3, cytoplasmic malic enzyme, tetrameric indophenol oxidase, and HL-A. Furthermore, evidence is presented that the loci for the human species-specific antigens are distributed over several chromosomes.**

Human chromosomes are lost preferentially in man–rodent somatic cell hybrids. The study of these hybrids has considerably facilitated the analysis of gene linkage in man (1). Two or more human markers are located on the same chromosome (syntenic) when they are present or absent simultaneously in hybrid cell populations. Most of the known human syntenic groups detected via the analysis of hybrid cells are connected with loci coding for enzymes, since most of the homologous enzymes of man and rodent origin can be distinguished by means of electrophoretic techniques. Besides enzymes, antigens of both parental genomes are expressed in the hybrid cells (2). The genes coding for antigens are therefore another class of markers which can be used in synteny studies, as has been shown by Puck (3).

In the present investigation the segregation patterns of 27 loci coding for human enzymes were studied and related to the expression of human species-specific surface antigens in man–Chinese hamster somatic cell hybrids. Furthermore, we examined whether the human locus for HL-A was segregating in close association with the locus for phosphoglucomutase 3 (PGM_3, EC 2.7.5.1) in these hybrid cells, since family studies have suggested that these loci are linked (4, 5).

MATERIALS AND METHODS

Hybrid somatic cell lines were obtained by fusion of mutant cell lines derived from the Chinese hamster DON cell line with normal or hypoxanthine-guanine phosphoribosyltransferase (HGPRT, EC 2.4.2.8) deficient human skin fibroblasts, or human leukocytes. Two Chinese hamster cell lines, a3 and a23, were thymidine kinase (EC 2.7.1.75) deficient, whereas the third line, wg3-h, was deficient in HGPRT. The diploid human parental fibroblast strain ms2 ($HGPRT^-$) was derived from a patient with the Lesch–Nyhan syndrome. Human white blood cells were obtained from peripheral blood of male and female donors. The details on production, isolation, and propagation of these hybrids were described earlier (6).

In the hybrid and parental cell populations the following enzymes were characterized electrophoretically on cellulose acetate gel (Cellogel, Chemetron, Milan, Italy): glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49); HGPRT; 3-phosphoglycerate kinase (PGK, EC 2.7.2.3); α-galactosidase (α-Gal, EC 3.2.1.22); lactate dehydrogenases (LDH-A, LDH-B, EC 1.1.1.27); 6-phosphogluconate dehydrogenase (PGD, EC 1.1.1.44); PGM_1, PGM_3; indophenol oxidases (dimeric IPO-A and tetrameric IPO-B); NAD-dependent cytoplasmic malate dehydrogenase (EC 1.1.1.37); NADP-dependent cytoplasmic isocitrate dehydrogenase (EC 1.1.1.42); glucose phosphate isomerase (EC 5.3.1.9); adenylate kinase (EC 2.7.4.3); glutamate oxaloacetate transaminase (EC 2.6.1.1); adenosine deaminase (EC 3.5.4.4); peptidases (Pep-A, Pep-B, Pep-C, Pep-D); cytoplasmic malic enzyme (ME_1, also known as malate oxidoreductase decarboxylating, EC 1.1.1.40); hexokinase (EC 2.7.1.1); hexosaminidases (Hex-A, Hex-B, EC 3.2.1.30); glyceraldehyde phosphate dehydrogenase (EC 1.2.1.12); and purine nucleoside phosphorylase (EC 2.4.2.1). The methods used for the characterization of these enzymes as well as for the preparation of cell lysates have been described elsewhere (ref. 7 and van Someren and Meera Khan, in preparation).

For serologic determination of the surface antigens, rabbit antisera against HeLa cells (Ra/HeLa) and Chinese hamster fibroblasts were prepared following the method described by Oda and Puck (8). The horse anti-human lymphocyte serum (ALS) was kindly supplied by Dr. H. Balner of the National Health Organization TNO, Rijswijk, The Netherlands. The antisera detecting the LA and FOUR series of the HL-A antigens were a generous gift from Dr. J. J. van Rood, Dept. of Immunology-Haematology of the University Hospital, Leiden, The Netherlands. They were defined according to

Abbreviations: HL-A, human histocompatibility antigens; PGM_3, phosphoglucomutase 3; ME_1, cytoplasmic malic enzyme; IPO-B, tetrameric indophenol oxidase; Ra/HeLa, rabbit antiserum against HeLa cells; ALS, horse anti-human lymphocyte serum; Hex-B, hexosaminidase B.

* Author to whom reprint requests should be addressed: Laboratory for Molecular Genetics, State University, Wassenaarseweg 64, Leiden, The Netherlands.

TABLE 1. *Linkage relationships between the enzyme markers PGM_3, ME_1, and IPO-B in man–Chinese hamster cell hybrids*

Primary hybrid cell lines

		PGM$_3$ +	PGM$_3$ −	IPO-B +	IPO-B −
ME$_1$	+	11	0	18	0
	−	0	16	1	14
IPO-B	+	12	1		
	−	0	16		

Primary and secondary hybrid cell lines

		PGM$_3$ +	PGM$_3$ −	IBO-B +	IBO-B −
ME$_1$	+	78	2	83	1
	−	5	168	5	156
IPO-B	+	75	6		
	−	4	189		

The data have been broken down to 2 × 2 format and tabulated in +/+, +/−, −/+ and −/− categories for particular pairs of markers. The results are given in absolute numbers.

van Rood (9) and the NIH method (10). The antisera detecting the species-specific antigens were heated to 56° for 30 min for complement inactivation. Guinea pig or rabbit serum was used as a source of complement. Prior to their usage the sera (except the HL-A antisera) were absorbed with Chinese hamster cells. All sera were stored at −70°.

For the immune-adherence test (11) monolayer cultures of parental and hybrid cells were incubated with antisera in various dilutions. Following incubation at 37° for 30 min the cultures were washed five times with a balanced salt solution to remove the unbound serum compounds. The detection of bound antibodies was carried out by incubating the cells with human O-erythrocytes and 2% complement at 37° for 30 min. Inversion of the plates caused the sedimentation of all free erythrocytes. The titer is expressed as the highest dilution which gave a positive hemadsorption as judged independently by two observers. In all experiments the hemadsorption on Chinese hamster cells was used as a control.

RESULTS AND DISCUSSION

The presence or absence of the 27 enzyme markers was studied in 33 primary clones and 241 subclones from 20 independent fusion experiments. The following sets of markers segregated concordantly: (*1*) the X-chromosomal markers HGPRT, G6PD, PGK, and α-Gal (12); (*2*) PGD, PGM_1, and Pep-C (13); (*3*) LDH-B and Pep-B (13), and (*4*) PGM_3, ME_1, and IPO-B. No demonstrable correlation between the other autosomal markers tested was observed. The electrophoretic patterns of the enzymes PGM_3, ME_1, and IPO-B are seen in Fig. 1. The data presented in Table 1 suggest that these loci are located on the same chromosome. In the primary hybrid clones only one clone was positive for IPO-B and negative for PGM_3 and ME_1. The table shows that there are more exceptions in the secondary clones. The exceptions may be caused by chromosomal breakages and rearrangements or may be the result of differences in the stability of these three enzymes and their detection limits. Our results confirm the finding of Chen (14) and Ruddle (1) that the human loci coding for ME_1 and IPO-B are syntenic in man–mouse hybrid cells.

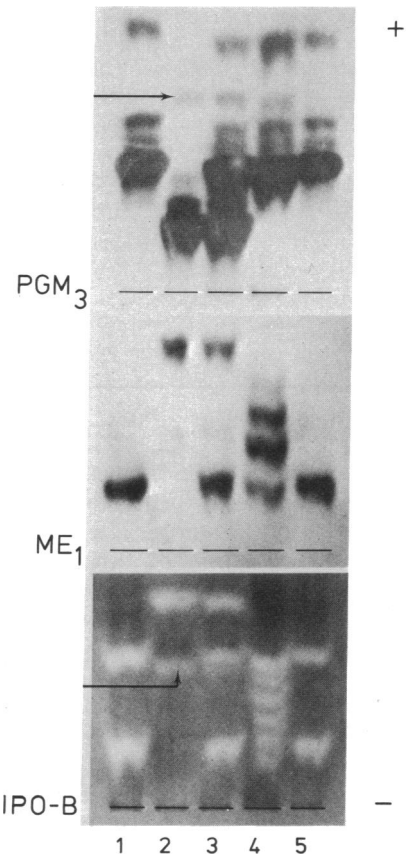

FIG. 1. Zymograms of PGM_3, ME_1, and IPO-B, comparing the fibroblast patterns of *1*, Chinese hamster; *2*, human; *3*, an artificial mixture of Chinese hamster and human; and *4*, *5*, man–Chinese hamster somatic cell hybrids. The human PGM_3 and IPO-B bands are indicated by *arrows*. The intermediate bands due to heteropolymeric enzyme molecules seen in ME_1 and IPO-B (channel *4*) are absent in PGM_3. The hybrid population in channel *5* has lost the human loci for PGM_3, ME_1, and IPO-B.

Our data suggest that the 27 enzyme markers tested behave as 19 asyntenic groups: 15 single loci and 4 sets of two or more loci.

The expression of human antigenic markers in hybrid clones was studied by the immune-adherence test (11). The results obtained with the cytotoxic test (9) on human fibroblasts and hybrid cells were not reproducible, irrespective of the fact that complement of different sources was tried and different dyes were used. To study the relationship between the amount of human genetic information retained in hybrid clones and the expression of human antigenic markers, the immune-adherence test was carried out on 46 hybrid clones having retained different numbers of human isoenzyme markers. These clones were collected from five independent fusion experiments. Table 2 shows that the number of human asyntenic enzyme markers present in the clones does not correlate with the highest dilution of the Ra/HeLa antiserum giving positive hemadsorption. Analogous results were ob-

TABLE 2. *The relationship between the titer obtained with Ra/HeLa antiserum against hybrid cell lines and the number of asyntenic enzyme markers, in these cell lines*

Ra/HeLa serum titer	Number of hybrid clones	Mean number of asyntenic enzyme markers
1:2	1	7
1:4	5	5 (2-9)*
1:8	3	5 (4-6)
1:16	4	6 (2-11)
1:32	3	7 (4-9)
1:64	0	—

* The range is presented in parentheses.

tained with the ALS antiserum. Four clones did not react with the Ra/HeLa and the ALS anti-human sera. This rare loss of human species-specific antigens has also been found in man–mouse somatic cell hybrids (2). Immediately after the isolation one clone gave positive reactions with both the anti-human sera. Following culturing in normal medium for several weeks, no human surface antigens and enzymes, except Hex-B, could be detected in this clone. Three other hybrid cell clones, having lost the human surface antigens before the first test was performed, retained only human Hex-B. Two hybrid cell lines having retained only the human X-chromosomal markers and Hex-B did react with the anti-human sera. The results suggest that the human chromosome(s) carrying the genetic information for Hex-B has no important share in the expression of human antigens on the cell surface which can be detected by our antisera, whereas, the human X chromosome contains one or more genes coding for these antigens. Apparently the genetic information for the human species-specific antigens at the cellular level is distributed over several chromosomes. From their data Weiss and Green (15) draw the same conclusion.

To study the linkage between the HL-A and the PGM_3 loci, described by Lamm *et al.* (4, 5) the segregation pattern of the HL-A marker in relation to the 27 human enzyme markers was analyzed in 38 hybrid clones obtained from five

TABLE 3. *Linkage relationships between HL-A and the three enzyme markers PGM_3, IPO-B, and ME_1 in secondary man–Chinese hamster hybrid cell clones from five independent fusion experiments with human leukocytes or diploid fibroblasts*

		PGM_3		IPO-B		ME_1	
		+	−	+	−	+	−
HL-A	+	19	2	18	2	16	2
	−	2	15	4	11	3	10

For explanation see Table 1.

fusion experiments. Although no absolute synteny was found between the HL-A locus and any of the loci coding for the tested enzymes, the data in Table 3 show a good correlation between the absence and presence of the HL-A, PGM_3, ME_1, and IPO-B markers.

There are several factors which may complicate linkage studies of HL-A antigens via somatic cell hybrids. For some hitherto-unknown reason the phenotypic expression of HL-A antigens in cultured cells sometimes disappears, probably without the loss of the genes coding for these antigens (16). The second factor which complicates this analysis is the appearance of antigens of an unexpected new type in man–Chinese hamster cell hybrids (Table 4). In addition to the LA and FOUR typing sera which react with the parental cell lines, used in the fusion experiments, some control sera gave occasionally positive reactions with hybrid cells. To study whether this phenomenon was characteristic of hybrid cells, or could also occur in primary fibroblast strains during culture, the expression of HL-A antigens was studied in eight fibroblastic cell strains. The typing data of these fibroblasts obtained with the immune-adherence test were completely in agreement with the data obtained by van Rood on the donor leukocytes using the cytotoxic test. The hybrid cell lines which were negative for all the HL-A antigens of the original parental cell were also negative for the control sera. Using a mixed agglutination test Kano *et al.* (17) described also in some man–mouse hybrid cells weak reactions with the control sera. Whether these new specificities are caused by hybrid

TABLE 4. *Immune-adherence test with HL-A typing sera on parental and hybrid cells*

	LA typing sera					FOUR typing sera						
Cells	1	2	3	9	10	5	7	8	12	13	W5	W15
ms2	−	−	+	−	−	−	−	+	−	−	−	−
a3-ms2 I 1 G		+	+					−	−			
a3-ms2 I 1 N		+	+	+	−	−	+	−	−	−	+	−
a3-ms2 I 1 AB		−	+				+	+	+	−		
a3-ms2 I 1 W	+	−	+	−	+	−	+	+	+	+	+	−
a3-ms2 I 1 X		−	−	−	−			−	−	−	−	−
a23-ms2 I 2 E	−	−	+	−	−	−	−	+	−	−	+	−
a23-ms2 I 2 azg		+	+	−	−	−	−	+	−	−	+	
lymf W	+	−	+	−	−	+	−	−	−	−	+	+
wg3-h/lymf I 1	−	−	−	−	−	−	−	−	−	−	−	
wg3-h/lymf I 1 J	−	−	+	−	−	+	+	+	−	−	+	
lymf K	+	−	+	−	−	−	−	+	−	−	−	−
a3/lymf I	+	−	+					+			+	
a3/lymf I 4		+	+					+				
a3/lymf I 6		−	−					−				

The tested sera did not react with the parental Chinese hamster cells. Human parental cells are in italics. Blank, not tested.

molecules between the man and Chinese hamster subunits of the antigen, or have another origin in these hybrid cells, has to be elucidated.

Our data on the segregation of the HL-A and enzyme markers in the hybrid cells together with the evidence for the linkage of the PGM_3 and HL-A loci from family studies present evidence for the synteny of the HL-A, PGM_3, ME_1, and IPO-B loci. Using the same man–Chinese hamster somatic cell hybrids, Jongsma *et al.* (18) have assigned the loci for PGM_3, ME_1, and IPO-B to chromosome C 6, which chromosome in that case will also bear the HL-A locus.

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