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Smith, M
Gold, P
Shuster, J
et al.

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CHROMOSOMAL ASSIGNMENT OF THE HL-A COMMON ANTIGENIC DETERMINANTS IN MAN-MOUSE SOMATIC CELL HYBRIDS*

MOYRA SMITH, P. GOLD, J. SHUSTER, N. TANIGAKI AND D. PRESSMAN

Division of Clinical Immunology and Allergy of the Montreal General Hospital and the McGill University Medical Clinic of the Montreal General Hospital Research Institute, Montreal, Quebec, Canada and Department of Immunology and Immunochemistry Research, Roswell Park Memorial Institute, Buffalo, New York

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SUMMARY

In the study presented here, man-mouse somatic cell hybrid clones were examined by means of radioimmunoassays for the presence of both β_2 -microglobulin (β_2 m) and the HL-A xenoantigenic determinant. In addition, the clones were examined for their karyotype and the expression of enzymes with known chromosomal assignments. The results obtained indicate that the gene coding for the HL-A xenoantigenic determinant is carried on chromosome 6. The data obtained provides a direct demonstration that the gene coding for β_2 m segregates independently of that coding for the alloantigenic polypeptide chain of the HL-A molecule, and that the gene coding for β_2 m is carried on chromosome 15.

INTRODUCTION

Recent studies have demonstrated that the HL-A molecule obtained after papain digestion of cell membranes consists of two polypeptide chains (Cresswell *et al.*, 1973; Tanigaki, *et al.*, 1973a, Tanigaki, *et al.*, 1974). The 11,000-dalton fragment constitutes one polypeptide chain while the remaining portion of the HL-A molecule, with a molecular size of approximately 33,000 daltons, constitutes the second polypeptide chain. This 33,000-dalton polypeptide chain carries the HL-A alloantigenic determinant (Nakamuro *et al.*, 1975; Tanigaki *et al.*, 1975). The 11,000-dalton portion of HL-A molecule has been demonstrated in free form in both serum and urine and has been identified as beta-2-microglobulin (β_2 m) (Grey *et al.*, 1973; Nakamuro *et al.*, 1973; Peterson *et al.*, 1974). The β_2 m polypeptide chain is structurally identical in all HL-A molecules, regardless of the alloantigenic specificity.

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Correspondence: Dr Moyra Smith, Division of Human Genetics, Department of Pediatrics, Mount Sinai Hospital, 100th Street & 5th Avenue, New York, N.Y. 10029, U.S.A.

HL-A molecules carrying different HL-A alloantigenic specificities share certain common antigenic structures that stimulate antibody production in heterologous animals. These common antigenic structures which have been designated HL-A common antigenic determinants, have been detected by use of rabbit antisera directed against HL-A molecules on both the 11,000-dalton and 33,000-dalton polypeptide chains, and they have been shown to be distinct from the HL-A alloantigenic determinants (Miyakawa *et al.*, 1973). In this study, the common antigenic determinant on the 11,000-dalton portion will be termed the β_2m determinant, while that on the 33,000-dalton portion will be termed the HL-A xenoantigenic determinant to distinguish it from the HL-A alloantigenic determinant. The xenoantigenic determinant, or a very similar one, has been shown to be present on the Rhesus monkey histocompatibility antigens, RhL-A, and has previously been referred to as the HL-A primate cross-reacting determinant (Katagiri *et al.*, 1974). Since this determinant has been located on the 33,000-dalton polypeptide chain carrying high HL-A alloantigenic activity (Nakamuro *et al.*, 1975), the HL-A common antigenic determinant apparently represents a part of the invariant portion of the product of the genes which code for HL-A alloantigenic chains. Further insight into the relationship between HL-A alloantigenic determinants, the HL-A xenoantigenic determinant and the β_2m determinant, may be obtained through genetic studies on the segregation of genetic markers in man-mouse somatic cell hybrids. Through such studies it is possible to establish the number of genes responsible for the production of the structural components of HL-A molecule, and more importantly, to clarify the interrelations of these genes in the antigenic expression of the HL-A molecule.

In the present study, man-mouse somatic cell hybrid clones were examined by means of radioimmunoassay for the presence of the β_2m determinant and for the presence of the HL-A xenoantigenic determinant. The cell clones were also analysed for both chromosomal composition and a variety of enzyme markers, and the results were correlated with those of the antigenic analyses. The data obtained indicate that the gene coding for the HL-A xenoantigenic determinant is syntenic with the genes for soluble malic enzyme (ME1) and tetrameric iodophenol oxidase (IPO B) on chromosome 6, the chromosome that also carries the genes coding for the alloantigenic determinants of the HL-A molecules (van Someren *et al.*, 1974; Jongsma *et al.*, 1973). Confirmation was obtained that the gene coding for the β_2m determinant is carried by chromosome 15 (Goodfellow *et al.*, 1975; Smith *et al.*, 1975). Moreover, it was shown that the production of HL-A, as determined by the presence of HL-A xenoantigenic determinant, occurs in hybrids in which no detectable human β_2m is produced.

MATERIALS AND METHODS

Cell types used

In this study the two human cell types used were peripheral blood lymphocytes isolated from two healthy donors and the established lymphoid culture line cells ODY and EB4. Each of these cell types were fused with RAG cells, a hypoxanthine-phosphoribosyl-transferase deficient mutant mouse cell line (Ruddle *et al.*, 1970).

Fusion

Fusion of human cells with mouse cells was performed using Sendai virus inactivated by ultra-violet light as previously described (Smith *et al.*, 1975).

Enzyme analyses

Lysates for enzyme studies were prepared by washing cells in Dulbecco's phosphate buffered saline (pH 7.2) and then subjecting the cells to one or two cycles of freezing and thawing. Lysates derived from different hybrid clones were examined for the presence of thirteen different murine, human and hybrid enzyme phenotypes by means of starch gel electrophoresis. The enzymes studied are listed in Tables 2 and 3. The enzyme detection systems and the chromosomal assignment for these enzymes are referenced in Table 3.

Chromosomal analyses

The methods used for chromosomal analyses have been previously described (Smith *et al.*, 1975).

Radioimmunoassay for HL-A and β_2m

The radioimmunoassays for the HL-A xenoantigenic determinant (Katagiri *et al.*, 1974; Nakamuro *et al.*, 1975), the HL-A2 alloantigenic determinant (Tanigaki *et al.*, 1973) and β_2m (Smith *et al.*, 1975) were performed as previously described. The β_2m activity and the HL-A activities were assayed in cell lysates. In solubilizing cells for use in the β_2m and HL-A assays, $3 \sim 5 \times 10^7$ cells/ml were treated with a 1% solution of the non-ionic detergent, IGEPAL CA-630 (GAF Corporation, New York). The lysates were centrifuged for 10 min at 1000 RCF and the supernatants were assayed. Activity was defined as the capacity of a specific quality (i.e. 50 μ l) of cell lysate to inhibit the binding of a defined amount of antibody to a defined amount of radioiodinated reference antigen.

In the HL-A assay systems, samples were considered positive if the inhibition of binding of labelled antigen to antibody caused by the prior addition of the test sample was over 10%. Control lysates from human cell clones showed 83–95% inhibition. In the β_2m assay system, samples were considered positive if the inhibition of binding of labelled antigen to antibody caused by the prior addition of the test sample was over 5%. Control samples from human cell clones showed 95–98% inhibition.

RESULTS

From the results of the radioimmunoassays for HL-A xenoantigenic activity and for β_2m activity, shown in Table 1, it will be seen that clones could be classified into four categories as follows: HL-A-positive and β_2m -positive; HL-A-positive and β_2m -negative; HL-A-negative and β_2m -positive; and HL-A-negative and β_2m -negative. In Tables 2 and 3, the segregation of human β_2m , HL-A and thirteen human enzyme markers are shown. A high degree of discordance was observed between the expression of human β_2m and HL-A. A similarly high degree of discordance was observed in the expression of HL-A and mannose phosphate isomerase (MPI) which maps on chromosome 15 (van Heynigen *et al.*, 1975).

A high degree of concordance was observed between the expression of HL-A and the expression of enzymes ME1 and IPO B which have been assigned to human chromosome 6 (van Someren *et al.*, 1974; Jongsma *et al.*, 1973). In the case of segregation of HL-A and ME1 two discordant clones were observed. Both of these clones were, however, positive for IPO B. From Table 4 it will be seen that in the case of segregation of IPO B and HL-A, the

TABLE 1. HL-A and human β_2 -microglobulin (β_2 m) expression in man-mouse somatic cell hybrid clones and control cell clones

Cell clones	Designation	HL-A*	β_2 m	Cell clones	Designation	HL-A*	β_2 m
		activity†	activity†			activity†	activity†
HL-A positive	RLyA3	59	97	HL-A negative	RLyA1	-3	93
β_2 m positive	RLyA12	22	93	β_2 m positive	RLyA4	-5	92
hybrid cell	RLyG	29	91	hybrid cell	RLyA7	-2	93
clones	RLyJ	50	94	clones	RLyA8	-2	93
	RLyMSAe	73	94		RLyA9	2	90
	RLyMSBc	61	80		RLyA10	0	92
	RLyMSBe	19	92		RLyA16	6	94
	RLyMSCd	26	96		RLyMSCg	4	94
	RLyMSCf	38	84		RLyMSNa	1	53
	RODYLb	42	91		RLyMSNb	-6	96
	REB4Cc	35	94		RLyMSNf	0	91
HL-A positive	RLyC9	38	0		REB4Cb	4	92
β_2 m negative	RLyC11	35	0		REB4Cd	-2	83
hybrid cell	RLyG8	39	1	HL-A negative	RLyG13	-2	-1
clones	RLyG10	25	-3	β_2 m negative	RLyM	7	-3
	RLyG12	33	1	hybrid cell	RLyMSAb	0	0
	RLyMSWb	59	-2	clones	RLyMSva	-7	-2
					REB4Bc	-2	-6
Control human	Chang	83	97	Control mouse	RAG	0	0
cell clones‡	Hep-2	83	97	cell clone§			
	HeLa	83	98				
	RPMI 1788	94	95				
	B46MC-1	95	97				

* HL-A as determined by the HL-A xenoantigenic activity.

† Activity is expressed as % inhibition.

‡ The first three are non-lymphoid cell clones and the others are lymphoid cell clones.

§ This is the parental mouse cell clone that was used for cell fusion.

concordant clones were almost equally distributed in the HL-A-positive and IPO B-positive clones, and in the HL-A-negative and IPO B-negative clones. Thus, the observed concordant segregation cannot be attributed to insufficient chromosomal loss from hybrid clones. In the case of segregation of ME1 and HL-A, a similar distribution of concordant clones was observed. A high degree of discordance was seen in the segregation of HL-A and the remaining enzyme markers examined. These markers have a known distribution of nine chromosomes (Table 3). Table 4 also shows a high degree of concordance in the expression of β_2 m and the expression of the enzyme MPI.

Chromosomal analyses were carried out on twenty-four of the thirty-five clones assayed for HL-A xenoantigenic activity. The results of the chromosomal analyses are shown in Table 5. It can be seen that HL-A xenoantigenic activity was expressed in all of the clones in which chromosome 6 was present. Similarly HL-A xenoantigenic activity was absent from clones in which chromosome 6 was absent. Furthermore, no other chromosome was found to be consistently present in HL-A-positive clones and consistently absent from HL-A-negative clones.

TABLE 2. Assessment of HL-A,* human β_2 -microglobulin (β_2 m) and human enzyme phenotypes in man-mouse hybrid clones

Clones	HL-A*	β_2 m	MPI	ME1	IPO B	IPO A	ADA	Pep A	Pep B	LDH B	LDH A	EST D	MDH(S)	G6PD	NP
RLyA3	+	+	+	+	NT	+	NT	-	+	+	NT	-	+	+	+
RLyA12	+	+	+	+	+	NT	NT	NT	NT	NT	NT	-	+	NT	+
RLyG	+	+	+	+	+	+	-	+	+	NT	+	+	+	+	+
RLyJ	+	+	+	+	NT	+	NT	-	-	-	+	+	+	NT	NT
RLyMSAe	+	+	+	+	+	+	-	NT	NT	-	-	-	NT	+	+
RLyMSBc	+	+	+	+	+	+	-	NT	NT	NT	NT	-	-	-	+
RLyMSBe	+	+	+	+	+	+	-	NT	+	+	-	-	-	+	+
RLyMSCd	+	+	+	+	NT	+	-	NT	+	NT	NT	-	+	NT	+
RLyMSCf	+	+	+	+	+	+	NT	+	+	+	NT	NT	+	+	+
RODYLB	+	+	+	+	+	+	NT	NT	NT	NT	NT	-	NT	NT	+
REB4Cc	+	+	+	-	+	+	NT	-	+	+	-	NT	NT	+	+
RLyC9	+	-	-	+	NT	+	NT	+	NT	+	NT	+	NT	+	+
RLyC11	+	-	-	+	NT	+	NT	+	NT	+	NT	+	-	+	+
RLyG8	+	-	-	+	+	+	+	-	NT	+	-	+	-	+	+
RLyG10	+	-	-	+	+	+	NT	+	+	NT	NT	NT	-	NT	+
RLyG12	+	-	-	+	+	+	-	+	+	+	NT	+	-	NT	+
RLyMSWb	+	-	-	-	+	-	-	-	-	-	-	-	-	NT	-
RLyA1	-	+	+	-	NT	+	+	+	-	-	+	-	+	-	+
RLyA4	-	+	+	-	-	+	NT	NT	+	+	+	-	-	NT	+
RLyA7	-	+	+	-	-	+	NT	+	+	+	+	-	+	-	+
RLyA8	-	+	-	-	-	-	-	+	NT	+	+	-	+	-	+
RLyA9	-	+	NT	-	NT	-	NT	+	+	+	+	-	+	-	+
RLyA10	-	+	+	-	-	NT	NT	NT	+	NT	NT	-	NT	NT	NT
RLyA16	-	+	+	-	NT	NT	NT	+	-	-	NT	-	NT	NT	+
RLyMSCg	-	+	+	-	-	+	NT	NT	-	NT	NT	-	NT	-	NT
RLyMSNa	-	+	+	-	-	+	-	-	-	-	-	-	+	+	NT
RLyMSNb	-	+	+	-	NT	+	NT	NT	NT	NT	NT	NT	NT	-	+
RLyMSNf	-	+	+	-	-	NT	-	+	-	-	NT	-	-	+	+
REB4Cb	-	+	+	-	NT	+	-	-	+	-	-	-	-	+	+
REB4Cd	-	+	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
RLyG13	-	-	-	-	-	+	+	+	+	+	+	+	-	+	+
RLyM	-	-	-	-	-	NT	NT	-	-	NT	+	-	NT	NT	+
RLyMSAb	-	-	-	-	-	-	-	NT	-	-	NT	-	-	-	+
RLyMSVa	-	-	NT	-	NT	NT	NT	+	-	NT	NT	-	+	NT	-
REB4Bc	-	-	-	-	-	+	-	-	+	NT	+	+	NT	+	-

* HL-A as determined by the HL-A xenoantigenic activity.

Abbreviations: β_2 m, Beta-2 microglobulin; MPI, mannose phosphate isomerase; IPO A and B, indophenoloxidas A and B; ADA, adenosine deaminase; Pep A and B, peptidas A and B; LDH A and B, lactate dehydrogenases A and B; EST D, esterase D; MDH, soluble malic dehydrogenase; ME1, malic enzyme; G6PD, glucose 6 phosphate dehydrogenase; NP, nucleoside phosphorylase; NT, not tested.

Several clones of RLYMS, derived from the fusion of RAG cells with the normal peripheral lymphocytes from an individual (MS) whose HL-A phenotype is known to be 2, 5, 7 and 12, were tested for HL-A2 alloantigenic activity by the radioimmunoassay. Two clones were positive for HL-A2 alloantigenic activity. The two clones, RLYMSAe and RLYMSWb, were also positive for HL-A xenoantigenic activity and had only chromosomes 6 and 10 in common.

TABLE 3. Segregation analyses of HL-A*, human β_2 -microglobulin (β_2m) and thirteen enzymes in man-mouse hybrid clones

Genetic Marker	Chromosome Assignment	Number of Concordant clones	Number of Discordant clones	Total number of clones examined	Reference of detection method	Reference of chromosome assignment
β_2m	15	16	19	35	Smith <i>et al.</i> , 1975	Smith <i>et al.</i> , 1975
MPI	15	15	16	31	Nichols <i>et al.</i> , 1973	Van Heyningen, <i>et al.</i> , 1975
ME1	6	32	2	34	Ruddle & Nichols, 1971	Van Someren <i>et al.</i> , 1974
IPO B	6	23	0	23	Brewer, 1967	Van Someren <i>et al.</i> , 1974
IPO A	21	18	10	28	Brewer, 1967	Tan <i>et al.</i> , 1973
Pep A	18	10	12	22	Lewis & Harris, 1967	Ruddle, 1974
Pep B	12	16	9	25	Lewis & Harris, 1967	Santachiara <i>et al.</i> , 1970
LDH B	12	14	8	22	Ruddle & Nichols, 1971	Santachiara <i>et al.</i> , 1970
LDH A	11	4	13	17	Ruddle & Nichols, 1971	Boone <i>et al.</i> , 1972
EST D	13	20	10	30	Hopkinson <i>et al.</i> , 1973	Van Heyningen, <i>et al.</i> , 1975
MDH(S)	2	11	13	24	Shows, 1970	Shows, 1970
G6PD	X	16	6	22	Ruddle & Nichols, 1971	Nabholz <i>et al.</i> , 1969
NP	14	17	13	30	Edwards <i>et al.</i> , 1971	Ruddle, 1974
ADA	20	7	9	16	Spencer <i>et al.</i> , 1968	Ruddle, 1974

* HL-A as determined by the HL-A xenoantigenic activity.

TABLE 4. Linkage relationships between HL-A* and human β_2 -microglobulin (β_2 m), MPI, ME1, and IPO B.

		HL-A *				HL-A *	
		+	-			+	-
β_2 m	+	11	13	MPI	+	10	10
	-	6	5		-	6	5
		HL-A *				HL-A *	
		+	-			+	-
ME1	+	15	0	IPO B	+	12	0
	-	2	17		-	0	11
		β_2 m					
		+	-				
MPI	+	20	0				
	-	2	10				

* HL-A as determined by the HL-A xenoantigenic activity.

DISCUSSION

The results of this study indicate that the HL-A xenoantigenic determinant can be assayed in man-mouse hybrid cells by means of radioimmunoassay and that the expression of this determinant in such hybrids is apparently dependent only upon the presence of human chromosome 6. The results also provide direct evidence that the human β_2 m gene segregates independently of the gene coding for both the HL-A alloantigenic and the HL-A xenoantigenic portions of the HL-A molecule. These results are, therefore, in agreement with previous reports on the chromosomal linkage relationships of HL-A antigens (Goodfellow *et al.*, 1975; Smith *et al.*, 1975).

Studies on the inheritance of HL-A alloantigens in families have revealed that the HL-alloantigens are determined by three closely linked structural gene loci (Thorsby, 1974), and that the genes determining HL-A alloantigens are linked to the gene for the enzyme phosphoglucosmutase (PGM 3) (Lamm *et al.*, 1971). Studies on man-Chinese hamster somatic cell hybrids have demonstrated that the PGM 3 gene and the genes determining ME1 and IPO B are located on chromosome 6 (van Someren *et al.*, 1974; Jongsma *et al.*, 1974). van Someren *et al.*, have also demonstrated a good correlation between the occurrence of HL-A alloantigens and the enzymes ME1, IPO B, and PGM 3 in man-Chinese hamster somatic cell hybrids. However, in their study, a number of discordant clones were observed. In six out of thirty-five clones examined, IPO B and HL-A alloantigenic activity segregated discordantly, while in five out of thirty-one clones, ME1 and HL-A allogenic activity segregated discordantly. In attempting to account for the observed discordance, they drew attention to the diffi-

TABLE 5. Human chromosome analyses in man-mouse hybrid clones

Clones	Expression of HL-A†β ₂ m		Human chromosomes •																								
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	
RLyA12	+	+					+	+	+		+		+		+	+			+								
RLyG	+	+					+	+	+	+		+	+	+	+	+			+	+			+		+		
RLyJ	+	+		+			+	+	+	+				+	+	+			+				+				
RLyMSAe	+	+						+	+		+				+	+			+							+	
RLyMSBc	+	+						+	+				+			+	+	+	+								
RLyMSCf	+	+						+	+	+			+			+			+	+			+				
RLyC9	+	-					+	+	+			+	+	+	+		+		+	+			+		+		
RLyC11	+	-					+	+	+	+			+	+	+		+	+	+	+			+	+	+		
RLyG8	+	-							+	+		+			+		+					+	+	+	+		
RLyG12	+	-					+	+	+	+		+		+	+	+			+	+			+		+		
RLyMSWb	+	-							+			+						+									
RLyA1	-	+		+			+	+				+			+	+			+			+	+	+			
RLyA4	-	+					+	+		+			+	+		+	+	+		+		+	+	+			
RLyA7	-	+		+			+	+		+		+	+	+		+	+	+	+	+		+	+	+			
RLyA9	-	+					+	+		+		+	+	+		+	+		+	+	+	+	+	+			
RLyA10	-	+						+		+		+		+		+	+	+		+	+	+					
RLyA16	-	+					+	+				+	+			+	+	+		+	+	+					
RLyMSNa	-	+		+						+							+	+			+		+		+		
RLyMSNb	-	+															+	+					+				
REB4Cb	-	+															+	+					+				
RLyG13	-	-					+			+		+	+	+	+	+		+	+	+						+	
RLyM	-	-											+			+		+	+		+		+				
RLyMSVa	-	-										+	+	+					+	+			+				
REB4Bc	-	-		+				+					+	+	+	+					+		+				

* In each case a minimum of 10 cells was examined.

† HL-A as determined by the HL-A xenoantigenic activity.

culties encountered in studying HL-A alloantigenic expression in hybrid cells, and ascribed this problem to the possibility of reduced expression of HL-A alloantigens after prolonged periods in cell culture. They also suggested the possibility that 'new' HL-A alloantigenic specificities might appear in hybrids due to the interaction between human HL-A and mouse H-2 polypeptide chains.

Studies on the linkage relationships of β_2m in man-mouse somatic cell hybrids by Goodfellow *et al.*, (1975) and by Smith *et al.*, (1975) indicated that β_2m is coded for by a gene carried on chromosome 15 rather than one on chromosome 6, the chromosome previously shown to carry the genetic constituents responsible for the synthesis of the HL-A alloantigens. In both of these studies, however, the independent segregation of β_2m and HL-A was demonstrated by indirect evidence based on the analysis of hybrid cells for β_2m activity, karyotypic composition and the production of enzymes with known chromosomal assignments, rather than by the direct measurement of HL-A activity in the hybrid cells. In the present study, we assayed man-mouse somatic cell hybrids for both the β_2m activity and the HL-A xenoantigenic activity and most directly showed the independent segregation of β_2m and HL-A.

The use of rabbit antisera directed against HL-A molecules greatly facilitated the demonstration of HL-A expression in hybrid cells having different HL-A phenotypes. Since the rabbit antisera contain antibodies directed to the antigenic determinant common to the alloantigenic polypeptide chain of HL-A molecule, it was possible to detect the presence of HL-A alloantigenic chains in clones derived from hybrid cells produced by the fusion of RAG cells with lymphocytes of different individuals, regardless of their HL-A phenotypes. However, even with this radioimmunoassay method for HL-A, the HL-A activity detected was extremely low in the hybrid clones tested, and the amount detected on hybrid cells was usually 1/100, or less, than that produced by human lymphoid cell lines (unpublished data). In order to overcome this difficulty, it was necessary to use cell lysates in high concentration.

The present data indicates that the HL-A alloantigenic chain, as detected by the presence of the HL-A xenoantigenic determinant, is expressed in cell hybrids that do not carry human chromosome 15 and, thus, do not produce any human β_2m . Similarly, hybrid clones were obtained that expressed β_2m in the absence of demonstrable HL-A xenoantigenic activity. However, in both types of clones, we have obtained evidence that hybrid molecules may be formed between human β_2m and mouse H-2 alloantigenic chains, and between mouse β_2m and human HL-A alloantigenic chains (unpublished data).

Recently, Ostberg *et al.* (1975) reported that a Burkitt lymphoma cell line, Daudi, does not produce β_2m and yet produces a polypeptide chain that is devoid of HL-A allospecificity but is very similar in the xenoantigenic and physicochemical characteristics to the HL-A alloantigenic polypeptide chains isolated from other human cell lines and suggested that β_2m and the HL-A alloantigenic chains are under separate genetic regulation. They also mentioned that Daudi cells have a deletion of chromosome 15.

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