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Studies of the linkage relationship of beta-2-microglobulin in man-mouse somatic cell hybrids

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The polymorphic human HL-A histocompatibility antigens, found both on cell surfaces and in the circulation, are associated in a bi-molecular non-covalent fashion with the beta-2-microglobulin molecule ($\beta 2\mu$) (Nakamuro, Tanigaki & Pressman, 1973). Although the function of $\beta 2\mu$ is unknown, it represents a chemically constant and well-defined portion of the known HL-A moieties (Nakamuro *et al.* 1973; Grey *et al.* 1973).

Family studies have revealed that variations seen in HL-A antigens are determined by multiple alleles at three structural gene loci (Thorsby, 1974) and that the human HL-A gene complex segregates in close association with the locus for the enzyme phosphoglucomutase (PGM_3) (Lamm, Svejaard & Kissmeyer-Nielsen, 1971). Studies in man-Chinese hamster somatic cell hybrids by Van Someren *et al.* (1974) indicate that the PGM_3 locus is syntenic with the loci for cytoplasmic malic enzyme (ME_1) and tetrameric indophenol oxidase (IPO_B). The loci for these three enzymes and thus for the *HL-A* gene complex, were shown by these workers to be on chromosome 6. Considerable difficulty was encountered in determining the HL-A phenotypes in hybrid cells (Van Someren *et al.* 1974). HL-A expression has been shown to be altered in fibroblasts cultured for prolonged periods (Goldstein, 1972).

Polymorphism of $\beta 2\mu$ has not yet been described, hence conventional family studies cannot be used to determine the linkage relationships of this molecule. Fortunately $\beta 2\mu$ is produced by most human cells even after prolonged periods of culture, and also by freshly isolated lymphocytes (Evrin & Nilsson, 1974). It was, therefore, possible for us to perform a study of the genetic linkage relationships of $\beta 2\mu$ in man-mouse somatic cell hybrids.

MATERIALS AND METHODS

Cell types studied. In this investigation four human cell types were used. These were Chang cells, a long-term fibroblastic line, normal peripheral lymphocytes separated from the blood of a healthy donor on Ficoll-Hypaque (Böyum, 1967) and the established lymphoid culture lines ODY and EB4. These human cells were fused with RAG cells, a hypoxanthine-guanine-phosphoribosyl transferase-deficient mutant mouse cell line isolated by Ruddle *et al.* (1970). The Chang cells and the RAG cells were maintained in McCoy's 5a medium supplemented with 10% foetal calf serum. Lymphoid lines were maintained in RPMI 1625 medium with 10% foetal calf serum.

Cell fusion. Cell fusions were carried out in suspension at pH 7.8 as described by Croce, Koprowski & Eagle (1972), using Sendai virus that had been inactivated by exposure to ultraviolet light according to the method of Harris & Watkins (1965). In the RAG Chang fusions, the

ratio of RAG cells to Chang cells was 100 to 1. In the RAG lymphocyte fusions, equal numbers of the two cell types were used. Equal numbers of cells were used in lymphoid fusions.

After fusion, cells were grown in the supplemented McCoy's 5a medium described above, but containing 10^{-4} M hypoxanthine, 10^{-5} M aminopterin, 4×10^{-5} M thymidine and 10^{-5} M glycine; this is the HAT selective medium described by Littlefield (1966). In the HAT selective medium, RAG cells and RAG-RAG fused cells were progressively eliminated. Normal peripheral lymphocytes and the lymphoid cell lines were non-adherent cells and were completely discarded during sequential changes of the selective culture medium. Thus, in the RAG cell-lymphocyte fusions and in RAG cell-lymphoid line fusions only hybrid cells grew in the selective HAT medium and adhered to the vessel surface. However, in the RAG-Chang fusions, hybrid cells had to be separated from parental Chang cells by means of cloning. All hybrid cells were cloned using the method described by Ham & Puck (1962).

Radioimmunoassay for $\beta 2\mu$. The concentrations of human $\beta 2\mu$ in spent culture media and in cell lysates were determined by means of a radioimmunoassay employing ^{125}I - $\beta 2\mu$ and a double antibody technique for the separation of antibody-bound from free ^{125}I - $\beta 2\mu$ (Hunter, 1973). Purified human $\beta 2\mu$ and rabbit anti-human- $\beta 2\mu$ antiserum were kindly provided by Dr M. D. Poulik.

Cell lysates for $\beta 2\mu$ assay were prepared by washing cultured cells in four changes of Dulbecco's phosphate-buffered saline (pH 7.2) and then subjecting them to three cycles of freezing and thawing. The lysates were centrifuged at 200 g and the supernatants were assayed for $\beta 2\mu$.

Studies of cell-surface-associated $\beta 2\mu$. The presence of $\beta 2\mu$ on the surfaces of viable cells was sought by means of an indirect immunofluorescence technique (Thomson & Alexander, 1973). Monolayers of cell clones were trypsinized, and the resulting single cell suspensions were incubated at 37° C. in unsupplemented McCoy's 5a medium for 2-4 hr. The cells were then incubated with a 1:10 dilution of rabbit anti-human $\beta 2\mu$ for 30 min., washed, and finally exposed to fluorescein-conjugated goat anti-rabbit IgG. In control experiments, cells were incubated with normal rabbit serum, washed and then exposed to fluorescein-conjugated goat anti-rabbit IgG. After washing, the cells were examined for surface fluorescence employing a Reichert fluorescence microscope with an incident light source as described by Thomson & Alexander (1973).

Enzyme analyses. The presence of 15 murine, human, and hybrid enzyme phenotypes was sought in cell lysates from individual clones by means of starch-gel electrophoresis. The enzyme markers in question are distributed on 12 of the human chromosomes. In preparing lysates for enzyme studies, cells were washed in Dulbecco's phosphate-buffered saline buffer (pH 7.2) and were then subjected to 1 or 2 cycles of freezing and thawing. The enzymes studied are listed, and the methods employed in their detection are referenced in Tables 1-3. For the enzymes selected for study, the human enzyme phenotypes differed clearly in electrophoretic mobility from those of the mouse

Karyotype analyses For karyotype analyses, cell cultures were treated with colchicine, at a final concentration of 2.5 $\mu\text{g./ml.}$ for 90 min. The cell monolayers were then trypsinized and subjected to hypotonic treatment for 15 min. in 0.075 M-KCl at 37° C. The resulting preparations were fixed in a solution consisting of 3 parts of methyl alcohol and 1 part of acetic acid for at least 2 hr. Air-dried drop preparations were made, and these chromosomes were then banded using a modification of the trypsin-Giemsa banding technique of Seabright (1971). Slides were placed in a 60° C. oven overnight. They were then cooled and immersed in a solution of 0.1 %

Table 1. *Assessment of human enzyme phenotypes and human $\beta 2\mu$ in 33 primary clones of man-mouse somatic cell hybrids*

(Hybrid cells were initially selected in HAT medium. Thereafter cloning was carried out in McCoy's medium with 20 % foetal calf serum.)

Clones	$\beta 2\mu$	G-6-PD	LDH A	LDH B	Pep B	PHI	ME 1	IPO B	IPO A	ADA	NP	Pep A	MPI	MDH(S)	ICD(S)	EST D
RC A ₂	+	-	-	+	+	-	NT	NT	-	-	+	-	-	-	-	+
RC A	+	+	-	+	NT	NT	+	+	NT	-	+	NT	NT	+	NT	NT
RC D	+	-	-	+	+	-	+	+	+	-	+	+	+	-	-	+
RC K	+	-	-	+	+	NT	+	+	+	-	+	-	-	+	NT	+
RC L	+	+	-	+	+	+	+	+	-	-	+	NT	-	+	+	+
RC N	+	-	-	+	NT	+	+	+	+	-	+	-	-	NT	NT	+
RC O	+	-	-	+	+	-	+	+	+	NT	+	NT	NT	+	-	NT
RC P ₂	+	-	-	+	+	-	+	+	+	NT	+	-	-	+	+	+
RC Q	+	-	-	+	+	-	+	+	+	NT	+	NT	-	-	+	+
RC Q ₂	+	NT	-	+	NT	-	+	+	-	NT	+	NT	-	NT	+	+
RC R	+	+	-	+	+	NT	-	+	+	NT	+	+	-	-	-	+
RC S	+	-	-	+	NT	+	+	+	+	-	+	NT	NT	-	-	+
RC Y	+	-	-	+	NT	+	+	+	+	-	+	+	-	-	-	+
RC D ₂	+	+	-	+	NT	-	+	NT	+	NT	+	NT	+	+	+	+
RC C ₂	+	-	-	+	NT	NT	+	NT	NT	NT	+	NT	NT	-	+	NT
RC Tb	-	-	-	+	+	-	+	+	+	-	+	+	-	-	-	+
RLy A	+	+	-	+	+	NT	-	-	-	NT	NT	+	+	+	+	+
RLy C	+	+	-	+	+	NT	+	+	+	NT	+	+	-	+	+	+
RLy E	+	+	-	+	+	NT	+	+	+	-	+	+	+	+	+	+
RLy F	+	+	-	+	+	NT	+	+	-	NT	+	+	+	-	-	+
RLy G	+	+	+	+	+	NT	+	+	+	-	+	+	+	+	+	+
RLy EB	+	NT	+	NT	+	NT	-	NT	+	NT	+	+	+	+	NT	+
RLy J	+	NT	+	-	-	NT	+	NT	+	NT	+	+	+	NT	NT	+
RLy M	-	NT	+	+	-	NT	-	-	NT	NT	+	-	-	NT	NT	-
RLy B	-	+	-	-	-	NT	-	-	+	NT	+	-	-	-	-	-
RLy H	-	NT	-	+	-	NT	-	-	-	NT	+	-	-	NT	NT	-
REB ₄ Bb	-	NT	NT	NT	NT	NT	-	NT	NT	NT	NT	NT	-	NT	NT	+
REB ₄ Bc	-	+	+	+	+	NT	-	-	+	-	-	-	-	NT	NT	+
REB ₄ Cb	+	NT	NT	NT	NT	NT	-	NT	NT	+	NT	-	+	-	NT	-
REB ₄ Cc	+	+	-	+	NT	NT	-	NT	+	NT	+	NT	+	NT	NT	+
RODy C	+	+	+	+	+	NT	+	+	+	-	+	-	+	NT	NT	-
RODy H	+	NT	+	+	NT	NT	+	+	NT	NT	+	NT	+	-	NT	+
RODy Lb	+	NT	NT	NT	NT	NT	+	NT	+	NT	+	NT	+	NT	NT	-

ABBREVIATIONS: $\beta 2\mu$, Beta-2-microglobulin; G-6-PD, Glucose-6-phosphate dehydrogenase; LDH A, LDH B, lactate dehydrogenases A and B; Pep A, Pep B, peptidases A and B; PHI, phosphohexose isomerase; ME 1, malic enzyme (soluble); IPO A, IPO B, indophenol oxidases A and B; MDH(S), soluble NAD malate-dehydrogenase; ICD(S), soluble isocitrate dehydrogenase; EST D, esterase D; NT, not tested.

trypsin in Hanks Balanced Salt Solution (HBSS) at pH 7.0 at 4° C. for 30–40 sec. The slides were then rinsed twice in HBSS at 4° C. Only the first rinse contained 1 % foetal calf serum. Such preparations were finally stained in 4 % Harleco Giemsa stain in Gurr's buffer, at pH 6.8 for 4 min., and then rinsed in the same buffer.

RESULTS

Classification of the hybrid cells by radioimmunoassay for $\beta 2\mu$ in spent culture media. The cells of hybrid clones were classified as $\beta 2\mu$ -positive or $\beta 2\mu$ -negative on the basis of whether or not $\beta 2\mu$ was detectable, by radioimmunoassay, in their spent culture media. Depending upon the age of the ^{125}I - $\beta 2\mu$, the radiolabelled product used in the assay, the lower limit of sensitivity of the radioimmunoassay for $\beta 2\mu$ ranged from 1 ng./ml. to 6 ng./ml. Fresh McCoy's 5a medium supplemented with 10 % foetal calf serum, identical medium which had been in contact with 10^6 RAG cells for 48 hr., and medium from RAG cells that had been exposed to the full fusion procedure in the absence of human cells, always gave assay values below the level of sensitivity of the procedure. Spent culture media which had been exposed to 10^6 Chang cells for 48 hr. gave $\beta 2\mu$ values of 100–200 ng./ml.

Man-mouse hybrid clones were considered to be $\beta 2\mu$ -positive if medium exposed to 10^6 cells for 48 hr. gave assay values for human $\beta 2\mu$ that were greater than 10 ng./ml. Man-mouse hybrid clones were classified as $\beta 2\mu$ -negative if the assay values for $\beta 2\mu$ were the same as, or less than, those obtained with medium exposed to RAG cells alone. On the basis of these criteria, 27 of the 33 primary hybrid clones studied were classified as $\beta 2\mu$ -positive, while 6 of the 33 primary clones examined were classified as $\beta 2\mu$ -negative (see Table 1 and Appendix). Of the 26 secondary RAG-lymphocyte hybrid clones, 17 were $\beta 2\mu$ -positive while 9 were $\beta 2\mu$ -negative (Table 2).

The absence of $\beta 2\mu$ from the spent culture media of $\beta 2\mu$ -negative clones may have been due to any of the following causes: (a) cessation of $\beta 2\mu$ synthesis by the cells in question; (b) continued synthesis of $\beta 2\mu$ by the cells in question with incorporation into the cell membrane, but failure of the material to be secreted into the culture medium; or (c) continued synthesis of $\beta 2\mu$ but with failure of the material to be either secreted into the culture medium or incorporated into the cell membrane. These possibilities were evaluated in the studies of cell lysates and in the immunofluorescence investigations of the hybrid cell clones under consideration.

Radioimmunoassay for $\beta 2\mu$ in cell lysates. The lysates of $2.5\text{--}5.0 \times 10^6$ RAG cells showed no evidence of $\beta 2\mu$ content. Similar results were obtained with the lysates derived from hybrid cell clones classified as $\beta 2\mu$ -negative. On the other hand, lysates derived from the same numbers of cells from hybrid clones which had been classified as $\beta 2\mu$ -positive gave assay values for $\beta 2\mu$ of 9–17.3 ng./ml.

Immunofluorescence studies. Immunofluorescence studies of viable cells derived from primary hybrid clones classified as $\beta 2\mu$ -positive demonstrated specific surface staining for $\beta 2\mu$. Cells of $\beta 2\mu$ -negative primary hybrid clones showed no such evidence for $\beta 2\mu$ on their surfaces.

Studies of enzyme phenotypes in hybrid clones. The 33 primary clones and the 26 secondary clones were examined for the expression of 15 different enzyme phenotypes. The presence or absence of the human enzyme phenotypes in each clone are shown in Tables 1 and 2; the segregation analyses of human $\beta 2\mu$ and the 15 human enzyme markers examined in the 33 primary clones are summarized in Table 3.

Table 2. Assessment of human enzyme phenotypes and human $\beta 2\mu$ in 26 secondary Rag-lymphocyte clones

Clones	$\beta 2\mu$	G-6-PD	LDH A	LDH B	Pep B	ME 1	IPO B	IPO A	ADA	NP	Pep A	MPI	MDH(S)	ICD(S)	EST D
RLy C ₁	+	NT	NT	NT	NT	NT	NT	+	NT	NT	NT	+	NT	NT	+
RLy C ₂	-	+	NT	NT	-	+	+	NT	NT	+	+	-	NT	NT	+
RLy C ₃	-	NT	NT	NT	NT	-	-	NT	NT	NT	+	-	-	NT	+
RLy C ₄	-	+	NT	NT	-	+	+	NT	-	+	+	-	NT	NT	+
RLy C ₅	-	NT	-	+	+	+	+	+	NT	+	+	-	NT	NT	+
RLy C ₆	-	+	-	-	-	-	-	NT	NT	-	+	-	NT	NT	-
RLy C ₇	+	+	-	-	-	-	-	NT	NT	NT	-	+	NT	NT	+
RLy C ₈	-	NT	NT	NT	+	-	NT	NT	NT	NT	-	-	NT	NT	+
RLy C ₉	-	NT	-	+	NT	+	NT	NT	NT	+	+	-	NT	NT	+
RLy C ₁₁	-	NT	-	+	+	+	NT	NT	NT	+	+	-	NT	NT	+
RLy C ₁₂	-	NT	NT	NT	-	-	-	NT	NT	-	-	-	-	NT	-
RLy A ₁	+	NT	NT	NT	-	NT	NT	+	+	+	+	+	NT	NT	-
RLy A ₃	+	+	NT	NT	+	NT	NT	NT	NT	NT	-	+	NT	NT	-
RLy A ₄	+	NT	-	+	+	-	NT	+	NT	+	NT	+	NT	NT	-
RLy A ₅	+	-	-	NT	NT	NT	NT	+	NT	-	NT	+	NT	NT	-
RLy A ₆	+	-	NT	+	NT	NT	NT	+	NT	NT	NT	+	NT	NT	-
RLy A ₇	+	-	-	+	+	-	NT	+	NT	+	NT	+	NT	NT	-
RLy A ₈	+	-	-	+	NT	NT	NT	NT	NT	+	NT	-	NT	NT	-
RLy A ₉	+	NT	-	+	+	-	NT	NT	NT	+	NT	NT	NT	NT	NT
RLy A ₁₀	+	NT	NT	+	+	-	NT	NT	NT	+	NT	+	NT	NT	-
RLy A ₁₁	+	-	-	NT	NT	-	NT	NT	NT	NT	NT	+	NT	NT	-
RLy A ₁₂	+	NT	NT	+	NT	NT	NT	NT	NT	+	NT	+	NT	NT	-
RLy A ₁₃	+	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	-
RLy A ₁₄	+	-	NT	NT	NT	NT	NT	NT	NT	NT	NT	+	NT	NT	-
RLy A ₁₅	+	NT	NT	NT	NT	NT	NT	+	NT	+	NT	+	NT	NT	-
RLy A ₁₆	+	-	-	-	-	-	NT	NT	NT	+	+	+	NT	NT	-

Table 3. Segregation analyses of $\beta 2\mu$ and 15 enzymes in 33 primary clones of man-mouse hybrids

Enzyme	Reference for enzyme detection	Reference for chromosome assignment	RAG-Chang hybrids		RAG- lymphocyte hybrids		RAG- lymphoid line hybrids		Cumulative data	
			No. of concordant clones	No. of discordant clones	No. of concordant clones	No. of discordant clones	No. of concordant clones	No. of discordant clones	No. of concordant clones	No. of discordant clones
G-6-PD	Ruddle & Nichols (1971)	Nabholz <i>et al.</i> (1969)	5	10	5	1	2	1	12	12
LDH A	Ruddle & Nichols (1971)	Boone <i>et al.</i> (1972)	1	15	4	5	2	2	7	22
LDH B	Ruddle & Nichols (1971)	Santachiara <i>et al.</i> (1970)	15	1	6	3	3	1	24	5
Pep B	Lewis & Harris (1967)	Santachiara <i>et al.</i> (1970)	8	1	9	1	1	1	18	3
PHI	Detter <i>et al.</i> (1968)	McMorris <i>et al.</i> (1973)	4	8	—	—	—	—	4	8
ME 1	Ruddle & Nichols (1971)	Van Someren <i>et al.</i> (1974)	12	2	8	2	5	2	25	6
IPO B	Brewer (1967)	Van Someren <i>et al.</i> (1974)	11	2	7	1	3	0	21	3
IPO A	Brewer (1967)	Tan <i>et al.</i> (1973)	10	4	6	3	3	1	19	8
ADA	Spencer <i>et al.</i> (1968)	Ruddle (1974)	1	8	0	2	2	1	3	11
NP	Edwards <i>et al.</i> (1971)	Ruddle (1974)	15	1	5	3	5	0	25	4
Pep A	Lewis & Harris (1967)	Ruddle (1974)	3	6	9	1	1	2	13	9
MPI	Nichols <i>et al.</i> (1973)	Van Heyningen <i>et al.</i> (1975)	4	8	9	1	7	0	20	9
MDH(S)	Shows (1970)	Shows (1970)	7	8	5	1	0	2	12	11
ICD(S)	Shows (1970)	Shows (1970)	4	8	5	1	—	—	9	9
EST D	Hopkinson <i>et al.</i> (1973)	Van Heyningen <i>et al.</i> (1975)	12	1	10	0	2	5	24	6

Table 4. Analyses of $\beta 2\mu$ and the enzymes MPI, ME 1, EST D, NP, Pep B and Pep A in 26 Rag-lymphocyte secondary clones

MPI	(a)	$\beta 2\mu$	ME 1	(b)	$\beta 2\mu$
	+	+		+	+
	13	0		0	6
	-	1		-	6
		9			3
EST D	(c)	$\beta 2\mu$	NP	(d)	$\beta 2\mu$
	+	+		+	+
	1	8		9	6
	-	15		-	2
		1			1
Pep B	(e)	$\beta 2\mu$	Pep A	(f)	$\beta 2\mu$
	+	+		+	+
	5	3		2	6
	-	3		-	2
		4			3

From the results obtained on examination of primary clones it was not possible to exclude linkage of human $\beta 2\mu$ to the following chromosomes: 6, 12, 14, and 13. Furthermore, although the discordance rates between $\beta 2\mu$ and both MPI (determined by chromosome 15) and Pep A (determined by chromosome 18) were high in the RAG-Chang hybrids, the discordance rates for $\beta 2\mu$ and these enzymes were low in the RAG-lymphocyte hybrids. In the RAG-lymphoid line hybrids a high degree of concordance was observed in the occurrence of human $\beta 2\mu$ and MPI. It is possible that the observed discrepancies between human $\beta 2\mu$ and MPI occurrence in the RAG-Chang hybrids could be due to chromosomal rearrangements in the long-term Chang cell line. For this reason, 26 secondary RAG-lymphocyte clones were examined. Segregation analyses of human $\beta 2\mu$ and the human phenotypes of the enzymes MPI, ME 1, Est D, NP, Pep A and Pep B in the secondary clones are summarized in Table 4. From this table it will be seen that a high degree of concordance was observed only between $\beta 2\mu$ and MPI.

Studies of hybrid clone karyotypes. Results of these studies are summarized in Table 5. Karyotypic analyses in the primary hybrid clones revealed a high degree of discordance between $\beta 2\mu$ production and the occurrence of chromosomes 1, 3, 4, 5, 7, 8, 9 and 10 for which no enzyme markers were examined. In 1 of the $\beta 2\mu$ -negative primary clones and in 4 of the $\beta 2\mu$ -negative secondary clones, chromosome 6 could be clearly demonstrated. Karyotypic studies have so far been done on 5 of the secondary RAG-lymphocyte clones; results support the enzyme studies in suggesting linkage of the gene determining $\beta 2\mu$ production to chromosome 15.

DISCUSSION

Results of our studies in RAG-lymphocyte and in RAG-lymphoid line hybrids show synteny of $\beta 2\mu$ and MPI. The gene determining human MPI production has been mapped to chromosome 15 (Van Heyningen *et al.* 1974). Chromosome studies in our hybrids support the results of enzyme studies and indicate localization of the genes determining $\beta 2\mu$ and MPI production to

Table 5. *Human chromosomes*

Clones	Primary cell clones																						Abnormal chromosomes
	1	2	3	4*	5*	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21†	22†	Y
RC R	.	.	.	.	.	.	.	.	+	.	.	+	+	+	.	.	+	+	+	.	+	.	.
RC K	+	+	.	.	.	+	.	+	+	.	.	+	+	+	+	+	+	.	.	.	+	+	.
RLy G	.	+	.	+	+	+	+	.	.	.	+	+	+	+	+	.	+	+	+	.	+	+	+
RLy F	.	.	.	+	+	+	.	.	.	.	.	+	+	+	+	.	+	+	+	.	+	+	.
RLy E	.	+	.	+	+	+	.	.	.	.	.	+	+	+	+	+	+	+	+	.	+	+	.
RLy J	.	+	.	+	+	+	+	.	.	.	+	+	+	+	+	.	+	.	.	.	+	+	.
RLy EB	.	+	.	+	+	.	.	.	.	.	.	+	+	+	.	+	+	+	.	.	+	+	.
RLy M	.	.	.	.	.	.	.	.	.	.	+	.	.	+	.	+	+	.	+	.	.	.	.
RLy B	.	.	.	.	.	.	+	.	.	.	+	.	.	+	.	.	.	.	.	.	.	.	+
RC Tb	.	.	+	.	.	+	+	.	.	.	.	+	.	+	.	+	.	+	.	.	+	.	.
Secondary cell clones																							Abnormal small metacentric
RLy C ₂	.	.	+	+	.	+	.	.	.	.	.	.	+	+	.	+	.	+	+	.	+	.	.
RLy C ₅	.	.	.	.	.	+	.	.	.	.	.	.	+	+	.	+	.	+	+	.	+	.	.
RLy C ₇	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	+	+	.	.	.	.	+	.
RLy C ₉	.	.	.	.	.	+	.	.	.	.	.	+	+	+	+	+	+	+	.	+	.	.	.
RLy C ₁₁	.	.	+	.	.	+	+	.	.	.	.	+	+	+	.	+	+	+	+	.	.	.	.

Minimum of 10 cells examined. Positive indicates at least 50 % of cells carry the chromosome in question.

In parental Chang cells 60-70 chromosomes were present. At least six abnormal chromosomes were present; the origin of these abnormal chromosomes was not clear.

* Chromosomes 4 and 5 could not easily be distinguished from each other.

† Chromosomes 21 and 22 could not easily be distinguished from each other.

chromosome 15. Our findings are therefore in agreement with those of Goodfellow *et al.* (1975), who recently assigned the $\beta 2\mu$ gene to chromosome 15. These workers used a cytotoxicity assay to determine the presence of human $\beta 2\mu$ in man-mouse somatic cell hybrids.

In RAG-Chang hybrids MPI and $\beta 2\mu$ apparently segregated independently. It is well known that chromosomal rearrangements occur in long-term fibroblast lines and this probably limits the usefulness of these lines in mapping studies. Chromosomal rearrangements apparently occur less frequently in lymphoid cell lines (Povey *et al.* 1973).

It is interesting to note that the clone RLY C was initially classified as $\beta 2\mu$ -positive, MPI negative. Two of the 11 secondary clones derived from this clone were $\beta 2\mu$ -positive, MPI positive, while 9 were $\beta 2\mu$ -negative, MPI negative. This indicates that the level of $\beta 2\mu$ production in these cells exceeds that of the MPI production, or that the detection system for human $\beta 2\mu$ is much more sensitive than the MPI detection system. Therefore, clones in which a low percentage of cells are positive for chromosome 15 could be positive for $\beta 2\mu$ and apparently negative for MPI.

The fact that the primary hybrid clones classified, during this study, as $\beta 2\mu$ -negative had lost the capacity to synthesize, rather than the ability to transport $\beta 2\mu$ was demonstrated by both the immunofluorescence studies and by radioimmunoassays performed on cell lysates. Cells of the $\beta 2\mu$ -negative clones failed to show the $\beta 2\mu$ molecule either in their membranes or in the supernatants of their lysates. Thus, the $\beta 2\mu$ -negative clones described here appear to lack the structural and/or the regulator gene for $\beta 2\mu$ production.

Discordant segregation of $\beta 2\mu$ and chromosome 6 was clearly demonstrated. If the assignment of the gene determining HL-A alloantigens to chromosome 6 is correct, our findings indicate that the gene responsible for $\beta 2\mu$ production is not syntenic with the gene responsible for HL-A alloantigen production.

SUMMARY

Beta-2-microglobulin ($\beta 2\mu$) production has been studied in 33 primary man-mouse hybrid clones and in 26 secondary man-mouse hybrid clones. These clones have also been examined for the presence of 15 human enzyme phenotypes. Karyotypic analyses have been carried out on clones. From these studies the following conclusions can be drawn:

(1) Gene(s) determining human $\beta 2\mu$ production in humans are apparently syntenic with the *MPI* gene on chromosome 15.

(2) Long-term fibroblast lines may be of limited use in mapping studies as chromosomal rearrangements frequently occur in these lines.

(3) The gene(s) determining $\beta 2\mu$ production in humans segregate independently of chromosome 6. If the assignment of the genes determining HL-A alloantigens to chromosome 6 is correct, our results imply that $\beta 2\mu$ and HL-A alloantigens are determined by genes carried on different chromosomes, despite the fact that $\beta 2\mu$ forms an integral part of the HL-A molecule.

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APPENDIX

*Concentration of Beta-2-microglobulin in spent culture medium**

Cell types	Concentration (ng./ml.)	Cell types	Concentration (ng./ml.)
RAG	ND†	RAG-lymphoid primary clones	
Chang	100-200	RODY C	100.0
RAG-Chang primary clones		RODY H	38.0
RC A2	28.5	RODY Lb	94.0
RC C2	27.0	REB ₄ Cc	62.0
RC D	26.8	REB ₄ Cb	25.0
RC D2	100.0	REB ₄ Bb	ND
RC F	28.4	REB ₄ Bc	ND
RC K	18.2	RAG-lymphocyte secondary clones	
RC L	72.0	RLy C1	25.0
RC N	11.4	RLy C2	ND
RC O	12.0	RLy C3	ND
RC P2	27.6	RLy C4	ND
RC Q2	100.0	RLy C5	ND
RC Q	32.0	RLy C6	ND
RC R	22.8	RLy C7	20.0
RC S	12.8	RLy C8	ND
RC Y	80.0	RLy C9	ND
RC Tb	ND	RLy C11	ND
RAG-lymphocyte primary clones		RLy C12	ND
RLy A	44.2	RLy A1	100.0
RLy C	28.4	RLy A3	100.0
RLy E	92.0	RLy A4	100.0
RLy F	100.0	RLy A5	35.5
RLy G	71.0	RLy A6	33.5
RLy J	49.0	RLy A7	100.0
RLy EB	11.7	RLy A8	57.0
RAG-lymphocyte primary clones		RLy A9	26.0
RLy B	ND	RLy A10	100.0
RLy M	ND	RLy A11	40.0
RLy H	ND	RLy A12	90.0
		RLy A13	63.0
		RLy A14	31.0
		RLy A15	29.0
		RLy A16	100.0

* Medium exposed to approximately 1×10^6 cells for 48 hr.

† ND: Not detectable values below level of sensitivity of assay.