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Regional localization of *HLA*, ME_S, and SOD_M on chromosome 6

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The four human HLA gene loci have been shown by family studies to be closely linked to each other and to the gene coding for phosphoglucomutase 3 (PGM_3).[1] PGM_3 and the genes determining the production of soluble malic enzyme (ME_S) and mitochondrial superoxide dismutase (SOD_M) were shown through studies in man-Chinese hamster somatic cell hybrids to be located on chromosome 6.[2] HLA alloantigenic activity was studied in the hybrid clones, but difficulties were encountered in determining HLA alloantigenic expression.[2] FRANCKE and PELLEGRINO[3] studied the expression of human HLA alloantigens and human PGM_3 and ME_S in hybrid cells produced by fusing Chinese hamster cells with human cells carrying a translocation between chromosomes 1 and 6, t(1;6) (p3200;p2100). From the results of this study, it was concluded that the HLA gene complex is located in the region 6pter→6p2100 of the short arm of chromosome 6, while the PGM_3 and ME_S loci map in the region 6p2100→6qter.

The HLA molecule released from cell membranes after papain digestion consists of two polypeptide chains: an 11,000-dalton chain (identified as beta-2-microglobulin) and a 33,000-dalton chain.[4] Each 33,000-dalton HLA polypeptide chain carries an alloantigenic determinant. Different HLA polypeptide chains carrying different HLA alloantigenic determinants share a common antigenic determinant which stimulates antibody production in heterologous animals.[5] This antigenic determinant has been referred to as the xenoantigenic determinant and is thought to represent the product of the invariable portion of the HLA

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genes.[5] Studies in man-mouse somatic cell hybrids showed that the gene determining the HLA xenoantigenic determinant in syntenic with SOD_M and ME_S and is carried on chromosome 6.[6] In the study described here, 12 different man-mouse hybrid clones, in which a deleted chromosome 6 was present, were used to determine the regional localization on chromosome 6 of the gene determining the HLA xenoantigenic determinant and the SOD_M and ME_S genes.

Cell lines used in these experiments were RAG, a hypoxanthine guanine phosphoribosyl transferase-deficient mouse cell line derived from a renal adenocarcinoma,[7] and Cali, a human lymphoid cell line obtained from Dr. J. FOGH, Sloan-Kettering Institute, Rye, New York. The Cali line, which was derived from a pleural effusion, grows in suspension and produces human immunoglobulins, as determined by immunoelectrophoresis. Twenty metaphases from the Cali line were examined, using conventional chromosome banding techniques; cells were found to have a 46,XY karyotype with no abnormal chromosomes.

Cells were fused using inactivated Sendai virus, according to methods previously described.[8] Following fusion, cells were grown in HAT selective medium, containing 4×10^{-7} M aminopterin, 4×10^{-5} M thymidine, and 10^{-4} M hypoxanthine. In this medium RAG cells were progressively eliminated and unfused Cali cells were discarded during medium changes. In one flask a vigorously growing colony was observed after 3 weeks; this colony was subsequently cloned in HAT medium. Cloning was achieved by seeding cells at low density (100 cells/5 ml/T25 flask) and subsequently isolating clones, which had apparently arisen from single cells, by means of stainless-steel cylinders. These clones were designated A, B, C, etc., and were considered to be primary clones, possibly not of independent origin. One primary clone, clone U, was found on karyotypic analysis to have several different cell populations and was recloned; the secondary clones were referred to as U1, U2, etc. Between the 5th and 10th passage, clones were studied to determine their chromosomal constitution, their expression of a number of different enzymes, and their expression of the human HLA xenoantigenic determinant.

Starch-gel electrophoresis was used to determine the mouse, human, and hybrid isozyme forms of 19 different enzyme systems. The enzyme systems studied and their human chromosomal assignments are listed in table I. The starch-gel electrophoresis methods used were those described by HARRIS and HOPKINSON,[9] and the human chromosomal assignments are those listed by them.[9]

Karyotypic analyses were carried out on banded metaphase chromosomes using methods previously described.[8] Alkaline Giemsa staining[10] was used to facilitate distinction of mouse and human chromosomes. A clone was considered to be positive for a specific human chromosome when (1) a human enzyme known to be determined by a gene on that human chromosome was identified in the hybrid and (2) if the specific human chromosome could be identified in at least 20% of the metaphases examined. Between 10 and 20 metaphases from each clone were examined. In the case of chromosomes 3, 4, 5, 7, 8, 16, 17, 22, and Y, the presence of the chromosome in the hybrid clone was determined by karyotyping alone.

Table I. Enzyme systems studied and chromosomal assignments.

Enzyme	E.C. No.	Human chromosome
Peptidase C	3.14.11 or 13	1
Alpha-fucosidase	3.2.1.51	1
Malate dehydrogenase (soluble)	1.1.1.37	2
Isocitrate dehydrogenase (soluble)	1.1.1.42	2
Malic enzyme (soluble)	1.1.1.40	6
Superoxide dismutase (mitochondrial)	1.15.1.1	6
Adenylate kinases 1 and 3	2.7.4.3	9
Glutamic oxaloacetic transaminase	2.6.1.1	10
Lactate dehydrogenase A	1.1.1.27	11
Lactate dehydrogenase B	1.1.1.27	12
Peptidase B	3.4.11 or 13	12
Esterase D	3.1.1.1	13
Purine nucleoside phosphorylase	2.4.2.1	14
Mannose phosphate isomerase	5.3.1.8	15
Peptidase A	3.4.11 or 13	18
Glucose phosphate isomerase	5.3.1.9	19
Adenosine deaminase	3.5.4.4	20
Superoxide dismutase (soluble)	1.15.1.1	21
Glucose-6-phosphate dehydrogenase	1.1.1.49	X

Radioimmunoassay for the HLA xenoantigenic determinant was carried out according to the method of NAKAMURO et al.[5] Cells used in the assay were solubilized with nonionic detergent; $1–3 \times 10^7$ cells/ml were treated with a 1 % solution of nonionic detergent. Activity was defined as the capacity of a specific quantity (50 μl) of cell lysate to inhibit the binding of an ^{125}I-labeled preparation of purified HLA antigen by rabbit antiserum specific for HLA.

In 8 of the 12 RAG/Cali hybrid clones examined, a deleted chromosome 6 was found. This chromosome was apparently deleted beyond the point 6q16. The deleted chromosome was shown to contain only human chromosomal material by Giemsa-11 staining. No mouse-human translocations were present in the hybrid clones, and no other abnormal human chromosomes were detected. These findings indicate that chromosome 6 material between 6q16 and 6qter was lost from the hybrid clones. All clones carrying the deleted chromosome 6 were positive for ME_S but negative for SOD_M, while clones in which chromosome 6 was apparently normal were positive for both of these enzymes (table II). Six of the RAG/Cali hybrid clones were selected for assay of the HLA xenoantigenic

Table II. Results of chromosome and enzyme studies.

Clone	Chromosomes[a]	ME_S	SOD_M
R/Cali B	1, 2, 5, 6, 10, 11, 12, 14, 15, 18, 19, 21, X	+	+
R/Cali C	1, 2, 6, 10, 11, 12, 14, 19, 21, X	+	+
R/Cali U5	1, 6, 10, 14, 21, X	+	+
R/Cali U8	1, 6, 10, 14, 19, 21, X	+	+
R/Cali U2	1, 6q−, 10, 14, 21, X	+	−
R/Cali I	1, 2, 5, 6q−, 10, 16, 21, X	+	−
R/Cali L	1, 2, 6q−, 21, X	+	−
R/Cali M	1, 6q−, 10, 13, 19, 21, X	+	−
R/Cali P	1, 6q−, 10, 13, 21, X	+	−
R/Cali O	1, 2, 6q−, 14, 16, 21, X	+	−
R/Cali S	1, 6q−, 10, 21, X	+	−
R/Cali T	1, 6q−, 10, 13, 21, X	+	−

[a] The chromosomal constitution of hybrid clones was determined by karyotypic analyses and enzyme electrophoresis.

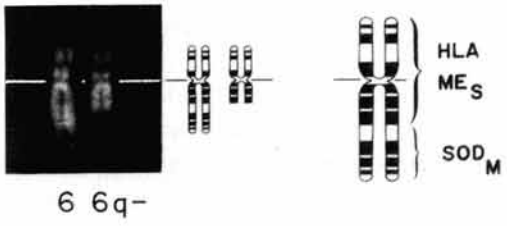

Fig. 1. Quinacrine fluorescence of a normal chromosome 6 and of the deleted chromosome 6 present in certain RAG/Cali hybrid clones, with a diagrammatic representation of the chromosome bands and regional localization of *HLA*, ME_S, and SOD_M.

determinant; in four of these clones a deleted chromosome 6 was present (fig. 1), while in two of the clones a normal chromosome 6 was present. All six clones showed expression of the HLA xenoantigenic determinant (table III).

It is of interest to note that the deleted chromosome 6 was not present in the 20 metaphases derived from the parent Cali line. It is possible that the deleted chromosome is present in a low percentage of cells of the parent line. Deletion may, however, have occurred during the fusion process. In either case, it is probable that the hybrid clones carrying the

Table III. HLA activity.

Category	Samples tested	HLA activity[a]
Controls	RAG (mouse)	2, 0
	RPMI 1788 (human)	99, 98
Hybrids 6+	R/Cali U5	58, 65
	R/Cali U8	55, 64
Hybrids 6q−	R/Cali S	53, 55
	R/Cali O	49, 50
	R/Cali I	50, 51
	R/Cali U2	56, 51
Hybrids 6−	R/RPMI1788 G2-3	2, 3
	R/RPMI1788 G2-9	1, 5

[a] Percentage inhibition; results of two assays.

deletion of chromosome 6 are not of independent origin. These clones, then, have most likely evolved from a single parent cell; however, they have diverged, so that they show differences in their chromosomal constitution.

Results of enzyme studies and radioimmunoassay of the HLA xeno-antigenic determinant on these clones indicate that SOD_M is carried on the long arm of human chromosome 6, distal to 6q16, while the genes coding for ME_S and HLA are carried either on the short arm of chromosome 6 or on the long arm, proximal to 6q16.

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