Chromosomal location of the genes for human immunoglobulin heavy chains

(mouse–human hybrids/gene regulation/human chromosomes/human isozymes)

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ABSTRACT   We have studied somatic cell hybrids between P3x63Ag8 mouse myeloma cells deficient in hypoxanthine phosphoribosyltransferase (EC 2.4.2.8) and either human peripheral lymphocytes or human lymphoblastoid or myeloma cells for the production of human immunoglobulin chains and for the expression of enzyme markers assigned to each of the different human chromosomes. Human chromosome 14 was the only human chromosome present in all independent hybrids producing μ, γ, and α human heavy chains. In two of the independent hybrids that produced human heavy chains, human chromosome 14 was the only human chromosome present in the hybrid cells. Loss of human chromosome 14 from these hybrids resulted in the concomitant loss of their ability to produce human immunoglobulin heavy chains. In view of these results, we conclude that the genes for human immunoglobulin heavy chains are located on human chromosome 14 in immunoglobulin-producing human cells.

Somatic cell hybrids between mouse and human cells have been extensively used to map human genes to their specific chromosomes (1–3). At present at least one gene has been assigned to each of the different human chromosomes (4). Recently Smith and Hirschhorn (5) have studied somatic cell hybrids between mouse RAG cells and human lymphoblastoid cells for the expression of human immunoglobulin and have concluded that the genes for human immunoglobulin heavy chains are located on human chromosome 6, probably on the region 6 pter → q16. These results were, however, rather surprising because it had been demonstrated that hybrids between fibroblasts and myeloma cells do not secrete detectable amounts of immunoglobulin (6). On the other hand, hybrids between different immunoglobulin-producing cells were found to produce antibody chains specific for both parents (7–10).

In this study we have produced somatic cell hybrids between P3x63Ag8 mouse myeloma cells producing IgG1 immunoglobulins (MOPC21) and deficient in hypoxanthine phosphoribosyltransferase (7, 9) and human lymphocytes obtained from the blood of different donors or from established cell lines (see Table 1). We have investigated expression of human immunoglobulin chains in these hybrids in order to establish which human chromosome(s) is necessary for the production of human immunoglobulins.

MATERIALS AND METHODS

Cell Hybridization. Hypoxanthine phosphoribosyltransferase-deficient P3x63Ag8 mouse myeloma cells were fused with either human peripheral lymphocytes or GM607 or GM1056 human lymphoblastoid cells or GM1500 human myeloma cells (Table 1) in the presence of polyethylene glycol

1000 (11), according to established procedures (9). Fused cultures were seeded in 24-well Linbro plates in the presence of selective medium. Hybrids between P3x63Ag8 cells and human lymphocytes were selected in hypoxanthine/aminopterin/thymidine (HAT) medium (12). Hybrids between P3x63Ag8 mouse cells and either human lymphoblastoid or myeloma cells were selected in HAT medium containing 0.1 mM ouabain (13). Cells growing as single independent colonies (clones) in the wells were picked up and grown continuously in nonselective medium. Hybrid clones producing human antibody chains were subcloned and subsubcloned as single cells in wells of 96-well Linbro plates.

Isozyme Analysis. Hybrid cells were studied for the expression of isozyme markers assigned to each of the different human chromosomes (chr) by starch gel or cellulose acetate gel electrophoresis (14–15): enolase 1 (ENO-1; EC 4.2.1.11) (chr 1); isocitrate dehydrogenase (IDH$_S$; EC 1.1.1.42) (chr 2); β-galactosidase (β-Gal; EC 3.2.1.23) (chr 3); phosphoglucomutase 2 (PGM$_2$; EC 2.7.5.1.) (chr 4); hexosaminidase B (HEX$_B$; EC 3.2.1.30) (chr 5); glyoxalase-1 (GLO-1; EC 4.4.1.5) and phosphoglucomutase 3 (PGM$_3$; EC 2.7.5.1) (chr 6); β-glucuronidase (β-GUS; EC 3.2.1.31) (chr 7); glutathione reductase (GSR; EC 1.6.4.2) (chr 8); aconitase (ACON; EC 4.2.1.3) (chr 9); glutamate oxaloacetic transaminase (GOT; EC 2.6.1.1) (chr 10); lactate dehydrogenase A (LDH$_A$; EC 1.1.1.27) (chr 11); lactate dehydrogenase B (LDH$_B$; EC 1.1.1.27) (chr 12); esterase D (ES-D; EC 3.1.1.1) (chr 13); nucleoside phosphorylase (NP; EC 2.4.2.1) (chr 14); mannosephosphate isomerase (MPI; EC 5.3.1.8) (chr 15); adenine phosphoribosyltransferase (APRT; EC 2.4.2.7) (chr 16); galactokinase (GK; EC 2.7.1.6) (chr 17); peptidase A (PEP-A; EC 3.4.11.-) (chr 18); glucose phosphate isomerase (GPI; EC 5.3.1.9) (chr 19); adenosine deaminase (ADA; EC 3.5.4.4) (chr 20); superoxide dismutase 1 (SOD-1; EC 1.15.1.1) (chr 21); arylsulfatase (ARS; EC 3.1.6.1) (chr 22); glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) (X chromosome) (4).

Analysis of the Immunoglobulins Produced by Hybrid Clones. Parent and hybrid cells were grown in leucine-deficient media containing 100 μCi of [³H]leucine (70 Ci/mmol) per ml (1 Ci = 3.7 × 10¹⁰ becquerels) for 24 hr. Cells were pelleted, and supernatants (500 μl) reacted with 25 μl of rabbit anti-human IgM, IgG, and IgA, rabbit anti-human μ, α, or γ heavy chain, anti-human κ or λ chain-specific sera, or rabbit anti-mouse gamma globulin for 1 hr in ice. One hundred microliters of a 10% suspension of fixed *Staphylococcus aureus* (16) was added to each reaction mixture for 30 min or 50 μl of goat anti-rabbit gamma globulin sera was added for 16–18 hr at 4°C. Double antibody precipitates were collected by centrifugation through a pad of 0.2 ml of 1 M sucrose. All immune precipitates were washed with 5% sucrose/15 mM Tris·HCl (pH 7.4)/0.5 M NaCl/5 mM EDTA/1% Nonidet P-40/2 mM phenylmethylsulfonyl fluoride. The immune precipitates were resuspended

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Table 1. Human parental cells used for hybridization

Human cells	Hybrid clones studied
DL peripheral lymphocytes*	14
OA peripheral lympocytes*	13
H peripheral lymphocytes*	3
NP peripheral lymphocytes*	4
GM1500 IgG-producing myeloma†	8
GM607 IgM-producing lymphoblastoid cell line†	7
GM1056 IgA-producing lymphoblastoid cell line†	8

* From blood.

† Obtained from the Human Genetic Mutant Cell Repository, Institute for Medical Research, Camden, NJ.

in Laemmli buffer (17) and analyzed on 10–11% polyacrylamide gels (17), embedded in 2,5-diphenyloxazole, and subjected to fluorography (18) for 5–14 days.

Karyologic Analysis. Metaphase chromosomes of hybrid cells were banded according to a modification (19) of the trypsin/Giemsa method of Seabright (20). Chromosomes of 10 clones containing either human chromosome 14 alone or chromosome 14 and an additional one or two human chromosomes were also stained by the G11 banding method (21).

RESULTS

Detection of human immunoglobulin chains produced by hybridomas

Mouse–human hybrids between P3x63Ag8 mouse myeloma cells and either human lymphocytes or human lymphoblastoid or human myeloma cells (Table 1) were labeled with [³H]leucine for 24 hr and the culture fluid was tested for the presence of human immunoglobulin chains by using rabbit antisera specific for human IgM, IgG, and IgA and human κ, λ, α, μ, and γ chains. As shown in Figs. 1–3, hybrid clones that produced the different human immunoglobulin chains were obtained. Hybrids between P3x63Ag8 mouse cells and peripheral human lymphocytes were found to produce predominantly human μ heavy chain (Fig. 1). Hybrids between P3x63Ag8 mouse cells and GM607, GM1056, and GM1500 human cells were found to produce human μ, α, and γ heavy chains, respectively (Figs. 1–3).
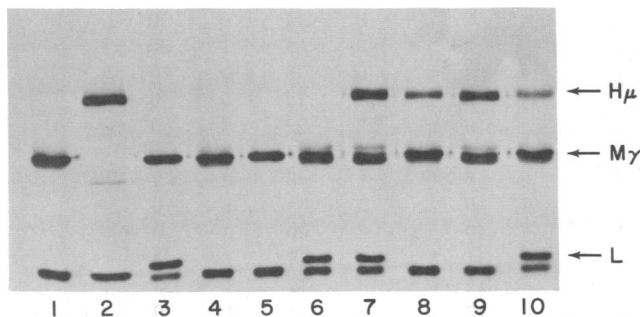

FIG. 1. Expression of human immunoglobulin μ chains in mouse–human hybridoma. Culture hybrids of parental and hybrid cells were precipitated by using antibodies specific for mouse IgG and human IgM. The immunoglobulin light (L) and heavy chains secreted by P3x63Ag8 mouse myeloma cells are shown in lane 1. The human immunoglobulin light and μ chains produced by human GM607 lymphoblastoid cells are shown in lane 2. Lanes 3–10 show the immunoglobulin chains produced by eight different mouse–human hybridomas, four of which (lanes 7–10) produce human μ chains. Four of the clones (lanes 3, 6, 7, and 10) also secrete human light chains. Hμ, human μ chain; Mγ, mouse γ chain; L, light chain.
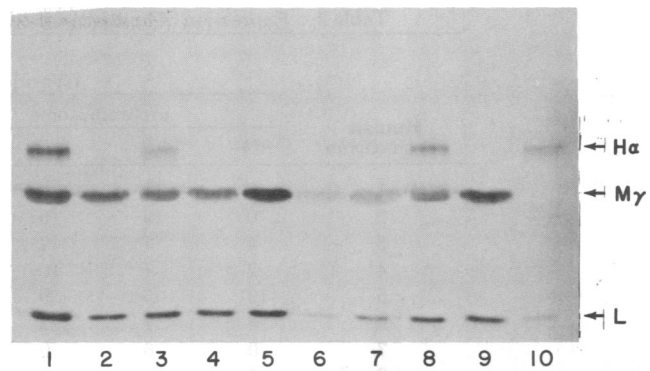

FIG. 2. Expression of human α chains in hybrids between P3x63Ag8 mouse myeloma cells and GM1056 human lymphoblastoid cells. Culture fluids were immunoprecipitated by using antibodies against mouse IgG and human IgA. Lane 9 shows the immunoglobulin chains secreted by P3x63Ag8 mouse myeloma cells. Lane 10 shows the immunoglobulin chains produced by GM1056 human lymphoblastoid cells. Lanes 1–8 show the immunoglobulin chains produced by eight independent P3x63Ag8 and GM1056 hybridomas. Hα, human α chains; Mγ, mouse γ chains; L, light chains.

Expression of human isozymes in hybrid clones

As shown in Table 2, human μ heavy chains were expressed in hybrid cells lacking human chromosome 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20. 21, 22, or X. Human chromosome 14 was the only human chromosome retained by all the hybrid clones producing human μ chain. Eleven out of 26 clones, however, were found to contain human chromosome 14 but did not produce human heavy chains. It is possible that some of these hybrids were produced by fusing P3x63Ag8 cells with human cells other than B lymphocytes (T cells or macrophages) or that these hybrid clones retained the human chromosome 14 that carried the excluded heavy chain allele (22). Analysis of clones between GM1500 human myeloma cells and P3x63Ag8 cells that were chosen on the basis of production of human γ heavy chains for the expression of human isozymes also indicated that human chromosome 14 was the only human chromosome present in all the hybrids producing human γ
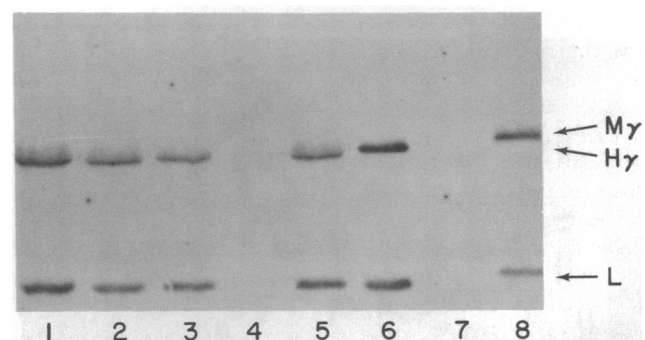

FIG. 3. Expression of human γ chains in mouse–human hybridomas. Culture fluids of parental P3x63Ag8 mouse and GM1500 human myeloma cells and hybrid cells were immunoprecipitated by using a rabbit anti-human IgG antiserum that was preabsorbed with a P3x63Ag8 ascitic fluid. Lane 8 shows the immunoglobulin chains secreted by P3x63Ag8 mouse cells that were precipitated by using a rabbit anti-mouse IgG antiserum. Lane 7 shows that the preabsorbed rabbit anti-human IgG antiserum does not react with P3x63Ag8 immunoglobulin chains. Lane 6 shows the human immunoglobulin chains secreted by GM1500 human myeloma cells. Lanes 1, 2, 3, and 5 show the immunoglobulin chains produced by different clones P3x63Ag8–GM1500 hybridomas. In lane 4 is a negative hybrid clone. Mγ, mouse γ chain; Hγ, human γ chain; L, light chain.

Table 2. Expression of human antibody heavy chains in mouse–human hybridomas

Human chromosome	Hybridoma clones						Hybridoma mass cultures	
	μ/chromosome				γ/chromosome		α/chromosome	
	+/+	+/−	−/+	−/−	+/+	+/−	+/+	+/−
1	2	11	0	12	2	6	1	4
2	0	13	0	8	0	8	0	5
3	4	7	7	1	4	2	1	0
4	2	7	10	3	0	8	0	2
5	10	5	9	0	3	3	2	1
6	6	7	2	0	0	6	2	1
7	0	6	0	2	1	5	0	1
8	5	9	4	6	0	7	4	1
9	2	15	3	6	0	8	0	3
10	3	18	0	2	0	8	4	1
11	11	11	11	1	0	8	0	4
12	2	20	7	5	0	8	2	2
13	3	18	2	0	0	8	0	4
14	15	0	11	0	8	0	4	0
15	5	10	7	7	0	8	2	0
16	4	9	1	1	1	7	4	1
17	3	11	8	4	0	7	0	5
18	3	18	5	6	1	7	4	1
19	3	19	0	14	3	5	4	1
20	1	13	1	12	0	8	3	2
21	8	7	2	10	0	6	2	1
22	0	5	0	2	0	6	ND	ND
X	6	6	2	2	0	6	ND	ND

ND, the hybrids were not tested for isozyme markers assignment.

heavy chains (Table 2). Two of these clones retained human chromosome 14 and no other human chromosomes (Fig. 4). Expression of human α heavy chains in a few hybrids between GM1056 IgA-producing human lymphoblastoid cells and P3x63Ag8 cells (Table 2) was also associated with the presence of human chromosome 14 (Table 2) and thus these results are also compatible with the hypothesis that the genes for human μ, γ, and α heavy chains are syntenic and are located on human chromosome 14.

In order to prove conclusively that the genes for human heavy chains are located on this human chromosome, we have subcloned six independent hybrid clones that were producing

human μ chain, and have studied the subclones for the production of human μ chain and the expression of human isozyme markers. As shown in Table 3, human chromosome 14 was the only human chromosome consistently present in all hybrid subclones producing human μ chain. Because only one subclone was found that had lost human chromosome 14 (this clone also lost the expression of human μ chain), three independent subclones derived from three different clones were subcloned and

FIG. 4. Metaphase chromosomes of a P3x63Ag8–GM1500 hybrid clone containing human chromosome 14 and no other human chromosome as determined by isozyme analysis is stained by the G11 banding method. Human chromosome 14 is the only human chromosome present in the hybrid (arrow).

Table 3. Expression of human μ chains in hybridoma subclones

Human chromosome	Hybridoma subclones, μ/chromosome			
	+/+	+/−	−/+	−/−
1	1	16	0	3
2	0	4	0	0
3	9	2	1	0
4	0	3	0	0
5	11	12	5	0
6	22	22	3	0
7	0	4	0	0
8	1	3	0	0
9	5	4	1	0
10	2	10	0	0
11	16	7	1	0
12	13	12	1	3
13	5	4	1	0
14	22	0	4	1
15	13	12	3	2
16	0	4	0	0
17	1	6	0	1
18	0	8	0	1
19	4	18	0	4
20	0	4	0	0
21	9	15	1	3
22	1	1	0	0
X	0	2	0	1

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Table 4. Expression of human μ chains in hybridoma subsubclones

Human chromosome	Hybridoma subsubclones, μ/chromosome			
	+/+	+/−	−/+	−/−
1	0	6	0	0
2	0	5	0	0
3	2	3	1	1
4	1	5	0	2
5	4	3	2	0
6	6	0	2	0
7	0	5	1	1
8	2	5	1	1
9	0	6	0	0
10	0	5	0	2
11	7	0	1	1
12	6	1	1	0
13	4	2	1	1
14	16	0	1	17
15	16	0	1	17
16	1	5	1	0
17	1	5	0	2
18	0	5	1	0
19	0	4	0	2
20	1	4	1	0
21	0	5	0	1
22	0	4	1	1
X	5	2	1	1

these subclones were studied for the expression of human μ chains and human isozyme markers. As shown in Table 4, only human chromosomes 6, 11, 14, and 15 were present in all the human μ-positive and absent in all μ-negative subsubclones tested. Loss of chromosome 14 from 17 subsubclones resulted in the loss of the production of human μ chains. Because Smith and Hirschhorn (5) have reported that the genes for human heavy chains are located on the region pter $\rightarrow$ q16 of human chromosome 6, and we did not find any positive correlation between human heavy chain production and human glyoxalase 1 expression [the gene for human glyoxalase is located on the region p21 $\rightarrow$ pter of human chromosome 6 (23)], we have studied eight independent hybrid clones for the expression of an additional isozyme marker coded for by a gene that is located on either the short or long arm of chromosome 6 very close to the centromere (23, 24). As shown in Table 5, three out of eight hybrid clones had lost the expression of human phosphoglucomutase 3 but had retained the expression of either μ or γ human heavy chains.

DISCUSSION

The results presented in this paper indicate that mouse–human hybrids between antibody-producing cells can be used to map the chromosomal location of genes involved in the production of human antibodies. Because human chromosome 14 was the only human chromosome present in all the hybrids producing human heavy chains and loss of this chromosome from the hybrids resulted in the loss of the expression of human heavy

Table 5. Expression of human phosphoglucomutase 3 and heavy chains in hybrid clones

Human μ or γ	Phosphoglucomutase 3	
	+	−
+	4	3
−	1	0

chains, we conclude that this human chromosome carries the genes for human heavy chains. These results disagree with those reported by Smith and Hirschhorn (5), because no correlation was found between the expression of human heavy chain genes and the retention of human chromosome 6.

Lack of expression of human heavy chains by 11 out of 26 independent hybrids and 4 out of 27 hybrid subclones that retained human chromosome 14 is not surprising because recombination between the genes for the antibody variable and constant region seems to occur on only one of the two homologous chromosomes in antibody-producing cells (22), and thus these hybrids may have retained the human chromosome that carries the embryonal nonrearranged immunoglobin constant and variable heavy chain genes, and not the homologous rearranged chromosome. The only possible alternative explanation of the results presented in this study is that the genes for immunoglobulin heavy chains are not chromosomal. If this is the case, presence of human chromosome 14 would be, however, necessary for the phenotypic expression of the genes. Further clarification of these possibilities may be achieved when the DNA probes specific for the variable and constant regions of the different human immunoglobulin heavy chains are available.

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