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Authors

Smith, Moyra

Krinsky, Adrienne

Arredondo-Vega, Francisco

et al.

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Moyra Smith⁺, Adrienne Krinsky,
Francisco Arredondo-Vega,
Ai-Lan Wang^o and Kurt Hirschhorn

Division of Genetics, Department of
Pediatrics, Mount Sinai School of
Medicine, New York

Confirmation of the assignment of genes for human immunoglobulin heavy chains to chromosome 14 by analysis of Ig synthesis by man-mouse hybridomas*

Hybridomas were produced by fusing the NS1 mouse myeloma line, which does not produce mouse heavy chain Ig, with human peripheral B lymphocytes from a normal individual. Two vigorously growing colonies from this fusion were found to secrete human Ig heavy chains and were recloned. Two secondary clones, which secreted human chains, were again recloned. Among the tertiary clones, two were identified which produced intracellular human Ig chains, but did not secrete immunoglobulin. These tertiary clones were recloned, generating 6 quaternary clones which failed to produce human Ig heavy chains, and 15 quaternary clones which produced intracellular Ig chains. Hybrid clones from each successive subcloning were examined for their human chromosomal content and only those clones which were found to be individually chromosomally distinct, a total of 56 clones in all, were used to analyze the segregation of human chromosomes and human Ig heavy chain synthesis. Results of this study indicate concordant segregation of human Ig heavy chain synthesis and chromosome 14. These studies therefore confirm the previous assignment by C. M. Croce et al. (*Proc. Natl. Acad. Sci. USA* 1979. 76: 3416) of the genes for human Ig heavy chains to chromosome 14.

1 Introduction

Several studies have been reported in which man-mouse somatic cell hybrids were used to obtain information on the chromosomal assignment of the genes for human immunoglobulin heavy chains (IgHC). Conflicting results have been reported, and these genes have been assigned to chromosome 6 [1], chromosome 8 [2], chromosome 6, 7 or 11 [3], chromosome 14 [4] and chromosome 15 [5].

Studies on the expression of Ig in somatic cell hybrids have proved problematical for a number of reasons. First, Ig production represents a differentiated function, and it appears that, for optimal expression of such functions in hybrid cells, both parental cell types used for fusion should express the differentiated function [6]. In hybrids produced by fusing human lymphoblastoid cells with nonmyeloma rodent cell lines [2, 3, 7, 8], only very low amounts of Ig are apparently produced. Furthermore, studies involving the use of fibroblast or epithelioid cell lines are complicated by the fact that receptors for the Fc portion of Ig occur on the surface of these cells, and these receptors may give rise to false positive results in immunoprecipitation and immunofluorescence experiments [9]. Second, in examination of Ig production by hybrid cells, it is necessary to take the phenomenon of allelic exclusion into account. It has been observed that in a single B cell, only one of the chromosomes carrying the gene for human IgHC under-

goes the Ig gene rearrangement and VJ joining which is required for differentiation and Ig chain expression [10, 11]. It is therefore possible that despite the failure to express human IgHC, a clone may contain one homologue of the pair of chromosomes which carries the human IgHC genes [12]. A third problem has arisen in certain studies on Ig mapping, because cells derived from patients with lymphoblastic leukemia were used as the human parental line for producing hybrids [3, 13]. Chromosome anomalies and, in particular, anomalies involving chromosome 14, are frequently found in these patients [14].

In the study reported here, somatic cell hybrids were produced by fusing the NS1 mouse myeloma line, (which does not produce mouse Ig heavy chains), with human peripheral B lymphocytes from a normal individual. Two primary clones were identified which secreted human IgH chains, and from these, secondary, tertiary and quaternary clones were generated. At each stage, only clones which synthesized human IgH chains (intracellular or secreted), were recloned. Following each recloning, at each successive stage, all clones were analyzed for their human chromosomal content, and only those clones which were found to be individually distinct, a total of 56 clones, were used to analyze the segregation of human chromosomes and human IgHC synthesis. Results of this study make it possible for us to confirm the previous assignment [4] of human IgHC genes to chromosome 14.

[I 3234]

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Correspondence: Moyra Smith, Department of Pediatrics, University of California, at Irvine, Irvine, CA 92717, USA

Abbreviations: IgHC: Immunoglobulin heavy chain(s) SDS: Sodium dodecyl sulfate FCS: Fetal calf serum

2 Materials and methods

2.1 Cell fusion and cell culture

Human cells used in fusion experiments, were obtained following leucopheresis of a normal individual. This lymphocyte-rich material was depleted of T cells by EA rosetting. The remaining B cell-enriched fraction was then grown in medium containing RPMI 1640, 15% fetal calf serum (FCS), penicillin (100 units/ml), streptomycin (100 µg/ml), and 10 µg/ml of pokeweed mitogen, for 4 days, to obtain a population of pro-

liferating B lymphocytes [15]. These cells were then fused with the NS1 mouse myeloma line [16], using polyethylene glycol, as previously described [16]. Following fusion, cells were resuspended in the HAT selective medium [17] and then seeded into 96-well microtiter plates (Costar Plastics, Cambridge, MA) at a density of 10^4 cells/well. Medium used for growing NS1 cells and for preparation of HAT selective medium was Dulbecco's minimum essential medium (MEM, Gibco, Grand Island, NY) containing 10–15% FCS, and gentamycin (60 $\mu\text{g}/\text{ml}$), penicillin and streptomycin as above. Parental mouse cells were killed in HAT medium, while parental human cells had a limited life-span, so that only mouse-human hybrid cells survived in the selective medium after 3 weeks. Vigorously growing colonies of man-mouse hybrid cells were obtained from 6 wells; two of these clones were found to be producing readily detectable amounts of human IgHC. These two clones were extensively subcloned, giving rise to secondary, tertiary and quaternary clonal populations (see Scheme 1). For cloning, cells were serially diluted until suspensions were obtained which contained approximately 1 cell/100 μl . This quantity of the suspension was then seeded into 96-well microtiter plates. All cloning was carried out in HAT medium, and subclones were maintained in this medium.

2.2 Characterization of human chromosomal content of hybrid clones by marker enzymes

Cell lysates of hybrid clones were examined by means of enzyme electrophoresis [18–20] for the presence of enzymes with known assignments to 21 of the 22 human autosomes. The enzymes analyzed included phosphoglucomutase 1 (chromosome 1), malate dehydrogenase (chromosome 2), aminoacylase (chromosome 3), phosphoglucomutase 2 (chromosome 4), hexosaminidase B (chromosome 5), malic enzyme (soluble form) and glyoxylase (chromosome 6), β -

glucuronidase (chromosome 7), glutathione reductase (chromosome 8), adenylate kinases 1 and 3 (chromosome 9), glutamic oxaloacetic transaminase (chromosome 10), lactate dehydrogenase A (chromosome 11), lactate dehydrogenase B and peptidase B (chromosome 12), esterase D (chromosome 13), nucleoside phosphorylase (chromosome 14), mannose phosphate isomerase (chromosome 15), phosphoglycolic phosphatase (chromosome 16), peptidase A (chromosome 18), phosphohexose isomerase (chromosome 19), adenosine deaminase (chromosome 20), superoxide dismutase (soluble form) (chromosome 21), mitochondrial aconitase (chromosome 22). The presence of chromosome 17 was determined only by cytogenetic studies.

2.3 Characterization of human chromosomal content of hybrid clones by karyotypic analyses

Following the preparation of metaphase spreads from hybrid clones [21], Giemsa 11 staining [22] was used to distinguish mouse and human chromosomes; this was followed by quinacrine dihydrochloride fluorescent staining of metaphase spreads to facilitate identification of specific human chromosomes [23].

2.4 Analysis of Ig production by hybrid cells

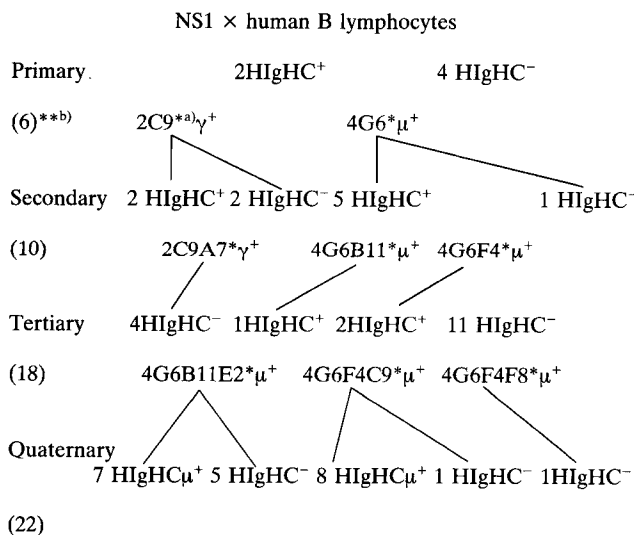
For biosynthetic labeling of cells, hybrid cells and controls (NS1 and P3 mouse myeloma cells, human lymphoblastoid cell lines), were grown in methionine-free Dulbecco's MEM without FCS, containing [^{35}S]methionine (20 $\mu\text{Ci}/\text{ml}$ = 740 kBq/ml), at a density of 5×10^5 cells/ml, for 5 h. Cells and spent medium were then separated by centrifugation, and cells were lysed using 0.5% Nonidet-P40 (BDH, Poole, GB) in 0.02 M Tris-HCl, containing 10 mM phenyl methyl sulfonyl fluoride [24].

Ig, present in spent medium or in cell lysates, was precipitated by the addition of appropriate amounts of rabbit anti-human IgM, anti-human IgG and/or anti-human IgA. In some experiments, carrier human Ig were added, and the immune complexes were separated on a sucrose gradient [24]. In other experiments, *Staphylococcus aureus* organisms (The Enzyme Center, Boston, MA) were used to precipitate human Ig-anti-human Ig complexes [25]. Control precipitations were carried out using normal rabbit serum in place of anti-human Ig. Material precipitated from spent media or lysates was dissolved in 6 M urea, and then subjected to sodium dodecyl sulfate (SDS) gel electrophoresis under reducing conditions, using the Laemmli discontinuous system and slab gels [26]. Following electrophoresis, gels were fixed, stained and dried. Autoradiography was carried out by exposing dried gels to Kodak X-Omat R (XR5) film for 24–72 h.

3 Results and discussion

Results are summarized in Scheme 1 and Table 1. Two out of six primary clones obtained from the fusion of the Ig-nonsecreting mouse myeloma line, NS1, with human peripheral blood B cells, were found to secrete Ig molecules containing human IgHC. One primary clone, 2C9, secreted human γ chains, while the other, 4G6, secreted μ chains, (Fig. 1). These

Scheme 1. Derivation of 56 chromosomally distinct NS1 (mouse myeloma) \times human B lymphocyte hybrid clones



a) * = Designation of clone.

b) ** = Number of clones.

two primary clones were then recloned. Only those clonally derived populations which could be shown to be individually chromosomally distinct were classified as clones. Seven of the secondary clones continued to secrete human IgHC, (Fig. 1b). Subcloning of 3 of these, including one γ -producing clone and 2 μ -producing clones yielded tertiary clones. None of the ter-

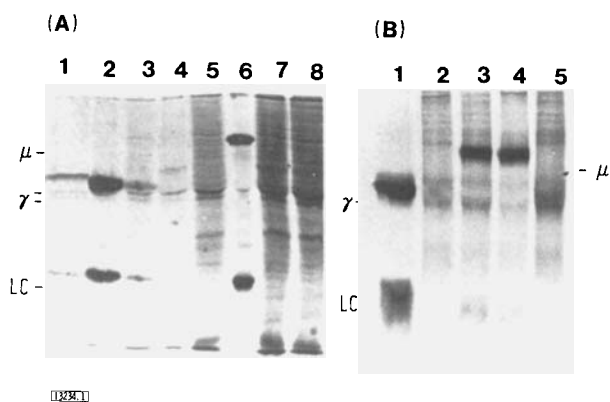


Figure 1. (a) SDS gel electrophoresis of immunoprecipitates from (1) spent media of human lymphoblasts: γ^+ , L chains⁺, (2, 3) P3 mouse cells: γ^+ , L chains⁺ and (4-8) hybrid clones. Primary clone 4G6 (6) is positive for μ and L chains. Clones 4-8 are negative for Ig H and L chains. (b) SDS gel electrophoresis of immunoprecipitates from spent media of P3 mouse cells (1) γ^+ , L chains⁺, and (2-5) secondary subclones of 4G6. Clones 4G6B11, (3) and 4G6F4 (4) are positive for μ and L chains while two remaining clones are Ig⁻.

ary clones secreted Ig. However, 3 of the clones continued to produce intracellular human μ chains. These 3 tertiary clones were then subcloned, yielding 15 chromosomally distinct quaternary clones, which produced intracellular human μ chains but did not secrete Ig molecules, and 6 clones which were negative for intracellular and secreted human Ig. At each stage of cloning, the human chromosomal content of Ig-positive and -negative clones was determined by cytogenetic and electrophoretic analyses. It may be seen from Table 2, that when data from tertiary and quaternary clones are taken into account, the only chromosome which is concordant with production of human IgHC is chromosome 14. Fig. 2 illustrates the mouse, human and hybrid isozymes of the chromosome 14 marker enzyme, nucleoside phosphorylase, in lysates of human and mouse control cultures and hybrid clones.

In B lymphocytes, the phenomenon of allelic exclusion occurs, and only one homologue of the pair of chromosomes carrying the genes for human IgHC is apparently expressed [10, 11]. Since allelic exclusion apparently also exists in somatic cell hybrids [4], the most informative clones for gene mapping studies, particularly among primary and secondary clones, are those which are positive for human Ig chain production. It is of interest to note that, as cloning proceeded from secondary to tertiary and then quaternary clonings, the incidence of concordance between human IgHC production and the presence of human chromosome 14 increased. Clones which were discordant for human IgHC production and human chromosome 14, included only clones in which IgHC synthesis was not detected despite the occurrence of chromosome 14. The fact

Table 1. Segregation of human chromosomes and human IgHC production in secondary, tertiary and quaternary hybrid clones (total 56). The presence of specific human chromosomes was determined by electrophoresis of 24 different marker enzymes, and these data were confirmed by cytogenetic analyses. In the case of human chromosome 17, only cytogenetic data were obtained

Chromosome	Chromosome/IgHC synthesis																			
	Secondary						Tertiary								Quaternary					
	+/+	-/-	+/-	-/+	C ^{a)}	D ^{b)}	+/+	-/-	+/-	-/+	C	D	+/+	-/-	+/-	-/+	C	D		
1	5	0	0	1	5	1	2	0	0	7	2	7	1	5	0	14	6	14		
2	0	0	0	5	0	5	1	0	1	1	1	2	0	5	0	15	5	15		
3	0	1	0	0	1	0	0	2	0	0	2	0	0	5	0	15	5	15		
4	0	2	0	0	2	0	0	11	0	3	11	3	0	5	0	15	5	15		
5	2	0	0	0	2	0	2	0	0	0	2	0	10	2	0	4	12	4		
6	6	2	0	1	8	1	3	9	6	0	12	6	2	1	2	10	3	12		
7	2	0	0	4	2	4	2	5	0	0	7	0	6	3	2	4	9	6		
8	2	0	0	0	2	0	2	0	0	0	2	0	12	5	0	4	17	4		
9	0	0	0	5	0	5	0	11	0	3	11	3	0	5	0	15	5	15		
10	4	0	1	0	4	1	1	0	11	1	1	12	0	5	0	15	5	15		
11	7	0	0	1	7	1	2	5	10	0	7	10	3	0	0	4	3	4		
12	7	0	1	1	7	2	2	6	9	1	8	10	6	3	0	3	9	3		
13	1	0	0	3	1	3	0	0	0	1	0	1	0	1	5	15	1	20		
14	7	0	2	0	7	2	3	12	3	0	15	3	15	5	1	0	20	1		
15	2	1	0	6	3	6	1	4	0	2	5	2	1	5	0	15	6	15		
16	1	0	0	5	1	5	2	0	0	1	2	1	1	5	0	7	6	7		
17	2	0	0	0	2	0	2	0	0	0	2	0	0	6	0	15	6	15		
18	4	0	1	0	4	1	1	0	3	2	1	5	4	3	0	10	7	10		
19	6	0	1	0	6	1	0	0	2	2	0	4	0	0	0	8	0	8		
20	5	0	0	0	5	0	2	0	1	0	2	1	9	0	0	7	9	7		
21	7	0	1	0	7	1	3	3	10	0	6	10	10	1	0	1	11	1		
22	2	0	0	4	2	4	1	0	0	1	1	1	2	3	0	4	5	4		

a) C = Concordant.

b) D = Discordant.

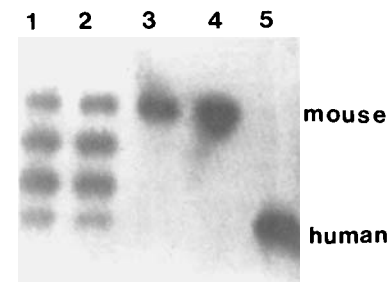


Figure 2. Electrophoresis of nucleoside phosphorylase in human (5), mouse (4) and hybrid clone lysates. Clones 1 and 2 are positive, and clone 3 is negative for chromosome 14.

that quaternary clones which produced intracellular human μ chains failed to secrete these, is of interest but cannot be readily explained. The mutation or mutations, which exist in the NS1 myeloma line, (originally derived from the IgG-secreting P3 mouse myeloma line) which lead to failure of H chain synthesis, and failure of secretion of mouse κ light chains in unfused NS1 cells, have not yet been defined [13].

Results of the study described here would therefore confirm the assignment of the genes for human IgHC to chromosome 14 [4]. The polymorphic marker coded by the human γ chain genes, Gm, is known to be linked to the gene for human α_1 -antitrypsin [27] through family studies. Recent studies on α_1 -antitrypsin synthesis by hybrid cells produced by fusing rodent hepatoma cells with human cells have demonstrated that human α_1 -antitrypsin production segregates with human chromosome 14*. These results provide further support for the assignment of the Gm-Pi linkage group to chromosome 14.

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