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Journal
Cytogenetic and Genome Research, 22(1-6)

ISSN
1424-8581

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Publication Date
1978

DOI
10.1159/000130953

Cytogenet. Cell Genet. *22:* 275–280 (1978)

Studies on the synthesis of immunoglobulins by man-mouse somatic cell hybrids

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Immunoglobulin synthesis has been studied in hybrid cells produced by fusing Rag mouse cells with human lymphoid cells. Hybrid clones were examined for the presence of cell surface immunoglobulins, and spent culture medium was analyzed to determine the presence of secreted immunoglobulins. Results of some of these studies have been briefly described previously.[1]

Cell lines used in this study were Rag, a hypoxanthine guanine phosphoribosyl transferase-deficient mouse cell line, derived from a renal adenocarcinoma,[2] and five different human lymphoid cell lines. The lymphoid cell lines did not appear to carry chromosomal rearrangements at the time of fusion. Cells were fused using inactivated Sendai virus according to methods previously described.[3] 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 human lymphoid cells were discarded during medium changes. Hybrid cells which survived and proliferated in the selective medium were subsequently cloned. In some cases cells were cloned in HAT medium, while in other cases cells were cloned in nonselective medium. After 10–20 passages, hybrid clones were usually recloned, giving rise to secondary clones.

Starch-gel electrophoresis was used to determine the human, mouse, and hybrid isozyme forms of 19 different enzyme systems. The enzyme systems studied and their human chromosomal assignments are listed in ref. 4. The starch-gel electrophoresis methods used were those described by HARRIS and HOPKINSON.[5] The human chromosomal assignments are listed in ref. 5. Karyotypic analyses were carried out on banded metaphase chromosomes using methods previously described. Between 10 and 20 metaphases from each clone were examined. A clone was

Supported by NIH grant GM 1993.

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. In the case of chromosomes 3, 4, 5, 7, 8, 16, 17, 22, and Y, the presence of the human chromosome was determined by karyotyping alone.

To detect the presence of immunoglobulins on the surface of viable cells, a double antibody technique was used. The first antibody consisted of rabbit anti-human immunoglobulin; anti-human IgA (alpha chain specific), anti-human IgG (gamma chain specific), anti-human IgM (mu chain specific) and, in some cases, antibodies against kappa and lambda light chains were individually used. After incubation with the first antibody, cells were washed several times and then treated with the second antibody, namely fluorescein-conjugated goat anti-rabbit IgG. All antibodies used were obtained from Behring Diagnostics. Following incubation with the second antibody, cells were washed, mounted, and examined under a fluorescence microscope.

To determine the presence of immunoglobulins in spent medium, cells were fed with serum-free medium containing ^{14}C-labeled amino acids (1 μCi/ml) and 0.5 % ovalbumin for a period of 1 week. Spent medium was then dialyzed for 48 h against successive changes of phosphate-buffered saline containing 0.1 % sodium azide. Medium was concentrated by lyophilization or by vacuum dialysis, to achieve a 100-fold concentration. Concentrated medium was then subjected to immuno-electrophoresis in agarose gels. Concentrated medium was loaded 3 times into wells, which were then loaded with carrier serum. Following electrophoresis, precipitin lines were developed using monospecific antisera against human immunoglobulins. Antisera used was obtained commercially from Behring Diagnostics, Meloy Laboratories, or Miles Laboratories.

Thirty-six clones were examined for surface immunofluorescence. Ten of these clones exhibited positive immunofluorescence when antisera against human heavy chain immunoglobulins were used as first antibody. The clones which exhibited positive immunofluorescence with anti-human IgA differed from clones which were negative in that they carried human chromosome 2. In this series of clones, no clone carried human chromosome 2 without human chromosome 6.

In most instances clones exhibited immunofluorescence with anti-human IgA but did not exhibit positive immunofluorescence when treated with anti-IgG or with anti-IgM antisera. Of considerable interest is the report by TEJLER and GRUBB[6] concerning a complex glycoprotein which occurs in free form in plasma and as complexes with IgA and with albumin. This glycoprotein, designated HC protein, has recently been reported to be present on the surfaces of all cells, including B and T lymphocytes, fibroblasts, and erythrocytes.[7] It seems possible that antisera produced against IgA may contain antibodies against HC protein. We recently

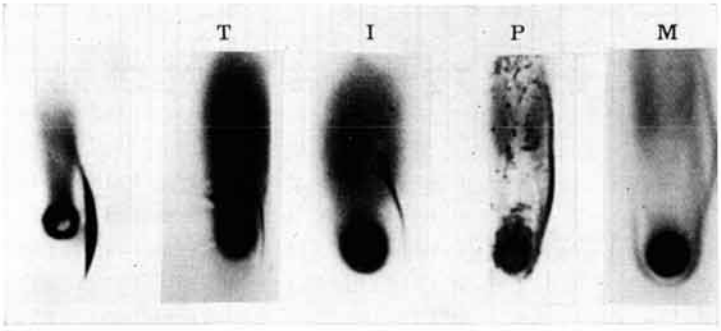

Fig. 1. Radioimmunoelectrophoresis of spent culture medium (concentrated 100×) derived from one of the parent lymphoid lines, Cali (far left), and from four Rag/Cali hybrid clones (T, I, P, and M). Following immunoelectrophoresis, agarose gels were washed, dried, and then exposed to No-Screen X-ray film for 17 days. Precipitin arcs were developed with anti-IgG (gamma chain specific).

noted that human fibroblasts exhibit positive immunofluorescence when rabbit anti-human IgA is used as the first antibody. Positive immunofluorescence was not observed when fibroblasts were treated with anti-human IgG or with anti-human IgM. Positive immunofluorescence with anti-human IgA antisera observed in man-mouse hybrid clones may therefore indicate: (1) that human IgA is attached to the surface of these cells or (2) that a cell surface molecule (HC protein?) occurs on these cells which reacts with antibodies present in the anti-human IgA antiserum. Studies are in progress to investigate these possibilities further.

Spent medium from 22 different hybrid clones was examined for immunoglobulins by radioimmunoelectrophoresis. Medium derived from 14 clones was found to produce a radiolabeled precipitin line when anti-human IgG (gamma chain specific) was added to the antiserum trough; two of these clones were also positive when anti-IgA or anti-IgM were used. Eight clones failed to produce radiolabeled precipitin lines when treated with antisera against human heavy chain immunoglobulins. Positive clones differed from negative clones in that the positive clones carried human chromosome 6 (see table I).

The concentrated spent medium from hybrid clones contains too little protein to produce a sharp precipitin line on immunoelectrophoresis. A carrier human serum was therefore used in all immunoelectrophoresis studies. One drawback of the use of this technique is that if radiolabeled

Table I. Chromosome and immunoglobulin (Ig) constitution of sampled hybrid clones.

Clone	Chromosome																							Surface Ig					Secreted Ig				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	α	μ	γ	$\varkappa$	λ	α	μ	γ	$\varkappa$	λ
R/ODY Lb 2					+	+	+							+	+	+				+	+		+		−	−	−	−	−	NT[b]				
R/ODY Lb 3					+	+	+		+		+			+	+	+				+	+		+		−	−	−	(±)	−	NT				
R/ODY Lb 11		+			+	+	+		+	+	+			+	+	+				+	+		+		+	−	−	+	−	+	+	+	+	−
R/ODY Lb 9						+			+					+		+				+	+		+		−	−	−	−	−	NT				
R/ODY Lb 12		+			+	+			+	+	+			+	+	+				+	+		+		+	−	−	+	−	NT				
R/ODY La 3						+			+			+	+	+				+	+	+	+				−	−	−	−	−	NT				
R/ODY La 2						+							+	+				+	+		+		+		−	−	−	−	−	−	−	+	−	+
R/ODY La 7						+			+			+	+	+				+	+		+		+		−	−	−	−	−	NT				
R/ODY La 10						+			+				+	+				+			+		+		−	−	−	−	−	−	−	+	−	+
R/ODY La 11						+			+			+	+	+					+		+		+		−	−	−	−	−	NT				
R/ODY La 16						+							+	+							+				−	−	−	−	−	NT				
R/ODY La 20						+			+			+	+	+							+				−	−	−	−	−	NT				
R/RPMI D		+	+	+		+	+				+	+	+	+		+		+	+		+		+		−	+	−	+	−	NT				
R/RPMI A		+				+	+				+			+		+		+	+		+		+		+	−	−	+	−	NT				
R/RPMI H	+								+		+		+	+				+	+						−	−	−	−	−	NT				
R/RPMI E5-7														+						+					−	−	−	−	−	−	−	−	−	−
R/RPMI E5-12	+					+						+							+				+		−	−	−	−	−	NT				
R/RPMI G2			+			+			+				+								+		+	+	−	−	−	−	−	NT				
R/RPMI G2-3			+										+								+		+	+	−	−	−	−	−	−	−	−	−	−
R/RPMI G2-9			+																		+		+	+	−	−	−	−	−	−	−	−	−	−
R/RPMI G2-1			+						+												+		+	+	−	−	−	−	−	−	−	−	−	−

Table I (continued)

R/NB 60 A1										+	+				+			+			−	−	−	−	±	−	−	−	−	−
R/NB 60 A3										+					+			+			−	−	−	−	−	−	−	−	−	−
R/NB 60 A8										+	+	+			+			+			−	−	−	−	±	−	−	−	−	−
R/NB 60 A8-3							+			+	+	+			+			+			−	−	−	−	±	−	−	−	−	−
R/NB 103 A		+		+			+	+	+	+		+						+		+	+	−	−	+	−	NT					
R/NB 103 C	+	+	+	+		+	+		+	+	+	+			+	+	+	+	+	+	+	−	−	+	−	NT					
R/NB 103 D		+		+		+	+	+	+	+	+	+	+		+	+		+	+	+	+	−	−	+	−	NT					
R/NB 103 F		+		+		+			+	+	+	+			+	+		+	+	+	+	−	−	+	−	−	−	+	NT		
R/NB 103 J				+		+	+		+	+	+	+			+			+	+	−	−	−	−	−	−	−	−	+	+	−	
R/Cali A	+	+			+	+		+	+	+		+	+		+	+		+	+	+	−	−	NT		NT					
R/Cali B	+	+		+	+	+		+	+	+		+	+		+	+		+	+	+	−	−	+	−	−	−	+	+	−	
R/Cali C	+	+			+			+	+	+		+				+		+	+	+	−	−	NT	−	NT					
R/Cali U	+				±[a]			+				+				+		+	+	−	−	−	−	−	+	−	+	−	−	
R/Cali I	+	+		+	±[a]			+						+				+	+	−	−	−	−	−	−	−	+	+	−	
R/Cali O	+	+			±[a]			+				+		+				+	+	−	−	−	−	−	−	−	+	−	−	
R/Cali S	+				±[a]			+										+	+			NT			−	−	+	−	−	
R/Cali L	+	+			±[a]													+	+			NT			−	−	+	+	−	
R/Cali P	+				±[a]			+			+							+	+			NT			−	−	+	−	−	
R/Cali M	+				±[a]			+			+				+			+	+			NT			−	−	+	+	−	
R/Cali T	+				±[a]			+			+							+	+			NT			−	−	+	+	−	

[a] In these clones a deleted chromosome 6 was present.
[b] NT = Not tested.

proteins occur in the medium which bind to immunoglobulin molecules or to immunoglobulin-containing complexes, the precipitin line which results from an antigen-antibody reaction may be radiolabeled. Positive results of the radioimmunoelectrophoresis experiments could therefore be due to (1) the presence of immunoglobulin molecules (e.g., γIg) in spent medium or (2) the presence of radiolabeled proteins in spent medium, which bind to certain immunoglobulins.

Experiments are being carried out to attempt to isolate material present in spent medium from hybrid clones carrying human chromosome 6, which gives positive results on immunoelectrophoresis autoradiographs.

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