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Genetics

Location of the genes for human heavy chain immunoglobulin to chromosome 6

(mapping/*in vitro* synthesis/differentiated function)

MOYRA SMITH AND KURT HIRSCHHORN

Department of Pediatrics, Mount Sinai School of Medicine of the City University of New York, 1 Gustave Levy Place, New York, New York 10029

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ABSTRACT **Immunoglobulin synthesis was examined in 31 man–mouse hybrid clones produced by fusing RAG mouse cells with human lymphoid cells. Cells were grown in serum-free medium containing [^{14}C]leucine and a ^{14}C-labeled amino acid mixture. Spent medium was dialyzed, concentrated, and subjected to radioimmunoelectrophoresis. Eighteen clones were found to produce material that gave a radiolabeled precipitin line with anti-human IgG (γ-chain specific). Production of material which was indistinguishable on radioimmunoelectrophoresis from human Ig γ heavy chain, was dependent on the presence in hybrid clones of human chromosome 6. The material was found to have the ion-exchange elution characteristics of human IgG. When radiolabeled spent medium from human lymphoid lines and from chromosome 6-positive hybrid clones was exposed to protein A-Sepharose and bound material eluted with 8 M urea was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis, three radiolabeled peaks occurred with molecular weights of approximately 55,000 (coinciding with that of Ig γ heavy chain), 40,000, and 25,000 (coinciding with that of Ig light chains). No similar peaks were detected in experiments where spent medium from RAG cells was treated identically. These studies lead us to conclude that certain RAG–human lymphoid hybrid clones produce human IgG and that the structural genes for γ heavy chains are located on human chromosome 6. These results also imply that the locus coding for human α_1-antitrypsin (Pi) is located on chromosome 6.**

Somatic cell hybridization techniques involving the fusion of human and rodent cells have been used extensively during the last decade to assign genes determining certain human proteins to specific human chromosomes (1). The majority of proteins that have been mapped represent constitutive proteins. Very few proteins that represent a differentiated function have been mapped. This failure is usually attributed to the facts that (*i*) differentiated functions are usually lost in cells cultured *in vitro* and (*ii*) even though differentiated functions may be maintained in the parent line, they are frequently lost when cells of this line are fused to other cell types (2). Immunoglobulin production represents a differentiated function that is maintained during prolonged *in vitro* culture of lymphoid cell lines developed from normal individuals (3). Orkin *et al.* (4) fused a mutant lymphoblastoid cell line, deficient in hypoxanthine phosphoribosyl transferase, with a mutant fibroblast cell line, deficient in thymidine kinase. Human λ light chain was the only immunoglobulin chain produced by the parent lymphoid line, as detected by immunofluorescence. Two independent hybrid clones obtained from this fusion carried human λ light chain on their cell surfaces and continued to do so after prolonged culture. One clone did not produce λ light chain detectable by surface immunofluorescence. Schwaber and Cohen (5) fused TEPC 15 mouse myeloma cells with human peripheral blood lymphocytes. They demonstrated by immunofluorescence studies and by immunoprecipitation of immunoglobulins in spent medium that hybrid cells synthesized both mouse and human immunoglobulins. Parkman *et al.* (6) found membrane bound immunoglobulins in hybrids produced by fusing human thymocytes with mouse fibroblasts. The immunoglobulins synthesized by the hybrid cells were apparently altered in several properties and were thought to be immunoglobulin fragments. In the study described here, the RAG mouse cell line, a hypoxanthine phosphoribosyltransferase deficient cell line derived from a mouse renal adenocarcinoma (7), and human lymphoid cell lines were fused. Hybrid clones were examined to define the chromosomal location of genes involved in immunoglobulin production in humans.

MATERIALS AND METHODS

Cell Fusion. The mouse parental line used for fusion was the RAG cell line (7). Six different human lymphoid lines were used: ODY, obtained from Michael Steel, Edinburgh, Scotland; RPMI 1788, from N. Tanigaki, Roswell Park Memorial Institute, Buffalo, NY; J. Fogh, Sloan–Kettering Institute, Rye, NY, supplied us with the Cali line; NB 60, NB 103, and NB 114 were lymphoid lines developed in this department by N. Beratis. Parental mouse and human cells were grown in RPMI 1640 medium containing 15% fetal calf serum. Fusion was carried out by using inactivated Sendai virus according to described methods (8, 9). After fusion, cells were grown in the hypoxanthine/aminopterin/thymidine (HAT) selective medium described by Littlefield (10). This medium favors selection of RAG–lymphoid cell hybrids, because RAG cells are killed in HAT medium and during successive medium changes, free growing lymphoid cells are progressively eliminated. Hybrid cells were then cloned using the technique of Ham and Puck (11). In some cases hybrid 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.

Determination of the Human Chromosomal Constitution of Hybrid Clones. Starch gel electrophoresis was used to determine the human, mouse, and hybrid isozyme forms of 19 different enzyme systems. The enzymes studied and their human chromosomal assignments are listed in Table 1. The starch gel electrophoresis methods and literature relating to the human chromosomal assignments are listed in refs. 1 and 12. Metaphase spreads for chromosomal analysis were prepared as described (9). Chromosomes were banded by using quinacrine hydrochloride fluorescence (13) and trypsin–Giemsa treatment (14). Staining with Giemsa 11 (15) was also used to distinguish mouse from human chromosomes. A clone was considered to be positive for a specific human chromosome when (*i*) a human enzyme known to be determined by a gene

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Abbreviations: $NaDodSO_4$, sodium dodecyl sulfate; P_i/NaCl, phosphate-buffered saline; RIE, radioimmunoelectrophoresis.

Table 1. Enzyme systems examined in man–mouse hybrids

Enzyme	Human chromosomal assignment
Phosphoglucomutase 1	1
Peptidase C	1
Malate dehydrogenase (soluble)	2
Isocitrate dehydrogenase (soluble)	2
Malic enzyme (soluble)	6
Superoxide dismutase (mitochondrial)	6
Glutathione reductase	8
Adenylate kinases 1 and 3	9
Glutamic oxalacetic transaminase	10
Lactate dehydrogenase A	11
Lactate dehydrogenase B	12
Esterase D	13
Nucleoside phosphorylase	14
Mannose phosphate isomerase	15
Peptidase A	18
Phosphohexose isomerase	19
Adenosine deaminase	20
Superoxide dismutase (soluble)	21
Glucose-6-phosphate dehydrogenase	X

The chromosomal assignments are according to ref. 1.

carried on that chromosome was identified in the hybrid and (*ii*) the specific human chromosome could be identified in at least 20% of the metaphases examined. In the case of chromosomes 3, 4, 5, 7, 16, 17, 22, and Y, the presence of the human chromosome was determined by karyotyping alone.

Radiolabeling and Concentration of Spent Medium. One day after trypsinization cultured cells were fed with serum-free, leucine-free minimal essential medium containing 0.5% ovalbumin, [^{14}C]leucine (0.5 μCi/ml, specific activity of 310 mCi/mmol), and a ^{14}C-labeled amino acid mixture (1 μCi/ml). Approximately 2.5×10^6 cells were incubated in 10 ml of medium for 6 days. Thereafter cell-free spent medium was dialyzed against three changes of 1000-fold volume excess of phosphate buffered saline (P_i/NaCl), containing 0.05 M potassium phosphate, 0.15 M sodium chloride, and 0.2% sodium azide. The dialyzed spent medium was then concentrated approximately 100-fold by vacuum dialysis in collodion bags. In some cases medium was concentrated by lyophilization after an initial dialysis against P_i/NaCl, then against a 1:2 dilution of P_i/NaCl and finally against a 1:10 dilution of this buffer. Lyophilized material was then reconstituted with distilled water to achieve 50- to 100-fold concentration.

Radioimmunoelectrophoresis (RIE). The method used was based on that described by Finegold *et al.* (16). Agarose gels (1% agarose in 0.05 M Veronal buffer, pH 8.6) were prepared on microscope slides that had been precoated with 0.5% agar in H_2O. Radiolabeled concentrated spent medium (approximately 10 μl) was applied in the sample well 2–3 times (a total of between 30,000 and 75,000 cpm of ^{14}C was applied). Carrier serum or, in some cases, purified IgG was applied to the sample well after application of radioactive material. After electrophoresis in Veronal buffer as described above, specific antiserum, e.g., rabbit anti-human IgG, was applied to the trough and precipitin lines were allowed to develop. Anti-human IgG antisera from four different sources were used in these experiments. Thereafter gels were soaked for 24 hr in P_i/NaCl and for an additional 24 hr in distilled H_2O. Gels were then dried and exposed to Kodak No-Screen x-ray film for 17–21 days. Autoradiographic films were developed according to the manufacturer's instructions.

DEAE-Sephadex Chromatography. The chromatographic conditions used were those described by Fahey and Terry (17) for isolation of human immunoglobulins from serum. DEAE-Sephadex was equilibrated in 15 mM Tris phosphate buffer, pH 8.0. Radiolabeled samples of concentrated spent medium were dialyzed against this same buffer before being applied to the column. After sample application, the column was washed with the equilibrating buffer. Two successive gradients were applied to the column, 0.15 M Tris phosphate (pH 8.0) and 0.3 M Tris phosphate (pH 8.0). When human serum was applied to the column under these conditions, IgG was eluted in the equilibrating buffer, while IgA and IgM were eluted with 0.15 M Tris phosphate.

Binding to Staphylococcus Protein A-Sepharose C14B Gel. The procedure used is based on that described by Moseley *et al.* (18) for assay of IgG. Staphylococcus protein A-Sepharose C14B (Protein A-Sepharose, Pharmacia) was purchased in freeze-dried form. The gel was reconstituted with P_i/NaCl (1 g per 3.5 ml). To determine direct binding of radiolabeled material in spent culture medium to this affinity gel, 100 μl of reconstituted gel was mixed with 400 μl of P_i/NaCl and 200 μl of concentrated spent medium containing between 2×10^5 and 10^6 cpm of ^{14}C, and the mixture was incubated for 30 min with occasional gentle agitation. In another series of experiments radiolabeled concentrated spent medium was incubated with rabbit anti-human IgG (25 μl of antiserum containing 0.5 mg of antibody per ml) for 30 min. Protein A-Sepharose was then added as in the direct-binding experiments. In both sets of experiments, after incubation with sample the protein A-Sepharose gel was washed three times with 5 ml of P_i/NaCl. After each wash, the gel was centrifuged at 1000 rpm for 3 min and the supernatant was removed. After the third wash, 0.5 ml of 8 M urea was added to the gel pellet, and this was allowed to stand at room temperature for 30 min. After centrifugation, aliquots of the supernatant were counted and subjected to sodium dodecyl sulfate ($NaDodSO_4$) polyacrylamide gel electrophoresis.

$NaDodSO_4$ Polyacrylamide Gel Electrophoresis. The procedure used is based on that described by Weber and Osborn (19). The standard $NaDodSO_4$ phosphate system was used. (0.1 M sodium phosphate, pH 7.2/0.1% $NaDodSO_4$/1% 2-mercaptoethanol). Samples electrophoresed included material eluted from protein A-Sepharose with 8 M urea, as described above (2000–4000 cpm ^{14}C was applied to each gel), and marker proteins IgG, (M_r of 55,000 for heavy chains and 25,000 for light chain) aldolase (M_r of 40,000) and chymotrypsinogen (M_r of 25,000). The marker proteins were electrophoresed separately from the radioactive samples; 1 μg of protein was suspended in 100 μl of urea. Radiolabeled samples and marker protein samples were mixed with an equal volume of 2% $NaDodSO_4$ in phosphate buffer as described above (containing 2% 2-mercaptoethanol), and then were boiled for 5 min. Fifty microliters of 80% (wt/vol) sucrose containing 0.05% bromphenol blue was added to 200 μl of boiled sample. Electrophoresis was carried out at 8 mA per gel. After electrophoresis, gels were measured, the position of the tracking dye was determined, and, in the case of gels to which radiolabeled samples had been applied, gels were cut into 2 mm slices and solubilized with 90% NCS solubilizer (Amersham/Searle). After 18 hr of incubation at 37°, acetic acid [50 μl of a 50% (vol/vol) solution] was added to 1 ml of solubilizer in each scintillation vial, followed by 10 ml of OCS scintillation fluid (Amersham/Searle). Gels with protein markers were stained with 0.1% Coomassie blue for 18 hr. Destained gels were remeasured and the R_F values for the protein bands were determined and these R_F values were related to those of the radiolabeled peaks.

RESULTS

RIE. A sample was considered positive for radiolabeled immunoglobulin if, when subjected to electrophoresis and autoradiography, a distinct radiolabeled arc was observed that coincided with the precipitin arc visible on protein staining. This radiolabeled arc had to be removed from the radiolabeled material that surrounded the sample loading well and extended into the anodal portion of the gel (see Fig. 1). This nonspecific radiolabeled material was not washed out of the gels despite prolonged soaking. Concentrated radiolabeled spent medium derived from 31 clones apparently contained I_g heavy chains, most frequently γ heavy chain (see Table 2). When radiolabeled concentrated spent medium from RAG cells was subjected to immunoelectrophoresis, no evidence was obtained to suggest that these cells produced material that reacted either with anti-human IgG or with anti-mouse IgG to produce precipitin lines.

Human Chromosomal Constitution of Hybrid Clones. The results of determination of the human chromosomal constitution of hybrid clones by a combination of karyotyping and studies of marker enzymes on starch gel electrophoresis are summarized in Table 2. By using the Giemsa 11 staining technique, we were able to clearly distinguish human from mouse chromosomes and to determine that no visible translocations between mouse and human chromosomes were present in the hybrid clones. The only human chromosome that was consistently present in hybrid clones that contained human heavy chain immunoglobulins in their spent media and that was consistently absent from clones that did not contain heavy chain immunoglobulins in their spent media, was chromosome 6. The enzyme markers for chromosome 6 were examined, soluble malic enzyme and mitochondrial superoxide dismutase (Table 3). In all 31 clones concordant segregation of γ-chain secretion and expression of human soluble malic enzyme occurred. In 24 clones γ-chain production and expression of mitochondrial superoxide dimutase were concordant. In the seven clones in which γ-chain production and mitochondrial superoxide dismutase expression were discordant, a deleted chromosome 6 occurred, (Fig. 2); this chromosome was deleted beyond 6q16.

DEAE-Sephadex Chromatography. When medium from five clones, classified as IgG heavy chain producers, was subjected to chromatography on DEAE-Sephadex and then to RIE, the radiolabeled material that reacted with anti-human IgG (γ-chain specific) on RIE was eluted from DEAE with the equilibrating buffer (0.015 M Tris phosphate, pH 8.0). In some cases material eluted with 0.15 M Tris phosphate, pH 8.0, reacted with anti-human IgA (α-chain specific) on immunoelectrophoresis. When material eluted from DEAE with 0.3 M Tris phosphate, pH 8.0, was concentrated, dialyzed and subjected to RIE, no radiolabeled arcs were detected using antisera against human immunoglobulin light and heavy chains. Medium from RAG cells, when subjected to the same procedures, produced no radiolabeled arcs. In control experiments when human serum was applied to the DEAE column, IgG was eluted almost entirely with the equilibrating buffer, (0.015 M Tris phosphate, pH 8.0) while IgA and IgM were eluted with 0.15 M Tris phosphate, pH 8.0.

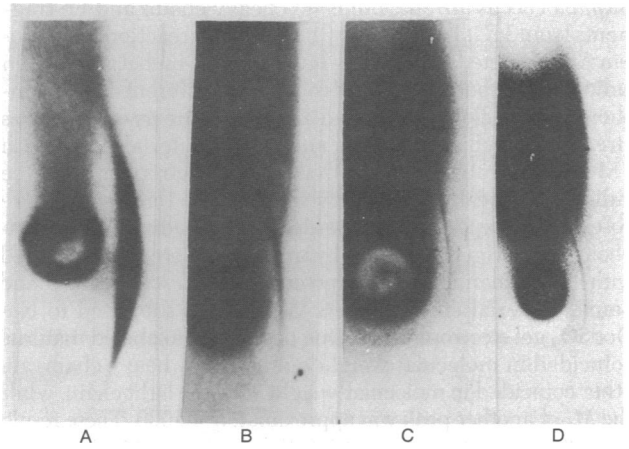

FIG. 1. RIE of concentrated spent medium from: (*A*) a human lymphoid line, Cali, and three RAG–human lymphoid hybrid clones; (*B*) R–Cali T; (*C*) R–ODY La 2; and (*D*) R–NB 103 J. After electrophoresis precipitin arcs were developed with anti-human IgG (γ-chain specific).

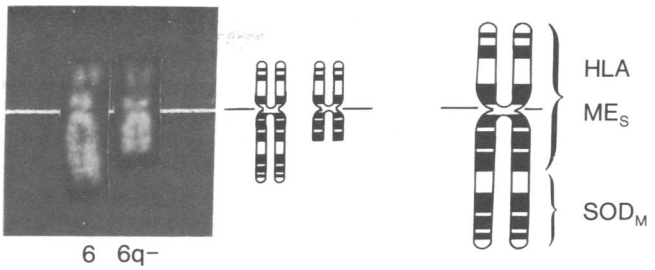

FIG. 2. Quinacrine hydrochloride fluorescence and diagrammatic representation of banding patterns on a normal human chromosome 6 and on the deleted chromosome 6 present in certain RAG–Cali hybrid clones. The regional locations on chromosome 6 of HLA, soluble malic enzyme (ME_s), and mitochondrial superoxide dismutase (SOD_m) are indicated (20).

Protein A-Sepharose Affinity and NaDodSO$_4$ Gel Electrophoresis. In the experiments in which radiolabeled concentrated spent media derived from human lymphoid lines and from four different IgG producing RAG–lymphoid hybrids were either directly applied to protein A-Sepharose or applied after prior incubation with rabbit anti-human IgG and bound material was subsequently eluted with 8 M urea, it was found that on NaDodSO$_4$ gel electrophoresis of this material, three peaks of radioactivity occurred. From comparison with the molecular weights of four marker proteins, IgG (γ chain and light chain), aldolase, and chymotrypsinogen, it was concluded that the three peaks had molecular weights of approximately 55,000, 40,000, and 25,000 (Fig. 3*A*). In experiments in which radiolabeled concentrated spent medium derived from RAG mouse cells was applied to protein A-Sepharose, a low percentage of ^{14}C-labeled material bound to the gel and was eluted with 8 M urea. No definite peaks were however observed on NaDodSO$_4$ gel electrophoresis of eluted material (Fig. 3*B*).

DISCUSSION

The combination of techniques of incorporation of radioactive amino acids into proteins and immunoelectrophoresis to determine the synthesis of immunoglobulins by cultured cells was first described by Hochwald *et al.* (21). It was noted that the sensitivity of RIE was greatly increased when carrier serum or specific carrier protein was applied to the sample well along with the radiolabeled culture medium. In our experiments the presence of carrier serum or carrier IgG was shown to be essential for the demonstration of radiolabeled arcs when concentrated spent medium or concentrated eluents from DEAE columns were subjected to RIE. The possibility that radiolabeled precipitin lines occurred as a result of nonspecific binding of radiolabel to carrier antigen was excluded by the fact that when concentrated spent medium from RAG cells and from hybrid clones that lacked chromosome 6 was subjected to RIE, no

Table 2. Chromosomal constitution as determined by enzyme and karyotypic analyses

Clones	Chromosomal constitution																						Ig heavy chain*		
	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
R-ODY Lb 11		+			+	+	+		+	+	+			+	+	+				+	+		+		+	+	+
R-ODY Lb 12		+			+	+	+		+	+	+				+	+				+	+		+			+	+
R-ODY Lb 10		+			+	+	+		+		+			+	+	+				+	+		+		+	+	+
R-ODY La 2						+							+	+				+	+		+		+				+
R-ODY La 10						+			+				+	+				+			+		+				+
R-RPMI E5-7														+						+							
R-RPMI G2-3			+						+				+								+	+	+	+	−	−	−
R-RPMI G2-9			+																		+	+	+	+	−	−	−
R-RPMI G2-1			+						+												+		+	+	−	−	−
R-NB 60 A1												+	+	+				+							−	−	−
R-NB 60 A3									+			+	+	+				+			+				−	−	−
R-NB 60 A8												+	+					+			+				−	−	−
R-NB 60A8-3												+									+				−	−	−
R-NB 103 C	+	+		+		+		+	+		+	+	+	+			+	+	+		+		+				+
R-NB 103 J						+		+	+		+	+	+	+				+			+		+				+
R-NB 103 F		+				+		+			+	+	+	+				+	+		+		+				+
R-NB 103C10		+						+			+		+	+									+		−	−	−
R-NB 103C2	+	+	+					+			+	+		+				+			+		+		−	−	−
R-NB 103C2G	+	+		+								+		+				+			+		+		−	−	−
R-NB 103C2N				+								+						+					+		−	−	−
R-NB 103C2B												+											+		−	−	−
R-NB 114A	+	+			+	+		+	+			+		+	+	+		+	+				+				+
R-Cali B	+	+			+	+	+			+	+	+		+	+			+	+		+		+				+
R-Cali U	+					+†				+				+							+		+		+		+
R-Cali I	+	+			+	+‡				+						+					+		+				+
R-Cali O	+	+				+‡				+				+		+					+		+				+
R-Cali S	+					+‡				+											+		+				+
R-Cali L	+	+				+‡															+		+				+
R-Cali P	+					+‡				+			+								+		+				+
R-Cali M	+					+‡				+			+								+		+				+
R-Cali T	+					+‡				+			+								+		+				+

* Immunoglobulins present in spent medium.

† In this clone both a deleted chromosome 6 and a normal chromosome 6 were present.

‡ In these clones a deleted chromosome 6 was present (6 q^-). See Fig. 2.

radiolabeled precipitin line was detected. Care was taken to ensure that the radiolabeled spent medium was adequately dialyzed prior to electrophoresis and that precipitin lines were developed in a position removed from the nonspecific radiolabeled material that surrounded the sample well and extended into the anodal portion of the gel. In an attempt to decrease the concentration of nonspecific radiolabeled material present in samples of spent medium and to define some of the characteristics of the material that produced a radiolabeled precipitin line with anti-human IgG, ion-exchange chromatography on DEAE was undertaken. The material from hybrid clones that produced a radiolabeled precipitin line with anti-human IgG was eluted from DEAE under the same conditions of ionic strength and pH as was human IgG. To exclude the possibility that the radiolabeled precipitin arcs obtained with anti-human IgG on RIE resulted from cellular production of material that bound to carrier IgG and to avoid the problems of nonspecific trapping of radiolabeled material during direct or indirect immunoprecipitation, experiments were undertaken in which radiolabeled spent medium from RAG–lymphoid hybrids was directly incubated with protein A-Sepharose. Forsgren and Sjöquist (22) showed that protein A, a cell wall constituent of *Staphylococcus aureus*, binds IgG heavy chains and Fc fragments from IgG. In the case of human IgG, reaction with protein A is restricted to the IgG1, IgG2 and IgG4 subclasses. When radiolabeled spent medium from three different RAG–lymphoid hybrid clones, classified as IgG producers on RIE, was directly exposed to protein A, and bound material eluted with 8 M urea was subjected to $NaDodSO_4$ gel electrophoresis, three radiolabeled peaks were observed. The same three peaks were observed in experiments in which spent medium from lymphoid lines or IgG producing hybrid clones were first incubated with anti-human IgG, then exposed to protein A Sepharose, and bound material eluted with 8 M urea was subjected to $NaDodSO_4$ gel electrophoresis. One peak of radiolabeled material coincided in molecular weight with the IgG heavy chain, another coincided in molecular weight with the light chain, while the M_r of another peak was approximately 40,000. These results strongly support the suggestion that certain RAG–lymphoid hybrid clones produce IgG, including γ heavy chain and light chain. It is not yet clear what the 40,000 M_r material represents. It may represent incomplete IgG γ chains or light-chain dimers.

Table 3. Segregation of immunoglobulin γ heavy chain production and the expression of soluble malic enzyme (ME_s) and mitochondrial superoxide dismutase (SOD_m) in 31 RAG–human lymphoid hybrid clones

		ME_s		SOD_m	
		+	−	+	−
Ig γ	+	18	0	11	7
	−	0	13	0	13

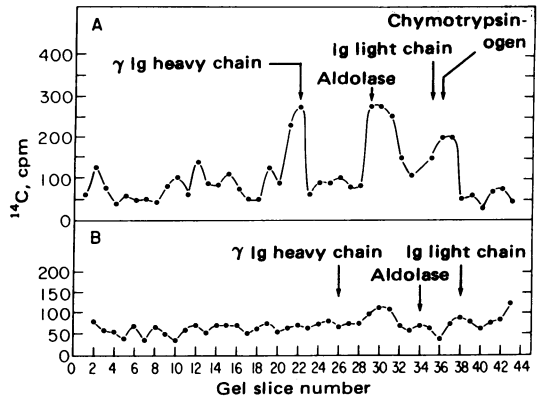

FIG. 3. Profile of $NaDodSO_4$ gel electrophoresis of material eluted from protein A-Sepharose with 8 M urea. Radiolabeled concentrated spent medium from (*A*) the RAG–lymphoid hybrid clone R–NB 114A and (*B*) the RAG mouse cell line was incubated initially with anti-human IgG (γ-chain specific) and then with protein A-Sepharose. The arrows indicate the relative positions of the marker proteins.

Results of these studies on the synthesis of immunoglobulin γ heavy chain by RAG–lymphoid hybrid clones, suggest that the structural genes for immunoglobulin heavy chains are carried on chromosome 6. From the results of studies on hybrid clones in which a deleted chromosome 6 was found, it seems probable that the genes for immunoglobulin γ heavy chains are carried on the short arm of chromosome 6 or on the long arm proximal to 6q16. It is known from family studies that the genes for immunoglobulin γ and α heavy chains are linked (23). These results imply therefore that at least two of the heavy-chain immunoglobulins are coded in chromosome 6. In our studies very few hybrid clones were found to contain IgA in their spent media. Using a double-antibody technique—i.e., treatment with rabbit anti-human IgA and subsequent treatment with goat anti-rabbit IgG that was fluorescein conjugated—positive surface immunofluorescence with anti-human IgA was demonstrated in RAG–lymphoid hybrid clones that carried human chromosome 2. Results of these studies have been described elsewhere (24, 25, *). In these experiments anti-human IgA from a single source was used. We recently determined that this antiserum produces surface immunofluorescence with human fibroblasts. No surface immunofluorescence on fibroblasts could be demonstrated with anti-human IgG or anti-human IgM. After absorption of this anti-human IgA with fibroblasts, immunofluorescence with mouse–human hybrid clones carrying chromosome 2 was no longer observed. Studies are now in progress to define the characteristics of the cell-surface component that reacts with antibodies present in certain anti-IgA antisera. In our studies very few clones have been found to contain IgM either on their cell surfaces or in their spent medium.

It has been found by family studies that the locus *Pi*, which codes for human α-1-antitrypsin, is linked to that for *Gm*, the polymorphic marker for IgG γ heavy chains (26). The present results therefore imply that the locus for *Pi* is also located on chromosome 6. The genes for the components of the major histocompatibility complex, including those for HLA, have been shown to be located on chromosome 6 on the short arm between bands 6p21 and 6p12 (27). Linkage of HLA and *Gm* has been excluded by Weitkamp *et al.* (28) who obtained negative lod scores for this combination (theta <0.3). Therefore even though the Ig γ heavy chain is on chromosome 6, it cannot be very close to the HLA region.

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