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Pattern of Immunoglobulin Synthesis and Assembly in a Human–Mouse Somatic Cell Hybrid Clone

(hybrid cells/hybrid immunoglobulin)

JERROLD SCHWABER AND EDWARD P. COHEN

La Rabida University of Chicago Institute and Departments of Theoretical Biology and Microbiology, University of Chicago, Chicago, Illinois 60649

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ABSTRACT **Fusion of human peripheral blood lymphocytes, not forming detectable immunoglobulins, with mouse myeloma cells (TEPC-15), secreting mouse immunoglobulin A with known antibody activity, yielded a somatic cell hybrid clone that secreted both human and mouse immunoglobulins. Analysis by sodium dodecyl sulfate–polyacrylamide gel electrophoresis indicated that human gamma, alpha, and light chains, as well as mouse alpha and light chains, were formed by the hybrid cells. To determine whether individual antibody molecules with both human and mouse components were secreted, medium from the hybrid cells was precipitated first with antibody against mouse immunoglobulin produced in rabbit, reduced, and alkylated, and then reprecipitated with antibody against human immunoglobulin produced in rabbit. Human gamma, alpha, and light chains were detected after electrophoresis of the immunoprecipitates on sodium dodecyl sulfate–polyacrylamide gels, indicating that antibody molecules containing both human and mouse components were secreted by the hybrid cells. These data indicate that this somatic cell hybrid clone synthesized human gamma and alpha heavy chains and human light chains, as well as mouse alpha heavy chains and mouse light chains. Some of these immunoglobulin components were assembled as hybrid antibody molecules.**

Immunoglobulin synthesis and secretion by individual antibody-forming cells is restricted to molecules of a single species, exhibiting one antibody specificity. Individual cells express only one of two codominant alleles of one immunoglobulin subclass. A cloned population of cells secreting multiple immunoglobulins may yield insight into the cellular basis of this restriction. Clones of such differentiated cells have not yet been isolated.

Somatic cell hybrids of immunoglobulin-forming cells may retain the potential of synthesizing and secreting immunoglobulins of both parental types. Such hybrids, however, either do not secrete immunoglobulins (1), or continue secreting only the single immunoglobulin formed before fusion (2, 3). Cotton and Milstein recently reported the isolation of a somatic cell hybrid of two immunoglobulin-forming cells that continued secretion of immunoglobulins of both parental types (4). We isolated previously a human–mouse somatic cell hybrid clone secreting mouse immunoglobulin (myeloma protein) (5). Human immunoglobulin synthesis and secretion also was initiated in this clone.

The somatic cell hybrid clone we reported resulted from the fusion of TEPC-15 mouse myeloma cells with human peripheral blood lymphocytes. Hybrid cells had a modal number of 73 chromosomes, including two metacentrics, as compared to 68 acrocentric chromosomes for the myeloma parent. Electrophoresis on sodium dodecyl sulfate–polyacrylamide gels of species-specific immune precipitates demonstrated that human and mouse immunoglobulins were secreted. By immunofluorescence, individual cells were found to synthesize both human and mouse immunoglobulins. Other clones of this hybrid pair that secrete human and mouse immunoglobulins have also been isolated and are being studied.

Possible restriction of immunoglobulin synthesis and secretion in this human–mouse somatic cell hybrid clone was investigated. Experiments were performed to determine the classes of human and mouse immunoglobulins that were secreted by the hybrid cells and to determine whether the human- and mouse-component immunoglobulin subunits (heavy and light chains) were assembled into hybrid immunoglobulin molecules.

MATERIALS AND METHODS

TEPC-15 mouse myeloma cells were maintained by serial intraperitoneal passage in BALB/c strain mice. They secreted mouse IgA ($\alpha_2\kappa_2$) with antibody activity to *Pneumococcus* C polysaccharide (6).

Normal human peripheral blood lymphocytes were obtained by sterile venipuncture from a healthy donor. The buffy coat cells from heparinized blood were incubated in plastic petri dishes for 1 hr at 37°. The cells that failed to adhere to the plastic were decanted. These cells consisted predominantly of small lymphocytes. By fluorescent antibody methods, approximately 40% of the peripheral blood lymphocytes obtained have membrane-bound antibody, but detectable amounts of human immunoglobulins are not secreted.

Human and mouse parental cells were fused with the aid of β-propiolactone-inactivated Sendai virus (7). Parental cells were cocultivated at ratios varying between 1:100 and 1:1000 (mouse myeloma cells to human lymphocytes) with 800 hemagglutinating units of Sendia virus. The cultures were grown in Eagle's minimum essential medium supplemented with 20% fetal-calf serum and 150 mM L-glutamine. The culture medium was changed completely every 4–5 days. Hybrid cell clone H1C11 was isolated as a single colony attached to plastic from a presumptive hybrid cell culture approximately 12 weeks after fusion (5).

Antiserum to human immunoglobulins was raised in a New Zealand White rabbit, injected biweekly with human serum immunoglobulins obtained by 50% $(NH_4)_2SO_4$ precipitation of whole human serum. Two to three milligrams of protein in

1 ml of phosphate-buffered saline, pH 7.2, was mixed in equal parts with Freund's complete adjuvant (Difco). The rabbit was bled monthly after the fourth immunizing injection. The gammaglobulin fraction of collected rabbit immune serum was obtained by precipitation with 50% $(NH_4)_2SO_4$. This fraction was absorbed once with TEPC-15 mouse myeloma protein, and then repeatedly absorbed with TEPC-15 cells. Antiserum to mouse immunoglobulins was obtained by immunization with mouse IgA obtained from ascites fluid of tumor-bearing (TEPC-15) mice by precipitation with 50% $(NH_4)_2SO_4$ followed by chromatography on DEAE-cellulose. The rabbit antiserum to TEPC-15 was absorbed once with human immunoglobulin, and then repeatedly absorbed with human lymphocytes. Antisera to human γ, α, and μ heavy chains were obtained from Meloy Laboratories and absorbed with TEPC-15 mouse myeloma protein and cells.

Experiments were performed to determine the specificity of the antisera. The species specificity of the antisera was tested with radioiodinated human or mouse immunoglobulin. Antiserum to mouse Ig was incubated with ^{125}I-labeled human immunoglobulin. An amount of antiserum to human Ig that was less than equivalence was then added (coprecipitation). This antiserum was considered species specific when no additional radioactivity was precipitable by the anti-mouse Ig, as detected by either direct radioactivity counting or radioactive analysis of Na dodecyl sulfate gel electrophoresis of the precipitates. The same criterion, with mouse immunoglobulin rather than human immunoglobulin, was used for antiserum to human Ig and the antisera specific for human heavy-chain classes. Reduced and alkylated, radioiodinated proteins were incubated with heterologous antiserum and analyzed by direct radioactivity determination and by determination of radioactivity in electrophoresis gels to ensure that there was no crossreactivity by a direct assay system. Since fetal-calf serum was present in the radioiodination mixture, tests were performed to determine whether the antisera might crossreact with fetal-calf serum proteins. Antiserum to human or mouse Ig was added to radioiodinated fresh culture medium. Antiserum to bovine-serum albumin was then added, and the resultant precipitates were examined for precipitation of proteins that did not precipitate with the antibody against bovine-serum albumin alone. Electrophoresis of the ^{125}I-labeled culture medium–anti-bovine-serum albumin precipitates demonstrated that the antisera to human or mouse Ig did not crossreact with proteins in the fetal-calf serum.

Samples of culture medium from the hybrid cells were isolated by $(NH_4)_2SO_4$ precipitation. The final precipitates were resuspended in an appropriate volume to obtain 1–3 mg of protein per ml. Similar concentrations of human serum immunoglobulins and mouse myeloma proteins were used as controls. To determine the classes of heavy chains that were synthesized by the hybrid clone, proteins from the culture medium were reduced with 0.2 M 2-mercaptoethanol and alkylated with 0.2 M iodoacetamide (8). After reduction and alkylation, the proteins were labeled with ^{125}I by the chloramine T method (10, 11). The proteins were then precipitated with the appropriate antiserum and the precipitates were analyzed subsequently by electrophoresis on Na dodecyl sulfate–polyacrylamide gels. Each class of heavy chains, as determined by electrophoretic migration, was confirmed subsequently by precipitation with the appropriate class-specific antiserum, with the presence of the single appropriate peak on Na dodecyl sulfate–polyacrylamide electrophoresis. To detect hybrid immunoglobulins, secreted hybrid cell protein was labeled with ^{125}I and precipitated with rabbit antiserum to either human or mouse Ig. The immune precipitate was dissociated into heavy and light chains by reduction and alkylation, and then reprecipitated with the antiserum not used for the first precipitation. As a control, mouse–human hybrid immunoglobulin was prepared in a cell-free system from whole human and mouse immunoglobulins. Equal parts of human immunoglobulin and TEPC-15 protein were reduced with 0.2 M 2-mercaptoethanol at pH 4.0 for 4 hr. The reduced proteins were then exhaustively dialyzed against 10 mM phosphate buffer, pH 7.2, and 0.15 M NaCl, allowing the human and mouse heavy and light chains to recombine randomly.

Reduction of proteins was performed in a solution with 0.2 M 2-mercaptoethanol, 80 mM NaCl, at pH 3.5–4.0. The proteins were alkylated after 4 hr by the addition of iodoacetamide to 0.2 M. The solution was then dialyzed against 10 mM phosphate buffer, pH 7, with 0.15 M NaCl, to remove the mercaptoethanol and to return the solution to neutral pH. This method is a modification of that reported by Criddle (9) to obtain soluble dissociated heavy and light chains. The protein that remained in solution at neutral pH consisted of dissociated heavy and light chains that were precipitable by antisera. Electrophoresis on Na dodecyl sulfate gels of the soluble and insoluble fractions after reduction and alkylation indicated that (*i*) 20–30% of the initial immunoglobulin was soluble in dissociated form and (*ii*) the dissociated heavy and light chains of different classes and species were present in the same proportions in the soluble fraction as in the insoluble fraction.

All immunoglobulins investigated were labeled with ^{125}I (10, 11). For each experiment, approximately 300–500 μg of sample was incubated with 200 μg of chloramine T and 1.0 mCi of Na^{125}I for 2–5 min. Three hundred micrograms of sodium metabisulfite was added to stop the reaction. Labeled protein was separated from free iodide by chromatography on Sephadex G-25. Labeled samples were frozen at $-20°$ and analyzed within 2 weeks.

Radioiodinated samples were directly precipitated at equivalence with the appropriate antiserum. The immunoprecipitates were resuspended in 10 mM phosphate buffer, pH 7.2, with 2% sodium dodecyl sulfate and incubated at 37° overnight. They were heated to 90° for 1 min, and then dialyzed against 10 mM phosphate buffer, pH 7.2, and 0.1% Na dodecyl sulfate before electrophoresis. Polyacrylamide gel electrophoresis was performed following the procedure of Maizel (12), using 10-cm gels, 5% in acrylamide with 0.1% Na dodecyl sulfate. Electrophoresis, with 10 mM phosphate buffer, pH 7.2, with 0.1% Na dodecyl sulfate, was performed at 6 mA per gel. Human or sheep hemoglobin served as the position marker: electrophoresis was stopped when the hemoglobin was 0.5–1.0 cm from the end of the gel. Human or sheep hemoglobin, bovine-serum albumin, and rabbit IgG were used as references of known molecular weight. For absorbance analysis, the gels were fixed and stained with Coomassie blue, destained, and scanned at 520 nm in a Gilford spectrophotometer equipped with a linear transport mechanism. For radioactivity determinations, 2-mm slices were placed in scintillation vials with 0.5 ml of water and incubated

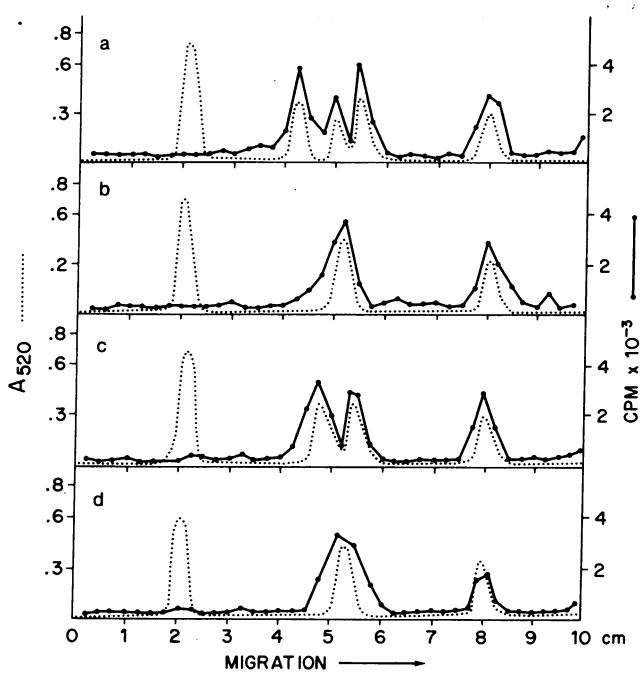

FIG. 1. Representation of Na dodecyl sulfate gel electrophoresis of reduced and alkylated, radioiodinated human serum immunoglobulins, TEPC-15 myeloma protein, and secreted hybrid cell protein precipitated with antisera specific for human Ig or for mouse Ig. (*a*) Human immunoglobulin, precipitated with anti-human Ig; (*b*) TEPC-15 myeloma protein, precipitated with anti-mouse Ig; (*c*) H1C11 hybrid cell protein, precipitated with anti-human Ig; (*d*) H1C11 hybrid cell protein, precipitated with anti-mouse Ig. Values for cpm have been multiplied by 10^{-3}.

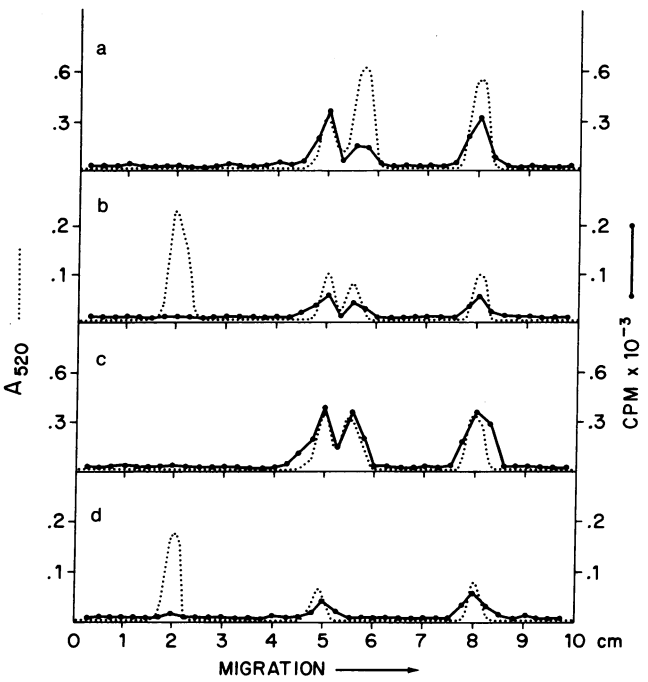

FIG. 2. Electropherograms of Na dodecyl sulfate gels of immune precipitates that formed after sequential precipitation of radioiodinated protein secreted by the hybrid cells. Hybrid H1C11 Ig: (*a*) precipitated with anti-mouse Ig; (*b*) precipitated with anti-mouse Ig, reduced and alkylated, then precipitated with anti-human Ig; (*c*) precipitated with anti-human Ig; (*d*) precipitated with anti-human Ig, reduced and alkylated, then precipitated with anti-mouse Ig.

at 37° for 48 hr. The radioactivity in the samples was determined in a Packard liquid scintillation counter with Bray's solution as scintillator.

RESULTS

The classes of human and mouse immunoglobulins secreted by the hybrid cells were determined by molecular size estimation of reduced and alkylated, radioiodinated proteins isolated by specific immunoprecipitation. Migration of reduced and alkylated TEPC-15 mouse myeloma protein on Na dodecyl sulfate–polyacrylamide gels yielded bands corresponding to light chains (23,000 daltons) and alpha chains (59,000 daltons) (Fig. 1*b*). Human serum immunoglobulins, also reduced and alkylated and then radioiodinated, contained bands corresponding to light chains (23,000 daltons), gamma chains (55,000 daltons), alpha chains (60,000 daltons), and mu chains (68,000 daltons) (Fig. 1*a*). Immunoglobulins from the hybrid cell culture medium were reduced and alkylated, radioiodinated, and precipitated with antiserum to either human Ig or mouse Ig. The precipitate that formed after incubation with antiserum specific for mouse Ig contained light chains and alpha chains (Fig. 1*d*). The precipitate from incubation with antiserum specific for human Ig contained peaks on electrophoresis corresponding to human light chains, alpha chains, and gamma chains (Fig. 1*c*). Since the antisera used to isolate these proteins were specific for the species of origin of the immunoglobulins, the hybrid cells from clone H1C11 secreted light chains and alpha chains of both human and mouse origin and gamma chains that were of human origin only.

Studies were performed to determine whether immunoglobulin molecules composed of mouse and human components were assembled by the hybrid cells. Radioiodinated proteins from the hybrid cell culture medium were precipitated with antiserum specific for human Ig. This precipitate was reduced and alkylated to dissociate the heavy and light chains. The separated chains were then reprecipitated with antiserum to mouse Ig. A similar experiment was performed, except the order of addition of specific antisera was reversed; i.e., whole radioiodinated immunoglobulins were precipitated with antiserum to mouse Ig followed by precipitation of the dissociated heavy and light chains with antiserum to human Ig. Using this sequential precipitation method, we detected hybrid antibody molecules containing components of mouse immunoglobulins assembled with human heavy and light chains. In the reverse experiment, we detected human immunoglobulin components assembled with mouse alpha and light chains.

Electrophoretic examination of the immunoprecipitates formed by sequential precipitation revealed that human and mouse components were assembled into hybrid immunoglobulin molecules. Precipitation of protein secreted by the hybrid cells first with antiserum to mouse Ig, followed by antiserum to human Ig, resulted in electrophoretic peaks corresponding to human gamma, alpha, and light chains (Fig. 2*a* and *b*). These chains had been assembled by the hybrid cells with mouse alpha or light chains. The peaks of human heavy and light chains represented 3–5% of the total

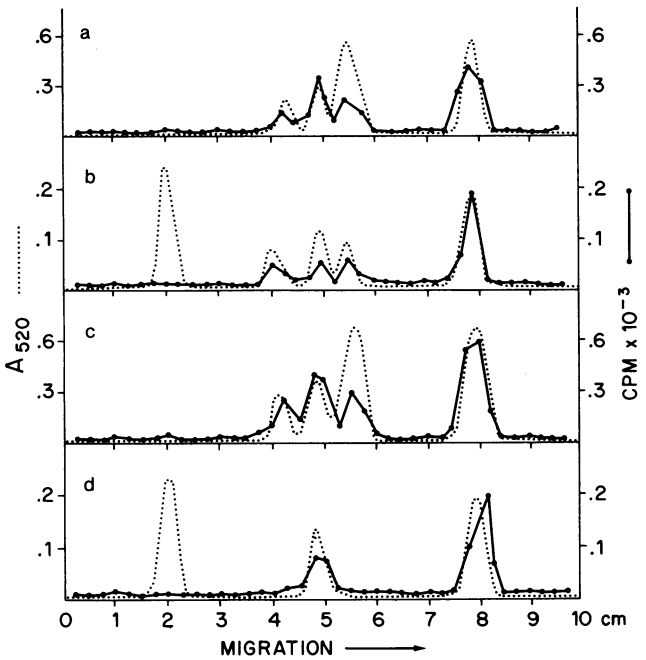

FIG. 3. Hybrid immunoglobulins made in a cell-free system were radioiodinated and then sequentially precipitated with antisera specific for human Ig or for mouse Ig. The patterns found after Na dodecyl sulfate gel electrophoresis of the immune precipitates are shown. (*a*) Precipitated with anti-mouse Ig; (*b*) precipitated with anti-mouse Ig, reduced and alkylated, then precipitated with anti-human Ig; (*c*) precipitated with anti-human Ig; (*d*) precipitated with anti-human Ig, reduced and alkylated, then precipitated with anti-mouse Ig.

radioactivity precipitable with antiserum to mouse immunoglobulin. The reverse experiment, precipitation first by antiserum to human Ig, followed by precipitation with antiserum to mouse Ig, resulted in peaks on electrophoresis represented mouse alpha and light chains (Fig. 2*c* and *d*). These mouse immunoglobulin components had been assembled with human heavy or light chains. The radioactivity precipitable with antiserum to mouse Ig represented 5% of the radioactivity precipitable with only antiserum to human Ig. Control experiments, in which complete radioiodinated human and mouse immunoglobulins were mixed in equal parts and then sequentially precipitated, indicated that this method is specific for hybrid immunoglobulin molecules.

The efficiency of hybrid molecule formation by the hybrid cells was compared with random assembly of human and mouse immunoglobulin components. Human and mouse immunoglobulins were reduced with 2-mercaptoethanol, allowed to recombine randomly, and then sequentially precipitated with the rabbit antisera to human and mouse Ig. Electrophoretic peaks corresponding to human light, alpha, and mu chains were found in the immunoprecipitate first precipitated with rabbit anti-mouse Ig and then with rabbit antiserum to human Ig (Fig. 3*a* and *b*). Hybrid molecules contained about 10% of the heavy chains reassembled in the cell-free system. This is somewhat higher than the hybrid molecules formed by the hybrid cells. Light chains were reassembled into hybrid molecules more efficiently than heavy chains: human light chains represented about 30% of the radioactivity associated with light chains in the precipitate formed with antiserum to mouse immunoglobulin. Precipitation first by rabbit antiserum to human Ig and then by antiserum to mouse Ig yielded similar results (Fig.3*c* and *d*). Mouse light chains were reassembled into hybrid molecules at a higher rate than mouse alpha chains.

To determine whether the assembly of hybrid immunoglobulin molecules was specific for the particular class of heavy chains, a sequential precipitation experiment was performed using antisera specific for the class of human heavy chain. Protein secreted by the hybrid cells was precipitated first with antiserum to either human gamma or alpha chains, and was reprecipitated with antiserum to mouse Ig. When hybrid cell protein was precipitated first with antiserum specific for human alpha chains, electrophoretic peaks representing mouse alpha and light chains were found. Human alpha chains and mouse alpha chains were assembled together in single hybrid immunoglobulin molecules (Fig. 4*b*). Human gamma chains, however, were assembled into hybrid molecules only with the mouse light chains (Fig. 4*a*). There was, then, specificity of assembly into hybrid immunoglobulin molecules of the human and mouse heavy chains.

DISCUSSION

Hybrid cell clone H1C11, resulting from the fusion of mouse myeloma cells with human peripheral blood lymphocytes, secreted immunoglobulins of both human and mouse origin. Examination of reduced and alkylated, radioiodinated hybrid cell protein by Na dodecyl sulfate–polyacrylamide gel electrophoresis revealed that mouse alpha and light chains and human gamma, alpha, and light chains were secreted by these cells. No fragments of heavy or light chains were detected.

The synthesis by the hybrid cells of multiple immunoglobulin chains of both parental types indicates that the usual limitations on immunoglobulin synthesis by nontransformed cells do not restrict the types of immunoglobulins synthesized by hybrid cells. We conclude that the failure of hybrids involving lymphoid cells and undifferentiated cell types (1–3 to synthesize multiple immunoglobulins results from a general restriction of synthesis of differentiated products in such cells, rather than from a specific restriction on the synthesis of multiple immunoglobulins. The synthesis of human gamma chains by the clone we examined indicates that the initiation of human immunoglobulin synthesis in this hybrid does not result from a class-specific mechanism of regulation.

The synthesis of multiple immunoglobulin heavy and light chains in individual hybrid cells results in the assembly of hybrid immunoglobulin molecules. To detect such hybrid molecules, we sequentially precipitated protein secreted by the cells with species-specific antisera. The antisera we used had no cross-species reactivity detectable in our assay system. The hybrid cell clone we examined secreted proteins that were precipitable sequentially by antisera to both human and mouse Ig. This hybrid antibody represented only 5% of the total of secreted immunoglobulin. Since free heavy or light chains were not secreted by the hybrid cells, we exclude the possibility that the hybrid immunoglobulin formed is the result of random extracellular assembly, although spontaneous dissociation and reassembly could not be excluded. However, mixed human and mouse immunoglobulins did not form hybrid molecules when subjected to the same conditions of collection, labeling, and precipitation as that used for the hybrid cell protein. The peaks found after sequentially precipitating hybrid cell immunoglobulin represent hybrid im-

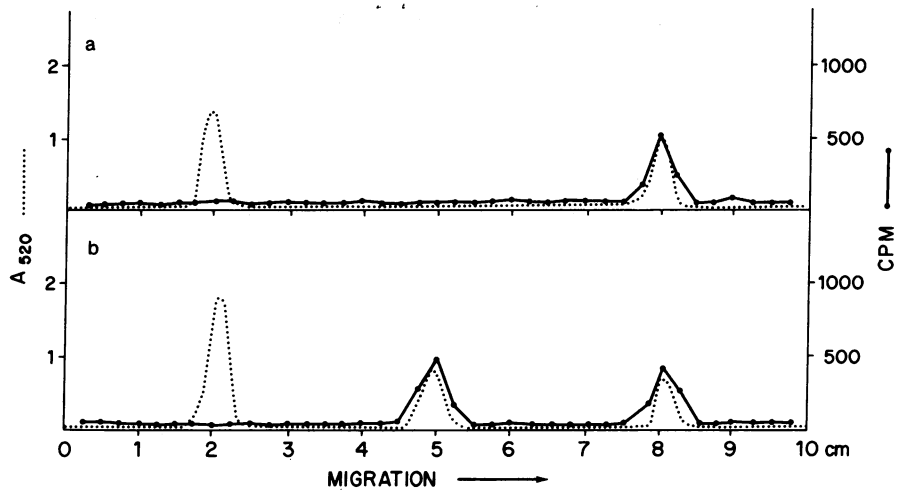

FIG. 4. Electrophoretic patterns of immune precipitates that formed after precipitation of hybrid Ig first with antiserum specific for human γ (*a*) or α (*b*) chains, reduction and alkylation, and then reprecipitation with antiserum specific for mouse Ig.

munoglobulins assembled intracellularly. Alpha chains of human and mouse origin were combined into single hybrid immunoglobulin molecules by the hybrid cells. There was class specificity of assembly in the hybrid cells: human gamma chains assembled with only mouse light chains, while human alpha chains assembled with both mouse light chains and with mouse alpha chains. This finding suggested that the assembly of hybrid immunoglobulin depended upon the formation of interchain disulfide bonds.

We compared hybrid immunoglobulin formation by the hybrid cells with cell-free random assembly. Cell-free recombination of human and mouse immunoglobulins indicated that light chains randomly recombined into hybrid molecules more frequently than occurred when the components were assembled by the hybrid cells. Heavy chains also recombined into hybrid immunoglobulins at a higher rate in the cell-free system than occurred with assembly by the hybrid cells.

We do not know whether the antisera we used had specificity for antigens associated with variable regions of heavy or light chains. Most interspecies antigenic markers reside in the constant region. Thus, it is unlikely that hybrid heavy or light chains, resulting from the peptide-bonded association of variable and constant regions of human and mouse origin, would be detected by the system we used.

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