Infectious Mononucleosis: Immunoglobulin Synthesis by Cell Lines

PHILIP R. GLADE and LAWRENCE N. CHESSIN

From the Laboratory of Clinical Investigations, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014

ABSTRACT Immunoglobulin synthesis by 16 long-term suspension cultures of mononuclear cells derived from peripheral blood of nine patients with heterophile-positive infectious mononucleosis (IM) has been demonstrated by radioimmunoelectrophoretic techniques. All cell lines synthesized molecules with IgG (γ) heavy chain specificity. 14 cell lines produced molecules with IgM (μ) heavy chain specificity and 11 cell lines produced molecules with IgA (α) heavy chain specificity. No detectable synthesis of molecules with IgD (δ) heavy chain specificity was observed by these cell lines derived from peripheral blood of patients with IM. 13 cell lines produced molecules with type K (κ) light chain specificity and 6 cell lines produced molecules with type L (λ) light chain specificity. Of interest, 9 of 16 lines produced IgG (γ), IgA (α), and IgM (μ) heavy chain molecules and 5 of these cell lines produced molecules with type K (κ) and type L (λ) light chain specificity as well.

Further characterization by combined polyacrylamide gel filtration, immunodiffusion, and radioautography indicated the presence of newly synthesized immunoglobulin molecules with both heavy and light polypeptide chains in close association as well as free light polypeptide chain synthesis. Investigation of the localization of immunoglobulin in single cells by immunofluorescent techniques revealed that 5–22% of cells in these lines were strongly reactive with a fluorescein isothiocyanate–conjugated rabbit antisera directed against the antigenic determinants of human IgG and cross-reactive with the determinants common to IgA and IgM. No heterophile antibody, heteroagglutinin, or hemolytic antibody could be demonstrated in these cell lines derived from peripheral blood of patients with heterophile-positive infectious mononucleosis.

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INTRODUCTION

Infectious mononucleosis (IM) is characterized by a striking lymphocytosis with large numbers of atypical lymphocytes in the peripheral circulation (1) and a remarkable increase in total serum immunoglobulins (2) with the appearance of heteroantibodies (3), autoantibodies (4), and heterophile antibody (5). The function of the circulating lymphocyte in this disease is essentially unknown (6) and the site of antibody production remains obscure. The frequent association of atypical lymphocytes in infectious (7), allergic (8), and immunologic (9) disorders, however, suggests that circulating cells in IM may play a significant role in the pathogenesis of this disease process. "Marked" synthesis of IgG, IgA, and IgM by peripheral leukocytes obtained during the 1st 10 days of clinical illness with IM has been noted by van Furth, Schuit, and Hijmans (10). Samples obtained after the 14th day of illness, however, displayed biosynthetic profiles identical to that of normal peripheral blood. Although unconfirmed, apparent synthesis of immunoglobulins with heterophile specificity has been suggested as well (11). Immunofluorescent studies, nevertheless,

usually fail to detect immunoglobulins in the peripheral lymphocyte in IM (12). At present these discrepancies remain unresolved and there is little evidence to link the circulating lymphocyte in IM with immunoglobulin and specific antibody synthesis.

In recent studies of the etiology and pathogenesis of infectious mononucleosis, we have demonstrated increased potential of peripheral blood leukocytes in IM for long-term in vitro proliferation (13, 14). 16 continuous suspension cultures of mononuclear cells derived from peripheral blood of nine patients have been established to date. With the techniques employed in this study, comparable collateral specimens from 18 individuals without clinical illness failed to proliferate in long-term culture (Table I). Furthermore, light microscope and ultrastructure analysis revealed that these lines are composed of a spectrum of lymphoid cells, some with close morphologic similarity to Downey cells (1). These findings suggested that the established lines may be related to the infectious mononucleosis state and afforded an unusual opportunity for repeated and controlled observations of the functional and biosynthetic capacities of lymphoid cells derived from peripheral blood of patients with infectious mononucleosis.

The present report concerns the biosynthesis of immunoglobulins by these cell lines derived from peripheral blood of patients with heterophile-positive IM. Newly synthesized protein was detected by isotopic labeling with amino acids-^{14}C and characterized by combined agar gel radioimmunoelectrophoresis and analytical multiphase zone electrophoresis in polyacrylamide gels. Investigation of antibody specificity for the newly synthesized immunoglobulins was undertaken as well.

TABLE I
Attempts To Establish Long-Term Suspension Cultures from Peripheral Blood Leukocytes

	No. of individuals	No. of specimens	Continuous suspension cultures engendered
No obvious clinical illness	18	24	0
Infectious mononucleosis	23	66	16

METHODS

Patients

Patients fulfilling clinical, hematologic, and serologic criteria suggested by Hoagland (15) for the diagnosis of IM were admitted for study to the Clinical Center of the National Institutes of Health. The patients ranged in age from 6–28 yr and had been in good health before the onset of IM.

Control patients were healthy volunteers of similar age who were screened for history of recent illness, immunizations, and consumption of specific medications.

Origin and maintenance of established cell lines

The establishment and characterization of continuous suspension cultures of lymphoid cells derived from peripheral blood leukocytes of patients with IM has been described previously (13, 14). All established cultures were maintained in Roswell Park Memorial Institute medium 1640 (16) supplemented with 20% heat-inactivated fetal calf serum, penicillin, streptomycin, and fresh *l*-glutamine in 8-oz screw cap prescription bottles and were regularly transferred into fresh medium at 3–4 day intervals. Before biosynthetic studies, a 3–4 day old culture was sedimented at 250 *g* for 30 min at 37°C in an International PR-2 centrifuge. The supernatant culture fluid was removed and the cells were resuspended in a small volume of Eagle's Spinner MEM (17) deficient in *l*-lysine and *l*-isoleucine.

Detection and characterization of newly synthesized immunoglobulins by continuous cell lines

Detailed description of the procedures for the detection and characterization of newly synthesized immunoglobulins has been reported previously (18).

A. In vitro production of ^{14}C-labeled proteins. Synthesis of immunoglobulins by cell lines was demonstrated with radioimmunoelectrophoretic techniques following the incorporation of amino acids-^{14}C into newly synthesized protein. A standard labeling medium was employed for this procedure consisting of Eagle's Spinner MEM deficient in *l*-lysine and *l*-isoleucine and supplemented with *l*-lysine-^{14}C (SA 198 mc/mmole, Schwartz BioResearch, Orangeburg, N. Y.), *l*-isoleucine-^{14}C (SA 160 mc/mmole, Schwarz BioResearch, Orangeburg, N. Y.), 10% heat-inactivated fetal calf serum, and fresh *l*-glutamine. The final concentration for each radioactive amino acid was 1 μc/ml. For biosynthesis 0.2 ml aliquots of washed cells (approx. 1.0×10^7) were added tc 1.8 ml of labeling medium in 16×125 mm screw cap disposable glass culture tubes. After incubation for 18 hr at 37°C in an upright stationary position, the cultures were frozen, thawed, and centrifuged at 15,000 *g* for 20 min. The supernatants were then dialyzed against 100 volumes of 0.014 M NaCl for 48 hr at 4°C to remove unincorporated amino acids. The dialysate was lyophi-

lized and reconstituted to 1/10 the original volume with distilled water. Several lines were studies at multiple cell concentrations and biosynthetic studies were repeated at multiple time intervals of continuous culture.

B. Radioimmunoelectrophoresis. Microimmunoelectrophoresis was performed in 1.5% agar with a barbital-acetate buffer, μ 0.05, pH 8.6 at 5 v/cm for 60 min. Precipitin arcs obtained by coprecipitation with human serum (19) were developed for 48 hr with appropriate antisera. Radioautography was performed by exposing Kodask medical, no screen, x-ray film to the dried plates for 1–2 wk. After radioautographs were developed the immunoelectrophoretic pattern was stained with fast green and the radioautographs were compared with the stained plates to determine electrophoretic mobilities of the labeled precipitin arcs.

C. Characterization and identification of newly synthesized proteins by multiphase zone electrophoresis in polyacrylamide gels. Further characterization of the newly synthesized protein by disc electrophoresis on polyacrylamide gels was performed according to the procedure described by Reisfeld, Lewis, and Williams (20) with a modified buffer system (21) without urea. Electrophoresis was carried out for 55 min in 5% acrylamide gel at a constant current of 2.5 ma/tube. Protein bands were localized by fixing the gel in 5% trichloroacetic acid or by staining with Coomasie blue. The R_f value of each band was determined and compared with the R_f values obtained for purified preparations of heavy (α, μ, γ) and light (κ, λ) polypeptide chains electrophoresed in parallel. The specificity of the protein bands was determined by embedding duplicate gels in agar, diffusion against specific antisera, and subsequent radioautography of the immunodiffusion patterns.

D. Antisera. Immunoglobulins were isolated from pooled normal serum (IgG), individual sera containing myeloma proteins (IgA, IgD), sera from patients with macroglobulinemia (IgM), and from individuals' urines containing Bence-Jones proteins (type K and L) according to methods of Fahey and McLaughlin (22). Antisera against pooled whole human serum (polyvalent), class specific for IgG, IgA, IgM, and IgD (specific for γ, α, μ, and δ heavy polypeptide chains) and type specific for L chain molecules (specific for light polypeptide chains κ and λ) were prepared in rabbits. Antisera were absorbed with hypogammaglobulinemic serum and appropriate purified immunoglobulin preparations to render them specific. The specificity of each reagent was determined by Ouchterlony and immunoelectrophoretic procedures against whole serum and purified immunoglobulin preparations.

E. Immunofluorescent staining. The localization of immunoglobulins in single cells was investigated by modification of the immunofluorescent techniques of Coons and Kaplan (23) with a fluorescein isothiocyanate–conjugated rabbit antiserum directed against the antigenic determinants of human IgG and cross-reactive with the determinants common to IgA and IgM prepared according to the method of Wood, Thompson, and Goldstein (24). Cell suspensions from 2 to 3 day cultures were washed three times in equal volumes of Eagle's MEM and resuspended in 20% bovine serum albumin in Eagle's MEM. Smears made on glass microscope slides were fixed in acetone for 10 min, air dried, and washed three times in 0.01 M phosphate-buffered saline, pH 7.4. The preparations were then incubated for 60 min at room temperature with optimal dilutions of the fluorescein-conjugated antisera proviously determined for each cell line. Direct blocking by prior incubation of duplicate preparations with the unconjugated antisera and purified immunoglobulins served as controls for each test. After incubation the preparations were washed three times in 0.01 M phosphate-buffered saline, pH 7.4, mounted under cover slips in 50% glycerolphosphate solution, and examined with an AO-Fluorstar microscope. A minimum of 500 lymphocytes were counted for each culture.

Investigation of heteroagglutinins and specific heterophile activity in established cell lines

3-day-old cultures containing 100 ml of standard medium with approximately $1–2 \times 10^6$ cells/ml were frozen, thawed, and sedimented at 15,000 *g* for 30 min. The supernatants were concentrated 50–100-fold by negative pressure ultrafiltration. Assay of heterophile agglutinin activity was performed according to the methods of Davidsohn and Walker (25) with 2% suspension of fresh-wash sheep red blood cells. In addition, heteroagglutinin activity for horse and beef red cells was investigated in similar fashion. All cell suspensions employed were tested simultaneously with previously titered IM sera and found to be highly reactive.

Attempts to demonstrate localized hemolysis in gel

Cells from continuous cultures were examined for their ability to produce localized hemolysis in gel against sheep, horse, beef, and pig red cells according to the methods of Jerne, Nordin, and Henry (26). Cells were examined for the production of IgM (19S) and IgG (7S) antibody. For detection of IgG (7S) antibody activity, plates were incubated with a specific rabbit antihuman IgG serum before addition of complement. Spleen cells derived from rabbits immunized with either sheep, horse, beef, or pig red blood cells served as positive controls for these studies. Spleen cells obtained from normal rabbits controlled for background hemolytic activity.

RESULTS

Radioimmunoelectrophoresis. Immunoglobulin synthesis was demonstrated in all 16 human cell lines derived from peripheral blood of nine patients with infectious mononucleosis. With the stationary culture technique employed, optimal biosynthetic capacity was dependent upon cell concentration and varied within limits for each cell line studied. The optimal cell density for the 2-ml

TABLE II

Immunoglobulin Biosynthetic Capacity of Lymphoid Cell Lines from Patients with Infectious Mononucleosis

Cell Line	Precipitating antisera specific					
		Heavy chains			Light chains	
	IgG (γ)	IgM (μ)	IgA (α)	IgD (δ)	K (κ)	L (λ)
PGLC 33H	+	+	+	Neg	+	+
PGLC 33J	+	+	+	Neg	+	+
PGLC 42D	+	+	+	Neg	+	Neg
PGLC 42F	+	+	+	Neg	+	+
PGLC 44B	+	+	Neg	Neg	+	Neg
PGLC 50B	+	+	+	Neg	Neg	Neg
PGLC 50D	+	+	Neg	Neg	Neg	Neg
PGLC 51D	+	+	+	Neg	+	+
PGLC 51E	+	+	+	Neg	+	+
PGLC 53B	+	+	Neg	Neg	+	Neg
PGLC 53D	+	Neg	+	Neg	+	Neg
PGLC 54B	+	Neg	+	Neg	+	Neg
PGLC 55B	+	+	+	Neg	Neg	+
PGLC 55E	+	+	Neg	Neg	+	Neg
PGLC 55F	+	+	Neg	Neg	+	Neg
PGLC 56B	+	+	+	Neg	+	Neg
Total Positive Synthesizers	16/16	14/16	11/16	0/16	13/16	6/16

standing culture ranged from 5.0×10^6 to 2.0×10^7 cells/2 ml culture. Newly synthesized immunoglobulins, however, were detectable at cell concentration below 1.0×10^6 and greater than 9.0×10^7 cells/2 ml culture.

The specificity of these newly synthesized proteins determined by radioimmunoelectrophoretic techniques is presented in Table II. All cell lines studied produced molecules with IgG (γ) heavy chain specificity. 14 cell lines produced molecules with IgM (μ) heavy chain specificity and 11 cell lines produced molecules with IgA (α) heavy chain specificity. No detectable synthesis of IgD (δ) heavy chain was observed by these cell lines. 13 cell lines produced molecules with type K (κ) light chain specificity and six lines produced molecules with type L (λ) light chain specificity. Of interest, 9 of 16 lines produced IgG (γ), IgA (α), and IgM (μ) heavy chains and five of these lines produced molecules with both type K (κ) and type L (λ) light chain specificity as well.

Studies of the electrophoretic heterogeneity of the immunoglobulins formed revealed that many cultures produced immunoglobulins with restricted mobilities. These appeared as focal labeling of the precipitin arc (Fig. 1). Lines PBLC 33H, PGLC 33J, and PGLC 56B showed focal labeling of the fast (anodal) region of the IgG (γ) precipitin arc, whereas PGLC 51D labeled the slow (cathodal) region of the IgG (γ) precipitin arc. Focal labeling of the slow region of the IgM (μ) precipitin arc was noted in line PGLC 55F. Focal labeling of the slow region of the IgA (α) precipitin arc was noted in line PGLC 56B. Employing an antiserum directed against the type K (κ) determinants, focal labeling of the slow region was detected in line PGLC 54B. In several of the cultures, the immunoglobulins formed displayed electrophoretic heterogeneity with radiolabel distributed over the entire precipitin arc.

Sequential study of several cell lines revealed qualitative differences in individual biosynthetic profiles with time (Table III). Cell concentration during these biosynthetic studies was maintained at approximately 1.0×10^7/2 ml. As seen in Table III, line PGLC 33H initially produced molecules with IgG (γ), IgA (α), and IgM (μ) heavy chain specificity and type K (κ) light chain specificity. Approximately 1 month later biosynthetic studies detected molecules with both type K (κ) and type L (λ) light chain specificity, as well as IgG (γ), IgA (α), and IgM (μ) heavy chain classes.

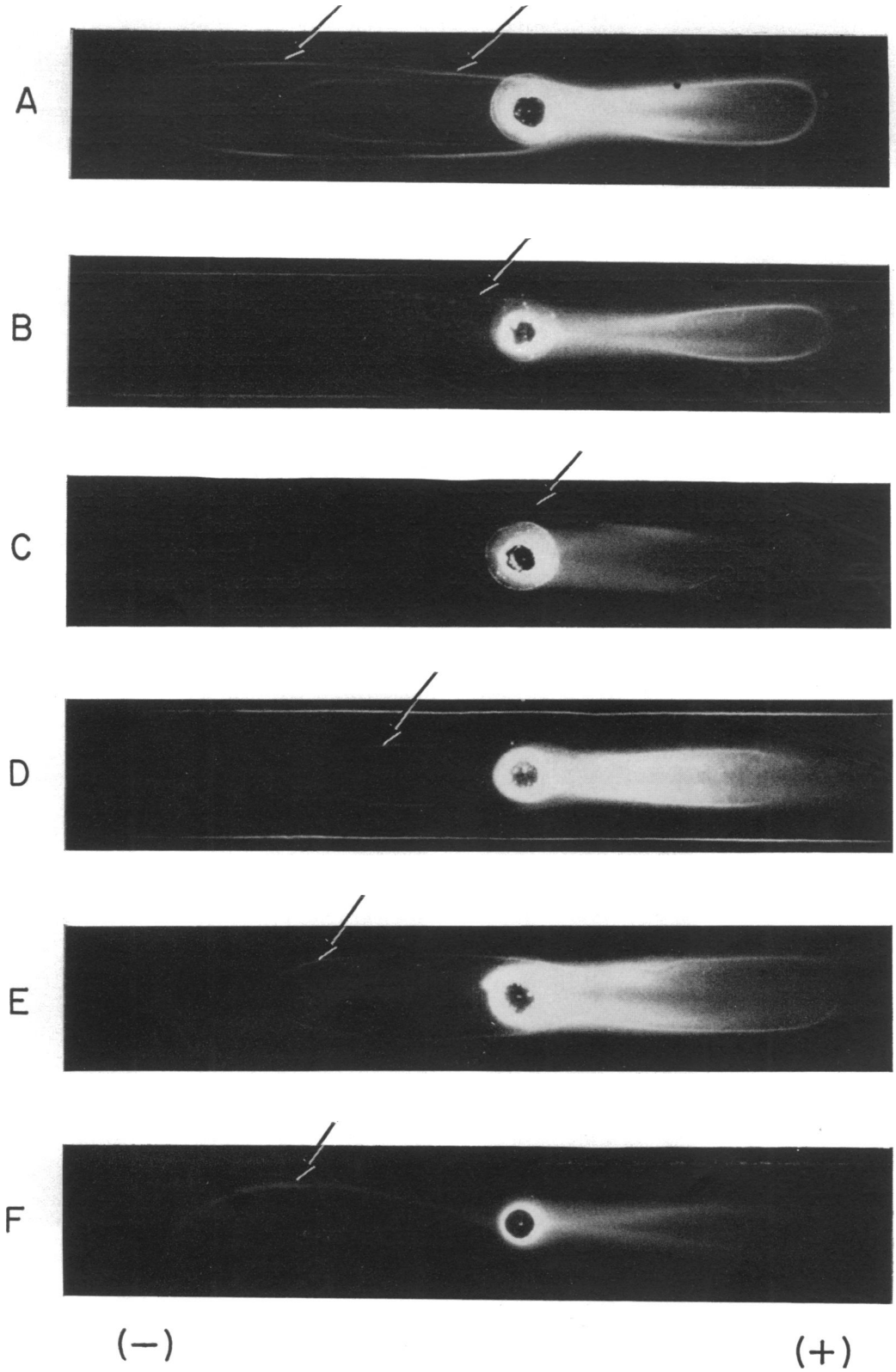

FIGURE 1 Focal labeling of radioimmunoelectrophoretic patterns of newly synthesized protein electrophoresed with human carrier serum and developed with polyvalent rabbit anti-human serum. A, Focal labeling of the IgG precipitin arc. IgM labeling is seen as well. Pattern of IgG labeling confirmed with specific antihuman IgG antiserum. B, C, IgG (γ) precipitin arc. D, IgM (μ) precipitin arc. E, IgA (α) precipitin arc. F, Type K (κ) precipitin arc.

Loss of previously detectable biosynthetic capacity was noted as well as additional synthesis of new molecular species. Line PGLC 44B initially synthesized molecules with IgG (γ) heavy chain specificity and type K (κ) light chain specificity. Biosynthesis studies performed 6 wk later revealed that this cell line synthesized molecules with IgG (γ) heavy chain specificity in the absence of detectable light chains. 3 months after the initial biosynthesis studies, newly synthesized molecules with IgG (γ) and IgM (μ) heavy chain specificity were demonstrated in the absence of detectable light chains. A similar occurrence was noted for line PGLC 51D as well. The time required for change in biosynthetic capacity may be short, perhaps less than the 2 wk noted for line PGLC 51D. The differences noted for these cultures were fairly unstable for the period of sequential study. Changes in line PGLC 33H, however, were stable for at least 3 wk.

Radioimmunoanalytic multiphase zone electrophoresis on polyacrylamide gels. The radioimmunoelectrophoretic studies have demonstrated that the cell lines elaborated molecules with electrophoretic mobility similar to the normal serum immunoglobulins and with sufficient antigenic determinants to react with class and type-specific antisera directed against purified human immunoglobulins. Further characterization by combined polyacrylamide gel filtration, immunodiffusion, and radioautography indicated the presence of newly synthesized immunoglobulin molecules with both heavy and light polypeptide chains in close association, as well as free light polypeptide chains. The R_f values obtained for some newly synthesized immunoglobulins on 5% polyacrylamide gels were similar to values obtained for duplicate preparations of purified whole immunoglobulin molecules (IgG, IgA, IgM) containing both heavy and light polypeptide chains. The specificity of these newly synthesized molecules was determined with heavy

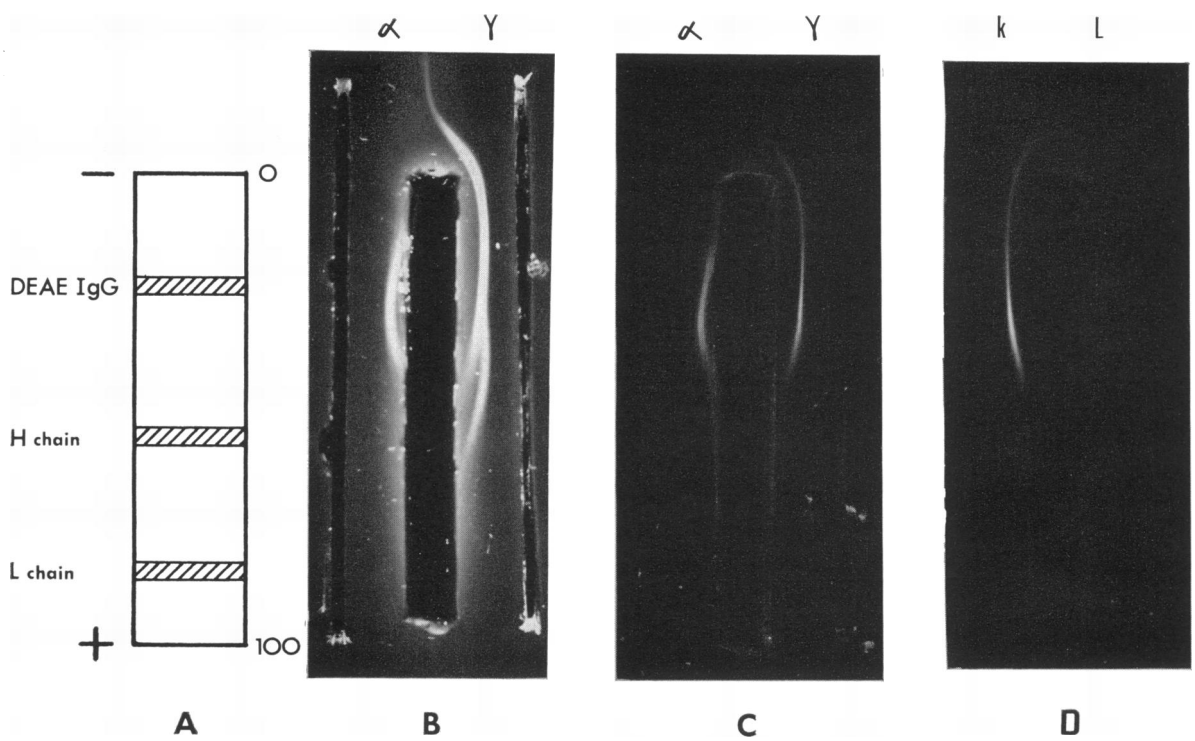

FIGURE 2 Confirmation of synthesis of immunoglobulin molecules with heavy and light polypeptide chains in close association by combined polyacrylamide gel filtration and radioimmunodiffusion. A, Diagram of the electrophoretic mobilities of of purified immunoglobulin and free heavy and light plypeptide chains. B, immunodiffusion pattern of pooled human immunoglobulins developed with antisera specific for IgA (α) and IgG (γ) heavy chains. C, D, Radioautographs of newly synth esized cellular protein electrophoresed in 5% polyacrylamide gel and developed with antisera specific for IgA (α) and IgG (γ) heavy chains and type K (κ) and type L (λ) light chains. Electrophoretic mobility of this material is similar to whole immunoglobulin.

TABLE III

Time Variation of Immunoglobulin Biosynthesis (RIE) by Lymphoid Cell Lines from Patients with Infectious Mononucleosis

Line	Date	Precipitating antisera specific: IgG (γ)	Heavy chains: IgM (μ)	Heavy chains: IgA (α)	Light chains: K (κ)	Light chains: L (λ)
PGLC 33H	6/ 5/67	+	+	+	+	Neg
	6/ 6/67	+	+	+	+	Neg
	7/ 6/67	+	+	+	+	+
	7/27/67	+	+	+	+	+
PGLC 44B	9/28/67	+	Neg	Neg	+	Neg
	11/14/67	+	Neg	Neg	Neg	Neg
	1/ 4/68	+	+	Neg	Neg	Neg
PGLC 51D	12/20/67	+	+	+	+	+
	1/ 4/68	+	+	Neg	Neg	Neg

chain class and light chain type-specific antisera. The R_f values determined from the midpoints of specific precipitin arcs correspond to values for known purified whole immunoglobulin molecules (Fig. 2). Synthesis of free light chain was confirmed for some lines by isolation of the light chain region of the gel, elution with 0.01 M phosphate-buffered saline, pH 7.4, and reelectrophoresis of the eluate in 5% polyacrylamide gel. The R_f value for this material corresponded to R_f values of light chains electrophoresed in parallel. Immunochemical analysis of this material by radioimmunodiffusion with type-specific antisera revealed that molecules with both type K (κ) and type L (λ) antigenic determinants were present. The isolated material did not react with specific antisera for the heavy chain classes (Fig. 3). Newly synthesized molecules with the electrophoretic mobility (as determined from R_f values) of free heavy chains have been observed in several cell lines as well. Studies are currently in progress to identify the physicochemical and immunochemical specificity of these molecules.

Immunofluorescence. Immunofluorescent anal-

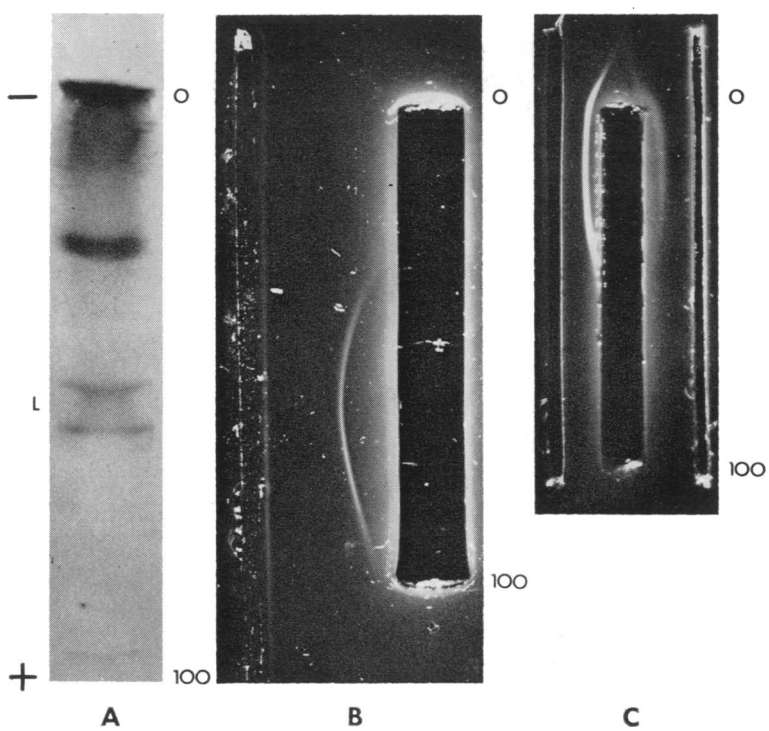

FIGURE 3 Confirmation of free light chain synthesis by IM cell lines. A, Radioautograph of newly synthesized cellular protein electrophoresed in 5% polyacrylamide gel and fixed with TCA. B, Immunodiffusion pattern of protein eluted from the light chain region of gel, electrophoresed in 5% polyacrylamide, and developed with antisera specific for type K (κ) light chain. C, Immunodiffusion pattern of purified immunoglobulin (DEAE IgG) handled in similar manner as B for comparison.

TABLE IV

Per Cent of Immunofluorescent Cells in Established Lines from Peripheral Blood of Patients with Infectious Mononucleosis

Cell line	% Cells/500 reactive with a fluorescein-conjugated antiserum specific for human immunoglobulins*
PGLC 33H	14
PGLC 33J	11
PGLC 42D	7
PGLC 42F	9
PGLC 44B	9
PGLC 50B	5
PGLC 50D	12
PGLC 51D	22
PGLC 53D	12
PGLC 54B	17
PGLC 55B	18

* IgG and cross-reactive with the determinants common to IgA and IgM.

ysis of the localization of molecules with immunoglobulin specificity in single cells in the continuous cell lines is presented in Table IV. All lines examined contained cells that reacted with the fluorescein isothiocyanate–conjugated antiserum directed against the antigenic determinants of human IgG and cross-reactive with the determinants common to IgA and IgM. 5–22% of cells in these lines were reactive and displayed globular or complete cytoplasmic fluorescence. "Signet ring" or speckled fluorescence was not observed in these cell lines. Diffuse weak cytoplasmic fluorescence was observed in an additional 20% of cells, but this type of fluorescence was not considered significant. In many of the cell lines localized membrane fluorescence was observed in absence of cytoplasmic staining. This could be abolished by preincubation with the unconjugated antiserum in direct blocking experiments, indicating that this fluorescence pattern was due to the binding of immunoglobulin molecules to the cell surface.

Attempts to demonstrate specific heterophile antibody, heteroagglutinins, and plaque-forming cells. Heterophilic and heteroagglutinin activity for sheep, horse, and beef red cells could not be detected in the concentrated supernatants from these cell lines. When individual cells were plaqued and examined for localized hemolysis in gel, neither 19S nor 7S hemolytic antibody for sheep, pig, horse, or beef red cells was detected.

DISCUSSION

The biosynthesis of immunoglobulins by normal human peripheral blood lymphocytes (10, 18), thoracic duct lymphocytes (27), and established lymphoid cell lines derived from peripheral blood of patients with leukemia and lymphoid tissue from patients with Burkitt's lymphoma (28–31) has recently been reported from several laboratories. Freshly harvested lymphocytes from peripheral blood and thoracic duct of man have been shown to synthesize immunoglobulin molecules with IgG (γ), IgA (α), IgM (μ), IgD (δ), type K (κ), and type L (λ) specificities. Time-course biosynthesis studies (18) have shown that lymphocytes retain their ability to synthesize these immunoglobulins in short-term culture as well. Employing Sephadex gel chromatography and immunoanalytic multiphase zone electrophoresis on polyacrylamide gels it was observed that for the 1st 72 hr of culture circulating lymphocytes synthesized molecules that were electrophoretically heterogeneous.[1] Cells maintained in culture for 168–192 hr, however, often synthesized immunoglobulin molecules that displayed restricted electrophoretic mobilities. Continuous cell lines derived from Burkitt's lymphoma tissue synthesize IgM (μ) heavy polypeptide chains predominantly (30). Lines derived from the peripheral blood of patients with leukemia produce several classes of heavy polypeptide chains, but usually only one type of light polypeptide chain was detectable (29, 31). In the present analysis of the biosynthetic capacities of cell lines derived from peripheral blood of patients with heterophile-positive IM, newly synthesized ^{14}C-labeled immunoglobulin molecules were identified by radioimmunoelectrophoretic techniques in all of the cell lines examined. Each of these lines displayed remarkable biosynthetic versatility, producing two or more heavy chain classes simultaneously. Nine of the IM lines studied produce immunoglobulin molecules with IgG (γ), IgA (α), and IgM (μ) heavy chain specificities. Five of these nine lines synthesized molecules with both light chain determinants, type K (κ) and type L (λ) as well. Combined

[1] Chessin, L. N., P. R. Glade, W. Terry, and R. Reisfeld. In preparation.

analytical multiphase zone electrophoresis on polyacrylamide gels and radioimmunodiffusion revealed that the immunoglobulins produced by these cell lines were similar to normal serum immunoglobulins with respect to size, molecular weight, charge, and antigenic determinants.

Despite the numerous classes and types of polypeptide chains produced by the lines derived from peripheral blood of patients with IM, immunofluorescent data suggest that only a small percentage of cells in these lines synthesized molecules with immunoglobulin specificity. Although it has been reported that single cells are capable of synthesizing more than one class of immunoglobulin (32, 33), it is unresolved whether single cells in these cultures are capable of synthesizing more than one heavy class or light type of polypeptide chain. Cloning of these lines should give more precise information concerning multiple immunoglobulin synthesis by single cells. It is of interest, however, that most cells in these lines derived from peripheral blood of patients with IM possess the requisite cytoarchitectural substructure for immunoglobulin production (34, 35). Most cells contain a well-developed Golgi apparatus, rough surfaced endoplasmic reticulum and aggregated polyribosomal complexes. Many cells in these lines, therefore, may have the potential for immunoglobulin synthesis.

Sequential study of several cell lines derived from patients with IM has revealed interesting individual biosynthetic profile differences with time. Qualitative loss of previous biosynthetic capacities as well as synthesis of additional classes of heavy and light polypeptide chains have been observed. Previous studies (30, 31) dealing with cells maintained in continuous culture for longer periods of time than the lines presently reported have found fairly stable biosynthetic profiles for each line with a gradual loss of synthetic capacity with prolonged culture. It is unclear at present what quantitative differences exist for these systems and what may have caused them. It is possible that occult changes in culture conditions, contamination with microbial agents or foreign proteins, somatic cell mutation, or subtle population changes might significantly alter the numbers and kinds of actively synthesizing cells and the types and classes of polypeptide chains produced by these cultures. This would suggest that in the early stages of culture cell lines may be capable of undergoing further differentiation and selection in vitro. Periodic study of newly established cell lines should provide data to answer questions concerning the role of differentiation and aging in the cellular production of immunoglobulins.

Whereas no lines derived from peripheral blood of patients with IM were observed to produce light chains in absence of heavy chain synthesis, several lines appeared to synthesize immunoglobulin molecules with heavy chain determinants in absence of light chain synthesis as determined by qualitative radioimmunoelectrophoresis. Other reports have suggested free heavy chain synthesis as well (29). Since 10% of light chains are not detected with present antisera directed against type K (κ) and type L (λ) molecules (36), it is possible that the heavy chains produced by these lines were associated with untypeable light chains. It is equally possible that subunits of light chains were produced lacking sufficient antigenic determinants to be detected by type-specific antisera in this system. The synthesis of free heavy polypeptide chains by these cell lines in absence of associated light chains is under current investigation by immunoanalytic multiphase zone electrophoretic techniques.

In vitro synthesis of immunoglobulins with antibody specificity has been described for lymph node suspensions (37–39), spleen cell suspensions (40), and peripheral blood lymphocytes (41–44). At present unconfirmed, McKinney (11) has reported heterophile antibody synthesis by phytohemagglutinin-stimulated peripheral blood lymphocytes from patients with IM. Apparent antibody synthesis has recently been reported for established Burkitt lymphoma cell cultures as well (45). 14 of the 16 cell lines derived from patients with heterophile-positive IM producing immunoglobulins with IgM (μ) heavy polypeptide chain specificity were examined for their ability to produce antibody. With the methods of detection employed, heterophile antibody, heteroagglutinins, and hemolytic antibody for sheep, beef, and horse red cells could not be demonstrated. It is possible that the progenitor of these cell lines lacked potential for synthesis of these specific antibodies. Based upon histochemical analysis Galbraith, Mitus, Gollerkeri, and Dameshek (12) suggested that the circulating cell in IM has no role in the production

of humoral antibody. Furthermore, pathologic material (46) and kinetic studies (47) argue for sequestration of the majority of cells involved in IM in fixed tissues where antibody production may occur. Chessin et al. (18), however, have demonstrated that progressive restriction of molecular species produced by human lymphoid cells in short-term culture often occurred. Since the initial signs of proliferation of the cell lines derived from patients with IM were observed at 21–78 days of culture and most lines were studied for heterophile activity after 3–4 months of culture, the inability to detect heterophile activity in the present study may have resulted from loss of cells with potential for antibody synthesis with time.

The inability to detect immunologic responses characteristic of infectious mononucleosis in cell lines derived from peripheral blood of patients with IM casts doubt upon the close relationship of these cells in culture to circulating cells in IM. A similar doubt has been raised for cell cultures derived from Burkitt's lymphoma tissue. Fahey and Finegold (48) have suggested that "Burkitt cell cultures" may originate from hematopoietic stem cells or from normal lymphoid cells established in long-term culture by a helper effect of tumor cells during the early days of explant growth. Long-term suspension cultures derived from peripheral blood of normal individuals have been established with special techniques by several investigators as well (16, 49). While it is impossible at present to clearly define the precursor of these cell lines, it is of interest that with the techniques and small blood volumes employed in the establishment of the cell lines derived from patients with IM, comparable collateral specimens from a control series of 18 normal individuals failed to proliferate in long-term culture. These findings suggest that our culture techniques may be selective for proliferative potentials above the normal range, and that there is an increased potential of circulating cells in patients with IM for long-term in vitro proliferation. This increased proliferative potential appears to be a function of the infectious mononucleosis state, disappearing with a return of clinical and laboratory parameters toward normal (50).

The precise relationship of continuous cell cultures derived from patients with infectious mononucleosis to circulating cells in IM requires further study. It is possible that factors present in IM may stimulate normal cells to proliferate in long-term culture. These lines, nevertheless, offer a useful tool for the investigation of the cellular production of immunoglobulins.

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