THE FIRST COMPONENT OF COMPLEMENT

A Quantitative Comparison of its Biosynthesis in Culture by Human Epithelial and Mesenchymal Cells*

By KATHERINE M. MORRIS, HARVEY R. COLTEN, AND DAVID H. BING‡

From the Center for Blood Research, Boston, Massachusetts 02115, The Department of Biological Chemistry, Harvard Medical School, and The Ina Sue Perlmutter Cystic Fibrosis Center, Children's Hospital Medical Center and The Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115

The first component of complement (C1)[1] circulates in plasma as a macromolecular complex of three distinct proteins designated C1q, C1r, and C1s (1). C1q, the largest subcomponent of the three (mol wt 410,000), consists of six subunits of three chains A, B, and C (mol wt 21,000–24,000) and is unique among the complement proteins with 40% of its amino acid sequence similar to collagen (proline, hydroxyproline, and hydroxylysine) (2, 3). Activation of the calcium dependent C1 complex occurs when C1q binds to the Fc portion of IgG or IgM (4, 5) presumably causing a conformational change in C1q (6). C1r, a protein with β-mobility (7), is present in plasma as a single chain protein in its zymogen form (mol wt 190,000 [8] or 166,000 [9]). It is postulated that activation of C1r to $C1\bar{r}$ (mol wt 95,000 [8] or 107,000 [9]) is initiated by the conformational change in C1q (6). A peptide bond is cleaved generating a two chain disulfide-linked molecule (mol wt 58,000 and 36,000 [8, 9]) with γ-mobility (7). A mechanism similar to C1r activation occurs when C1s (mol wt 87,000) binds to $C1\bar{r}$ and is activated by cleavage of the peptide chain generating the two-chain (mol wt 58,000 and 29,000 [8, 9]) serine esterase $C1\bar{s}$.

The sites of biosynthesis of most of the complement proteins have been identified. However, because of conflicting findings in studies of C1 biosynthesis (10–21), the cell type primarily responsible for the synthesis of this component has not been clearly identifiable. To resolve the apparent discrepancies in these findings we compared the capacity of human fetal intestine columnar epithelium, bladder transitional epithelium, skin and lung fibroblasts, blood monocytes and several primary cell cultures from biopsies of the urogenital tract to synthesize C1. All three cell types (epithelia, fibroblasts, and monocytes) tested synthesized hemolytically active C1, but epithelial cells produced 400–3,700 times more active C1 than either fibroblasts or monocytes. Radiolabeled C1q, C1r, and C1s were detected in culture media from both epithelial

* Supported in part by the National Institutes of Health U. S. Public Health Service grants CA 17376, AM 16392, and AI 12791, and by The Ina Sue Perlmutter Research Fund.

‡ Recipient of American Heart Association Established Investigatorship 76 222.

[1] *Bull. W. H. O.* 1968. **39**:935. Terminology for the complement system: complement proteins are designated C1 through C9. The activated form of a given component is designated by a bar across the top, e.g. $C\bar{1}$ is the activated form of C1. E stands for sheep erythrocytes, A is antibody to E.

and mesenchymal cells. However, only columnar epithelial cells produced C1q with a subunit structure similar to reported values for C1q in serum (2, 3). C1q from the other lines had a larger apparent molecular weight than serum C1q and did not dissociate like serum C1q. C1s from all cell types tested had an apparent molecular weight greater than serum C1s.

Materials and Methods

Media and Reagents. Dulbecco's minimal essential medium (D-MEM),[2] medium 199 (M199), medium 199 special (M199 special; lacking valine, leucine, isoleucine, lysine, and glutamine), Earle's minimal essential medium (E-MEM), fetal bovine serum, mycoplasma free (FBS), glutamine, nonessential amino acids (NEAA), penicillin (10,000 U/ml)-streptomycin (10,000 μg/ml)-fungizone (25 μg/ml) (antibiotics), Hanks' balanced salt solution (HBSS), trypsin-versene, and guinea pig serum were purchased from Microbiological Associates, Walkersville, Md. Sheep erythrocytes (E) were purchased from Scott Laboratories, Fiskeville, R.I. Bovine serum albumin (BSA), phosphorylase a, phenylmethyl sulfonyl fluoride (PMSF), insulin, and cycloheximide were purchased from Sigma Chemical Co., St. Louis, Mo.; creatine kinase and glutamate dehydrogenase were purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind.; β-galactosidase and carbonic anhydrase were purchased from Worthington Biochemical Corp., Freehold, N.J. A tritiated mixture of 15 amino acids consisting of: 80 μCi L-alanine, 70 μCi L-arginine, 80 μCi L-aspartic acid, 125 μCi L-glutamic acid, 40 μCi glycine, 15 μCi L-histidine, 50 μCi L-isoleucine, 140 μCi L-leucine, 60 μCi L-lysine, 80 μCi L-phenylalanine, 50 μCi L-proline, 40 μCi L-serine, 50 μCi L-threonine, 40 μCi L-tyrosine, and 80 μCi L-valine, and Biofluor were purchased from New England Nuclear Corp., Boston, Mass.

Antisera. Goat antiserum to human C1q, C1s, and IgG were obtained from Atlantic Antibodies, Westbrook, Maine. Initially, rabbit antiserum to human C1r was a gift from Dr. Robert Ziccardi, Research Institute of Scripps Clinic, La Jolla, Calif. In later experiments, goat antiserum to human C1r was obtained by adsorption of a crude antiserum to C1r (Atlantic Antibodies), with Sepharose 6B-linked pseudoglobulin, C1s and C3. The globulin fraction of each antiserum was obtained by precipitation with 18% sodium sulfate at 37°C. The precipitate was dissolved in 100 mM KCl-50 mM Tris(hydroxymethyl)aminomethane (Tris), pH 7.5 (Tris-KCl buffer), dialyzed against the Tris-KCl buffer and centrifuged (10,000 *g*) before use. Euglobulin and pseudoglobulin serum fractions, which served as carriers for immunoprecipitations, were obtained by overnight dialysis of human serum against 100 vol of 0.065 ionic strength sodium acetate pH 5.5 at 4°C. The euglobulin precipitate was removed from the supernate (pseudoglobulin) by centrifugation (10,000 *g*, 20 min) and the euglobulin was dissolved in 0.1 the serum volume with 150 mM NaCl. Precipitin curves of antiserum to C1q, C1r, and C1s against euglobulin and anti-IgG with pseudoglobulin were done to determine the amount of antibody and antigen giving 30 μg of precipitate at equivalence.

Cell Cultures. The methods for culture of primary transitional epithelial cells have been briefly described (21). Surgical samples in HBSS and antibiotics were obtained from Dr. Murray M. Bern, New England Deaconess Hospital, Boston, Mass. Transitional cells were carefully scraped from the tissue and washed in HBSS with antibiotics. Viability was estimated by trypan blue exclusion and a maximum of 5×10^5 viable cells seeded into 25 cm^2 T-flasks (Falcon Labware Div., Becton-Dickinson, Oxnard, Calif.) containing 10% E-MEM (vol/vol) heat-inactivated (56°C, 30 min) FBS, glutamine, and antibiotics, and incubated at 37°C in a

[2] *Abbreviations used in this paper:* BSA, bovine serum albumin; C1, first component of complement; D-MEM, Dulbecco's minimal essential medium; D-MEM complete, D-MEM + 15% FBS + glutamine + insulin + NEAA; EDTA, ethylene diamine tetra-acetic acid disodium salt; EDTA buffer, Veronal-buffered 1 mM EDTA with gelatin; em, effective molecules; E-MEM, Earle's minimal essential medium; FBS, fetal bovine serum; HBSS, Hanks' balanced salt solution; IgG-Sepharose, *p*-azo-benzyloxy-ethylsulfonoethyl-Sepharose 6B; M199, Medium 199; NEAA, nonessential amino acids; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethyl sulfonyl fluoride; ppt, parts per thousand; Rf, ^{3}H-protein migration divided by dye marker migration times 100; SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; VBS, Veronal-buffered saline; VBS sucrose, isotonic Veronal-buffered sucrose with gelatin, calcium, and magnesium.

humidified 5% CO_2/95% air atmosphere. Cultures were monitored daily and fed with fresh medium at least twice weekly, and were maintained for at least 8 wk before transfer to allow establishment of the monolayer. If present, fibroblasts were selectively removed by a 2-min trypsin-versene treatment as described by Owens et al. (22). Because of nonspecific lysis which occurs in the hemolytic assay where bacterial or fungal products were present, contaminated cultures were not used for any experiment.

The human fetal intestine, Hs0074, fetal intestine-agammaglobulinemia, Hs0677, normal bladder transitional epithelium, Hs0767, and normal lung fibroblasts, Hs0907, were provided by contract E-73-2001-N01 within the Special Virus-Cancer Program, National Institutes of Health, Public Health Service through the courtesy of Dr. Helene S. Smith, Peralta Cancer Research Institute, Oakland, Calif. These cells were fed twice weekly with D-MEM, 10% (vol/vol) heat-inactivated FBS, 10 μg/ml bovine insulin, and NEAA. Monolayers of ≈7.5 × 10^5 cells/75 cm^2 flask formed weekly and were transferred 1:2 with trypsin-versene. All cultures were in passage numbers from 4 to 30. The adult human skin fibroblast cultures in passages 4 and 5 were maintained in M199, 15% FBS, and antibiotics at 37°C, 5% CO_2/95% air. Blood monocyte cultures were established as described by Einstein et al. (23) and maintained for 4 wk in vitro before use.

Medium from primary cultures was removed at various intervals, concentrated 5- to 10-fold by dialysis against sucrose and dialyzed overnight against 1 mM Veronal-buffered saline containing 1 mM $MgCl_2$ and 1.5 mM $CaCl_2$, pH 7.35 (VBS). Samples were either assayed for C1 hemolytic activity within 24 h, or stored at −80°C.

For radiolabeling experiments, cells were grown to ≈75% confluency in the appropriate culture medium. The D-MEM complete medium from the fetal intestine (Hs0074 and Hs0677), bladder transitional epithelium (Hs0767), and lung fibroblast (Hs0907) cultures was removed and replaced with D-MEM containing 10% (vol/vol) HBSS, 10 μg/ml insulin, and from 10.0 to 100 μCi/ml of ^{3}H-amino acids. M199 special containing antibiotics and 100 μCi/ml of the ^{3}H-amino acid mixture was used for the fibroblast and monocyte cultures. All cultures were supplemented with 10% (vol/vol) heat-inactivated FBS which had been dialyzed against four changes of 100 vol of deionized distilled water and two changes of 100 vol of 150 mM sodium chloride and sterilized by Millipore filtration. At timed intervals medium was decanted, made 2 mM in PMSF by using a 200-mM stock solution of PMSF, and assayed within 24 h or stored at −80°C.

Assay of Culture Medium for C1 Hemolytic Activity. Samples of unconcentrated medium from bladder and intestinal epithelium, monocyte and skin and lung fibroblast cultures or concentrated dialyzed primary culture medium were assayed for $C\bar{1}$ by a modification of the $C\bar{1}$ assay (24). Dilutions of culture medium were made in isotonic Veronal-buffered sucrose with gelatin, calcium, and magnesium, pH 7.35 (VBS sucrose). Equal vol (0.2 ml) of EAC4 (1.5 × 10^8 cells/ml) and culture medium (or dilutions) were incubated at 30°C for 10–60 min with shaking. VBS sucrose (1 ml), warmed to 30°C, was added, the cells centrifuged, washed again with 1 ml warmed VBS sucrose, and centrifuged. The pellet was resuspended in 0.4 ml VBS sucrose and 0.2 ml transferred to a clean tube. Purified guinea pig C2 (1.0 ml, 50 effective molecules/cell) in VBS sucrose was added and incubated with shaking at 30°C for 10 min. Terminal complement components (0.3 ml), C3–C–9, were supplied by adding guinea pig serum diluted 1:35 (vol/vol) with isotonic Veronal-buffered 1 mM EDTA with gelatin, pH 7.35 0.3 ml EDTA buffer. After 1 h at 37°C, 1 ml of ice-cold 150 mM NaCl was added, the mixture centrifuged and the $A_{412}^{1\,cm}$ of the supernate read. Effective molecules (em) of C1 were calculated by the method outlined by Borsos and Rapp (25).

Measurement of C1 activation in culture medium was performed with a slight modification of a previously described procedure. EAC4 (1.5 × 10^8 cells/ml) were added to an equal vol of 1:10 dilutions of culture medium and incubated at 30°C with shaking. At timed intervals 0.8 ml of EAC4 and medium from each sample flask were removed and washed twice with 2.0 ml of warmed VBS sucrose. The pellet was resuspended in the original volume of VBS sucrose (0.8 ml) which was divided equally among four tubes. The assay for C1 activity was then performed as described above.

Isolation of $C\bar{1}$ from IgG-Sepharose. The procedure of Assimeh et al. (26) as modified by J. M. Andrews (unpublished data) using IgG coupled to *p*-azo-benzyloxyethyl-sulfonoethyl-Sepharose-6B (IgG-Sepharose) was used to purify C1. Each 10 ml of fivefold concentrated dialyzed

medium from various time periods was added to 1 g of IgG-Sepharose and stirred for 1 h at 4°C. Medium was removed from the resin on a Buchner funnel, the resin was suspended in VBS and poured into an appropriate sized column. The resin was washed first in a volume of VBS equivalent to the starting volume of medium and the $C\bar{1}$ was eluted with 200 mM 1,3-diaminopropane-200 mM borate-1,000 mM NaCl, pH 7.4 (diamine eluate). After dialysis against two changes of 100 vol of VBS the eluates were tested for $C\bar{1}$ hemolytic activity as described.

Inhibition of Synthesis by Cycloheximide. Three cultures of bladder epithelium (Hs0677), seeded with equal cell numbers, were grown for 3 days. At that time the medium was decanted and replaced with 10 μCi/ml of ^{3}H-amino acids. Two cultures were also exposed to 5 μg/ml of cycloheximide. 24 h later all media were removed, the cultures washed with HBSS and fresh medium containing 10 μCi/ml of ^{3}H-amino acids was added. One of the cycloheximide cultures was again incubated with 5 μg/ml of cycloheximide. Media were removed at 48 h, and all were assayed for hemolytic activity as described previously, and subcomponent levels were determined by immunoprecipitation.

Assay of Total Protein Synthesis. 5-μl samples of each of radiolabeled culture medium were precipitated on Whatman 3 MM filter paper discs with 10% trichloroacetic acid-10 mM leucine and counted in a Searle Mark III Liquid Scintillation Spectrometer (Searle Diagnostics Inc., subsid. of G. D. Searle & Co., Des Plaines, Ill.), according to the procedure of Roberts and Patterson (27) as modified by Hall and Colten (28).

Immunoprecipitation. A modification of the procedure of Hall and Colten (29) was used for precipitation of C1q, C1r, C1s, and IgG from culture media. Each reaction mixture consisted of 100–700 μl of radiolabeled culture medium made 10 mM with EDTA, the appropriate amounts of euglobulin or pseudoglobulin, and either anti-C1q, anti-C1r, anti-C1s, or anti-IgG antiserum. This mixture was incubated at 37°C for 3 h and overnight at room temperature. The immune precipitates were washed three times in cold 50 mM Tris-100 mM KCl pH 7.5 and twice in cold 150 mM NaCl. Total radioactivity was estimated by scintillation spectrometry after dissolving in 200 μl of 0.1 N NaOH and transferring the solution to a scintillation vial containing 5 ml Biofluor (New England Nuclear Corp.). Molecular weights and subunit structures were estimated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) as described below.

SDS-PAGE. The procedure of Weber and Osborn (30) was followed for the preparation and electrophoresis of 5.0 and 7.5% polyacrylamide-SDS gels. Immune precipitates dissolved in sample buffer were boiled for 2 min, reduced by boiling for 2 min in 2% 2-mercaptoethanol, and alkylated with 40 μl/100-μl samples of 0.5 M iodoacetic acid for 30 min at 37°C. Gels were sliced into 1.5-mm slices with a Savant autogel divider (Savant Instruments, Inc., Hicksville, N.Y.) and dispensed into scintillation vials containing 5 ml Biofluor in an LKB 2112 Redirac fraction collector (LKB Instruments, Inc., Stockholm, Sweden). The vials were counted by liquid scintillation spectrometry and the counts were plotted as R_f, the ^{3}H-protein migration divided by the dye marker migration times 100. R_f values for standard markers β-galactosidase (130,000), phosphorylase a (94,000), BSA (68,000), glutamate dehydrogenase (54,000), creatine kinase (40,000), and carbonic anhydrase (29,000) were estimated from Coomassie Blue stained gels and were used for calculating molecular weights of the radiolabeled peaks.

Results

Production of C1 by Primary Cultures. Transitional epithelial cells were obtained from biopsies of bladder, renal pelvis, and ureter. Primary cultures of each synthesized and secreted hemolytically active C1 while under similar conditions a lymphoma, kidney epithelium, and hypernephroma failed to produce detectable C1 activity (Table I). The transitional epithelial cell cultures secreted 2×10^8–1.8×10^{10} effective C1 molecules (em)/culture per day. In two separate transitional epithelial cell cultures obtained from renal pelvis it was possible to precisely calculate the rate of active C1 synthesis per cell; i.e., 5.7×10^3 and 2.9×10^3 em/cell per h, respectively, during the first 2 days in vitro.

TABLE I

Production of Hemolytically Active C1 in Primary Cell Cultures

Culture	em/culture $\times 10^{-10}$	em/culture per 24 h $\times 10^{-10}$	em/cell per h $\times 10^{-3}$
Transitional epithelium:			
Renal pelvis: 1			
Day 0–2	3.31	1.66	5.70
Day 8–15	12.60	1.80	
Day 16–19	1.51	0.50	
Renal pelvis: 3	0.45	0.23	
Ureter: 4			
Day 0–2	1.79	0.60	1.93
Ureter: 1	3.40	1.10	
Ureter: 6			
Day 0–2	0.04	0.02	1.01
Kidney parenchyma	0.01	<0.003	
Tumor cell cultures:			
Renal pelvis: 2			
Day 0–4	4.44	1.11	2.88
Bladder epithelium	<0.001	—	
Hypernephroma: 8	0.003		
Lymphoma	<0.001		

TABLE II

Biosynthesis of Hemolytically Active C1 and Total Protein by Epithelial and Mesenchymal Cells in Culture

Culture	em/culture per 24 h $\times 10^{-10}$	Cell number $\times 10^{6}$	em/cell per h $\times 10^{-3}$	Total protein (TCA ppt cpm) per culture per 24 h $\times 10^{-6}$
Bladder transitional epithelium (Hs0767)	57.9	0.5	483.33	2.30
Fetal intestine epithelium (Hs0474)	75.9	0.5	633.33	2.08
Skin fibroblasts 1	0.04	1.0	0.17	3.40
Skin fibroblasts 2	0.07	1.0	0.29	2.06
Lung fibroblasts (Hs0907)	0.22	0.7	1.15	2.00
Blood monocytes 11	0.02	1.0	0.83	0.80
Blood monocytes 21	0.29	1.0	1.21	0.43

A comparison of the capacity of several epithelial and mesenchymal cell cultures (low passage and primary) to synthesize C1 and total protein is shown in Table II. Bladder transitional epithelial and columnar epithelial cells from fetal intestine synthesized and secreted 400–3,700 times as much hemolytically active C1 as fibroblasts from skin and lung. Monocytes synthesized approximately three to five times less total protein (TCA precipitable [ppt] cpm) and 400–760 times less active C1 compared to the epithelial cell cultures.

Activation of C1 in Culture Medium. As shown in Fig. 1, medium from bladder transitional epithelial cells contained both $C\bar{1}$ and C1. By 2 min, there were 2.3×10^{10} em/culture in the bladder medium which increased to 5.2×10^{11} em/culture by 60 min. This activity did not increase after 60 min and was inhibited by addition of anti-C1 antiserum to the initial incubation mixture (data not shown). Although

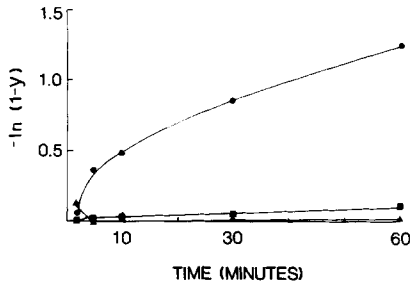

FIG. 1. Activation of C1 in culture medium. ●, transitional epithelium; ▲, fibroblast; ■, monocyte.

TABLE III

Cycloheximide Inhibition of C1 and Protein Synthesis

Bladder transitional epithelium*	em/cell per h $\times 10^{-6}$	cpm in 30 μg specific immunoprecipitate		
		C1q	C1r	C1s
Medium/medium	1,260/1,700‡	4,150/5,300	3,250/4,200	2,700/3,500
Cycloheximide/cycloheximide	575/720	0/0	150/150	0/200
Cycloheximide/medium	800/1,420	0/2,700	0/2,700	0/1,300

* Cultures were grown in medium containing 5 μg/ml cycloheximide and 10 μCi/ml ^{3}H-amino acids. At 24 h the medium was removed, the monolayer washed with HBSS and replaced with fresh medium, with or without 5 μg/ml cycloheximide, and 10 μCi/ml ^{3}H-amino acids and grown for 48 h before collection of medium. Assays were performed as reported in the Materials and Methods section.

‡ The first number equals 24 h and the second number, 48 h after washing and replacing with fresh medium containing 10 μCi/ml ^{3}H-amino acids and containing or lacking cycloheximide.

medium from fibroblast and monocyte cultures contained hemolytically active C1 (2.7×10^8 and 3.4×10^8 em/ml) there was no detectable zymogen form of C1 in these culture media. The possibility that fibroblast and monocyte cultures synthesized C1r and C1s but no functionally active C1q was tested. Accordingly, 10 μg purified C1q was incubated at 32°C for 15 min with several dilutions of fibroblast and monocyte culture medium and then assayed for C1 activity in the usual manner. This did not result in generation of detectable hemolytic C1 activity in any of the mixtures. A positive control consisting of purified C1q, C1r, and C1s, assayed in the identical manner yielded 2.52×10^{10} em/ml of C1 activity.

Inhibition of de novo Protein and C1 Synthesis by Cycloheximide. The data in Table III show that addition of cycloheximide to transitional epithelial cell cultures resulted in a decrease in C1 hemolytic activity. In addition, only the cultures without cycloheximide synthesized C1q, C1r, and C1s. The inhibitory effect of cycloheximide was reversible.

Isolation of Hemolytically Active C1 from Culture Medium on IgG-Sepharose. The results of isolation of C1 from culture medium on IgG-Sepharose (as described in Materials and Methods) are presented in Table IV. Hemolytically active $C\bar{1}$ from bladder and intestinal epithelial cell cultures bound to the IgG-Sepharose and was eluted with diamine. A similar binding and elution of authentic $C\bar{1}$ in normal serum was also observed in agreement with previously reported findings (26). Media from two hypernephromas, a lymphoma, a bladder carcinoma, and HeLa cells with no demonstrable hemolytically active $C\bar{1}$, were fractionated in an identical manner to determine whether $C\bar{1}$ was present in quantities only detectable in a purified (concentrated)

TABLE IV
Isolation of C1 Hemolytic Activity on IgG-Sepharose

C1 Source	Fraction	em × 10^{-10}
Bladder epithelium (Hs0767)	Medium	5.58
	Nonadsorbed	0.008
	Diamine eluant	21.9
Fetal intestine (Hs0677)	Medium	129
	Nonadsorbed	0
	Diamine eluant	167
Normal serum	Euglobulin fraction	9,300
	Nonadsorbed	275
	Diamine eluant	11,000

form or was masked by an inhibitor. No functionally active $C\bar{1}$ was detected.

Identification of Subunits of C1q, C1r, and C1s on SDS-PAGE under Reducing Conditions. C1q subunits synthesized by fetal intestine epithelium (Hs0074) (Fig. 2a) had a size (mol wt 20,000–24,000) and subunit structure similar to those reported for serum C1q (2, 3). Analysis on SDS-5.6% PAGE of reduced and alkylated C1q from bladder epithelium (Hs0767) revealed major peaks of approximately 175,000, 81,000, and 72,000 daltons. A minor peak was also evident at 105,000 daltons (Fig. 2b). In monocyte culture medium (Fig. 2c) two major peaks (78,000 and 30,000–36,000 daltons) were present. C1q isolated from fibroblast medium had a molecular size of approximately 185,000 daltons (Fig. 2d). In addition, a heterogeneous mixture of labeled protein specifically precipitated with anti-C1q antiserum was distributed over a broad range (35,000–120,000 daltons). Radiolabel in control immunoprecipitates (consisting of IgG anti-IgG) distributed uniformly throughout the gel without recognizable peaks, in this and other experiments (Figs. 2–4).

As illustrated in Fig. 3a the radiolabeled C1r synthesized by fetal intestine epithelium (Hs0677) distributed in two major peaks with apparent mol wt of 145,000 and 90,000. These corresponded in size to the dimer and monomer C1r. On SDS-7.5% PAGE, the major peaks for C1r from monocyte culture medium approximated 85,000 and 33,000 (Fig. 3b). C1r isolated from fibroblast and transitional epithelial cell culture medium had similar distributions in gels (data not shown).

SDS-PAGE analysis of C1s isolated from monocyte medium illustrated in Fig. 4a, showed a broad major peak in the area from 20,000 to 40,000 with a smaller peak at 80,000. Patterns with the broad major peak in this range were obtained for C1s from fetal intestine and bladder epithelium culture medium. Fibroblast cultures produced a molecule with subunits corresponding to those reported for C1s (7) but also consistently contained a larger molecular species of ≈130,000 daltons.

Discussion

Early studies on the biosynthesis of complement involved the removal or ablation of an organ and measurement of the reduction of whole complement levels in serum (31–33). Improvement in tissue culture techniques and development of methods for detection of individual complement proteins allowed studies of complement synthesis in vitro. Colten et al. (10, 11) used the hemolytic assay for C1 (27) to identify biologically active C1 in short term organ and cell cultures of guinea pig and human intestine. Although they could detect incorporation of ^{14}C-amino acids into a molecule

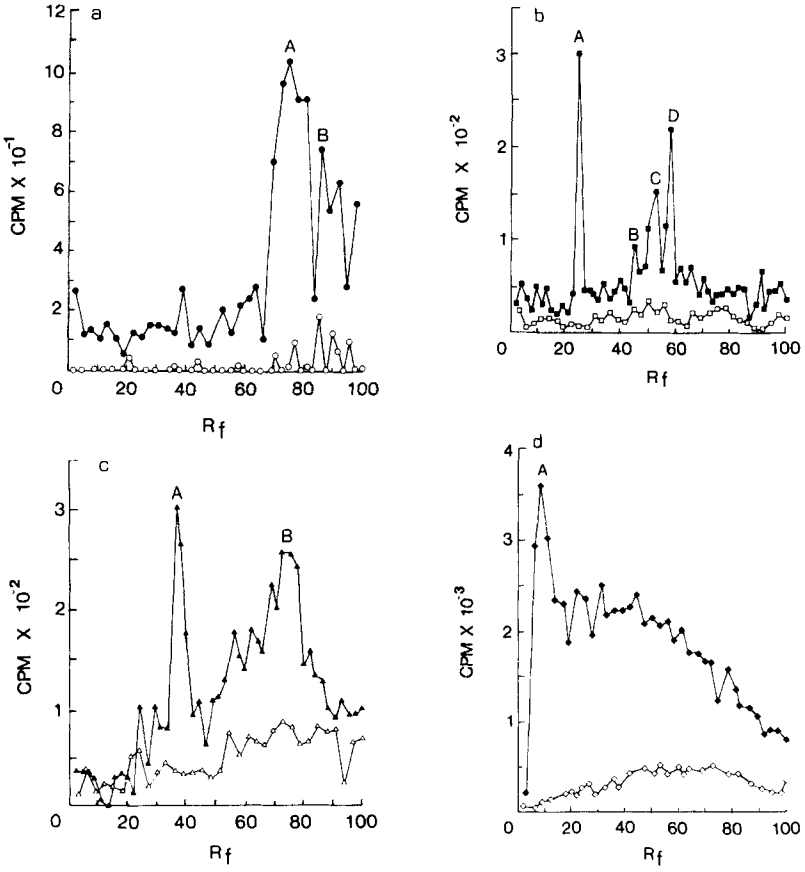

FIG. 2. SDS-PAGE of immunoprecipitates of reduced and alkylated C1q. (a) Fetal intestine columnar epithelial cell culture medium (10 μCi/ml ^{3}H-amino acids; 24 h); 5.0% polyacrylamide gel; peak A: 27,000–32,000, peak B: 19,000–21,000. ●, anti-C1q; ○, anti-IgG. (b) Bladder transitional epithelial cell culture medium (10 μCi/ml ^{3}H-amino acids; 24 h); 5.0% polyacrylamide gel; peak A: 175,000, peak B: 105,000, peak C: 81,000, peak D: 72,000. ■, anti-C1q; □, anti-IgG. (c) Monocyte culture medium (100 μCi/ml ^{3}H-amino acids; 24 h); 7.5% polyacrylamide gel; peak A: 78,000, peak B: 35,000. ▲, anti-C1q; △, anti-IgG. (d) Fibroblast culture medium (100 μCi/ml ^{3}H-amino acids; 24 h); 7.5% polyacrylamide gels; peak A: 185,000. ◆, anti-C1q; ◇, anti-IgG.

functionally similar to C1, the cell type responsible for synthesis was not identified. The development of a modification of the Jerne plaque technique led to the finding that the columnar epithelial cell produced biologically active C1 (12). Additional evidence that C1 found in epithelial cell cultures was synthesized and not merely the result of release of preformed protein was obtained in studies of long-term primary suspension cultures of human colon, adenocarcinoma, and transitional epithelial cells of the urogenital tract (13).

With the use of autoradiography of precipitin bands on radioimmunoelectrophoresis, Stecher et al. (14) found labeled C1q in monocyte cultures. They realized that although microgram quantities of newly synthesized protein could be detected technical limitations such as nonspecific binding could occur and neither net synthesis nor biological function could be demonstrated by this technique. Using similar

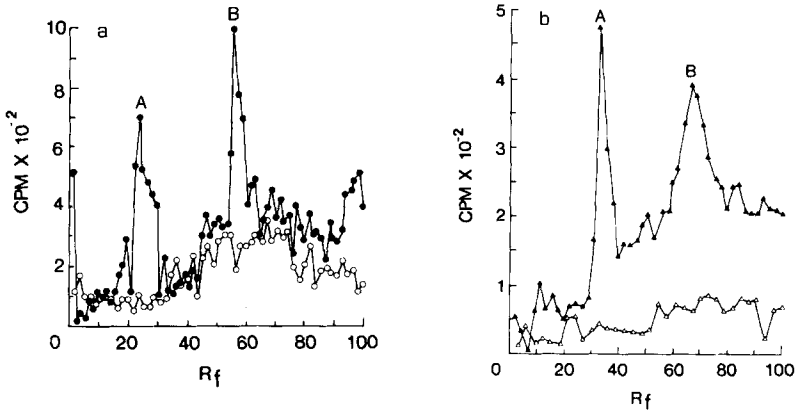

FIG. 3. SDS-PAGE of immunoprecipitates of reduced and alkylated C1r. (a) Fetal intestine columnar epithelial cell culture medium (10 μCi/ml ^{3}H-amino acids; 24 h); 5.0% polyacrylamide gels; peak A: 145,000, peak B: 90,000. ●, anti-C1r; ○, anti-IgG. (b) Monocyte culture medium (100 μCi/ml ^{3}H-amino acids; 24 h); 7.5% polyacrylamide; peak A: 85,000, peak B: 33,000. ▲, anti-C1r; △, anti-IgG.

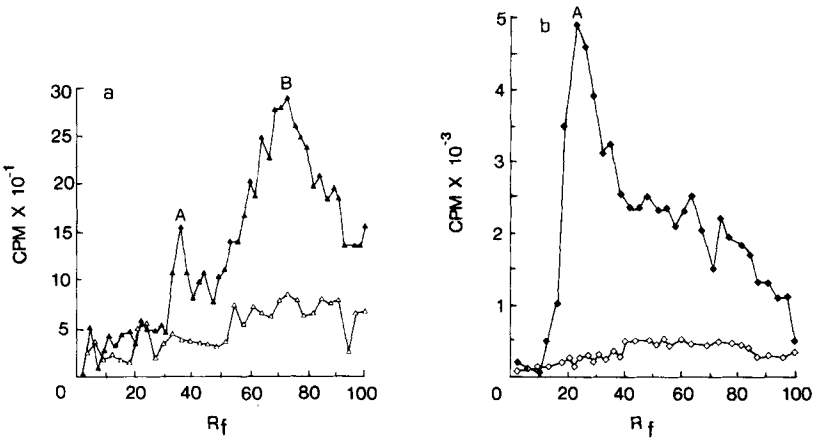

FIG. 4. SDS-7.5%-PAGE of an immunoprecipitate of reduced and alkylated C1s. (a) Monocyte culture medium (100 μCi/ml ^{3}H-amino acids; 24 h); peak A: 80,000, peak B: 13,000–40,000. ▲, anti-C1s; △, anti IgG. (b) Fibroblast culture medium (100 μCi/ml ^{3}H-amino acids; 24 h); peak A: 130,000. ◆, anti-C1s; ◇, anti-IgG.

techniques others have also suggested that monocytes or macrophages synthesize C1q (15, 16). Loos et al. (17) provided equally convincing evidence that macrophages were sites of $C\bar{1}$ synthesis. Recently, Al-Adnani and McGee (18) demonstrated synthesis of C1q by lung and skin fibroblasts by immunoprecipitation of culture medium and with an immunohistochemical method demonstrated C1q on the surface of these cells. Reid and Solomon (19) provided additional evidence for synthesis of C1 by fibroblasts. They detected both functionally active C1 and identified the radiolabeled protein and subunits on SDS-PAGE analyses of immunoprecipitates of each of the subcomponents.

We have compared the capacity of these different cell types to synthesize C1 to resolve these apparent differences. Epithelial cells synthesized and secreted 400–3,700

times more hemolytically active C1 than fibroblasts or monocytes. Synthesis of C1 and its subcomponents by transitional epithelial cells, as assessed functionally or immunochemically, was inhibited by cycloheximide. Moreover, the C1 produced by transitional or columnar epithelial cells bound to and eluted from IgG-Sepharose in a manner analogous to serum C1. Immunochemical and physicochemical analyses provided evidence that C1q from columnar epithelial cell cultures and C1r from each of the cell types tested had subunit structures similar to serum C1q and C1r. The C1s produced by epithelial cells and monocytes had subunits approximating those of human C1s ($C1\bar{s}$). C1q excreted by all cultures except columnar epithelium had an apparent molecular size greater than serum C1q (2). $C1\bar{s}$ from fibroblast medium had subunits with larger apparent molecular sizes than the reported subunit sizes of C1q or $C1\bar{s}$ isolated from serum (2, 7).

The $C\bar{1}$ hemolytic activity in medium from primary transitional epithelial, parenchymal, and tumor cell cultures from biopsy samples was similar to that found in low passage epithelial cell cultures. C1 hemolytic activity produced by three different fibroblast cultures ranged from 17–121 effective C1 molecules/cell per h. Reid and Solomon (19) reported similar rates of C1 production (50–230 em/cell per h). These values are close to the lower limit of the hemolytic assay. In contrast, fetal intestinal columnar epithelial cells produced 4.83×10^4 em/cell per h. These results agree with those reported by Bing et al. (13) and Colten et al. (10) for transitional and columnar epithelial cells. Production of hemolytically active C1 by blood monocytes (29–83 em/cell per h) can only be compared to other cultures by accounting for their decreased protein synthesis (Table II). They synthesized 3.6 times less total protein (TCA ppt cpm/culture) than epithelial cells and 4.4 times less than fibroblasts. However, monocytes produced approximately 400–760 times less $C\bar{1}$ than epithelial cells and three to seven times as much $C\bar{1}$ as fibroblasts. C1 was present in both the activated and zymogen form in epithelium culture medium. To determine if an inhibitor of C1 hemolytic activity was present in fibroblast culture medium, the ability of medium from two different skin fibroblast cultures to inhibit C1 hemolytic activity was measured. No inhhibition was observed. The possibility that C1r and C1s but not C1q were being synthesized was also tested. Addition of 10 μg purified C1q to fibroblast or monocyte medium produced no C1 hemolytic activity ($<2.1 \times 10^7$ em/culture). These results were different than those of Reid and Solomon (19) who found that addition of C1q to fibroblast culture medium with low hemolytic activity ($<1 \times 10^8$ em/ml) increased the level to $1\text{–}5 \times 10^9$ em/ml. Addition of purified C1s to monocyte or fibroblast culture medium did not generate additional hemolytic activity.

Unlike all other cell types tested, intestinal columnar epithelial cells synthesized and secreted C1q with a subunit structure analogous to serum C1q. C1q isolated from bladder transitional epithelium, fibroblast and monocyte culture media contained radiolabeled peaks on SDS-PAGE gels with molecular weights higher than the subunits of serum C1q. A peak of approximately 180,000 daltons was found in transitional epithelial and fibroblast media while a 78,000 dalton subunit was present in monocyte culture medium. These could represent multimers of C1q (A-B dimer, etc.), precursor, pro-C1q molecules or a molecular species not normally found in serum. These results were similar to the findings of Reid and Solomon (19) of C1q subunits with larger apparent molecular weights. C1r and $C1\bar{r}$ from all cell types

tested had apparent molecular weights (Fig. 3) which corresponded to published reports (6, 7). The subunit structure of newly synthesized C1s ($C1\bar{s}$) was similar in all cell types tested; peaks corresponding to published reports plus a heterogeneous peak of lower molecular weight subunits suggested degradation. This same explanation could account for some of the heterogeneous patterns observed for C1q.

These results indicated that epithelial cells were the major site of hemolytically active C1 biosynthesis in vitro. The capacity of columnar and transitional epithelial cells to produce functional C1 was not approached by any mesenchymal cell culture tested (two to three logs lower). Fetal intestine columnar epithelial cell cultures synthesized subcomponents of C1 similar to those of serum C1. All other culture types tested produced proteins with apparent molecular weights both larger than and similar to serum subunits of C1q and C1s. The hemolytic, immunochemical, and physicochemical evidence clearly demonstrated that one cell type can synthesize and secrete C1q, C1r, and C1s. However, caution must be exercised in interpreting in vitro synthetic studies as normal in vivo cellular regulatory mechanism are lacking. This lack could lead to synthesis or loss of regulation of synthesis by proteins which are not normally produced by certain cell types in vivo. As Al-Adnani and McGee (18) reported, fibroblasts synthesized and secreted C1q in vitro but attempts to detect C1q in vivo were negative until a pathological condition (a silica granuloma) was induced. The results of our in vitro studies indicated that columnar or transitional epithelial cells should be useful for studies of regulation of biologically active C1 synthesis, but transitional cells, fibroblasts, or monocytes are appropriate for investigating the precursors of C1q, C1r, and C1s.

Summary

Epithelial and mesenchymal cells synthesized and secreted all three subcomponents of the first component of complement (C1): C1q, C1r, and C1s. Quantitatively, however, columnar and transitional epithelial cells secreted 400–3,700 times more hemolytically active C1 than monocytes or fibroblasts. Only columnar epithelial cells synthesized C1 subcomponents with subunit structures similar to their serum counterparts. Transitional epithelial cells, fibroblasts, and monocytes produced C1q and C1s with subunits of apparent molecular weights larger than reported values. C1r from all cell lines was physicochemically similar to serum C1r.

The authors would like to thank Dr. Murray M. Bern for obtaining the biopsy material, Dr. Helene S. Smith for the epithelial and lung fibroblast cultures, and Dr. Robert Ziccardi for the anti-C1r antiserum. We would also like to thank Phyllis S. Gorin and Leila Rodwan for their expert technical assistance and Rachelle A. Rosenbaum for her excellent secretarial and editorial assistance.

Received for publication 22 June 1978.

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