SYNTHESIS AND ASSEMBLY OF IMMUNOGLOBULINS BY MALIGNANT HUMAN PLASMACYTES

I. Myelomas Producing γ-Chains and Light Chains

By SUSAN ZOLLA,* Ph.D., JOEL BUXBAUM,‡ M.D., E. C. FRANKLIN,§ M.D., and M. D. SCHARFF,‖ M.D.

(From the Department of Medicine, New York University Medical Center, New York 10016, and the Departments of Cell Biology and Medicine, Albert Einstein College of Medicine, Bronx, New York 10461)

(Received for publication 11 March 1970)

The basic features of γ-globulin synthesis and assembly have been elucidated in hyperimmune rabbit lymph nodes and malignant tissues of the mouse (1–12). These studies indicate that heavy (H) and light (L) chains are synthesized independently on ribosomes of different sizes (3, 4) and that L chains are then released from their ribosomes into an intracellular pool (5). No equivalent pool of soluble (released) intracellular H chains has been demonstrated (6). In at least four mouse tumors, L chains combine with nascent H chains on polyribosomes before their release (3, 6, 7). The assembly of γ-globulin occurs intracellularly (8, 9), and there is a 20 to 30 minute lag between the synthesis of chains and their secretion (9). During this period, the attachment of the major part of the carbohydrate moieties takes place (11).

The relative amounts of H and L chains synthesized and the patterns of assembly differ in several of the immunoglobulin synthesizing tissues studied to date. Some malignant and hyperimmune cells appear to be balanced with respect to the number of L and H chains secreted while others secrete excess free light chains. There is also evidence that the H-L dimer serves as an intermediate in the assembly of γ-globulin by some mouse plasmacytomas while others are assembled primarily through an H-H dimer (6, 9, 12).

Gamma globulin synthesis by malignant tissues from humans with multiple myeloma has been examined (13, 14) but a systematic study of this process in various types of human plasma cell tumors has not yet been performed. The

* Supported by National Institute of Health Postdoctoral Fellowship No. 5, F02 AI 23,369.

‡ Supported by National Institute of Health Postdoctoral Fellowship No. 5, F2 AM 32,687.

§ Supported by National Institute of Health Grants Nos. AM 02594, AM 01431 and AM 05064 and New York City Health Research Council I-274.

‖ Supported by National Institute of Health Grants Nos. AI 5231 and AI 4153. National Science Foundation Grants Nos. GB 6364 and GB 4751. American Cancer Society Grant No. E-379. Career Development Award Fellow of the U.S. Public Health Service.

present study was designed to investigate three aspects of γ-globulin synthesis: first, to examine the synthesis, assembly, and secretion of γG globulins and their constituent polypeptide chains by bone marrow cells from patients with multiple myeloma; second, to compare the results of these studies with those previously performed with mouse plasmacytomas; and third, to compare the γ-globulin components found in the cytoplasm and secreted material of cells in short-term culture with those found in the patient's serum and urine. This is of interest since proteins in the serum and urine reflect not only the synthetic but also the catabolic activities of a number of tissues. This report will consider only tumors producing γG and/or Bence Jones proteins. Subsequent papers will consider the synthesis of γA and γM proteins and the additional problems imposed by the formation of larger polymers of the basic H_2L_2 subunit.

Materials and Methods

Bone marrow samples from 18 patients undergoing diagnostic studies for multiple myeloma were aspirated through a large bore needle into a heparinized syringe. The aspirates were transferred to heparinized test tubes containing 20 ml of Eagle's minimal essential medium (Microbiological Associates Inc., Bethesda, Md.) containing $\frac{1}{20}$ the normal concentration of valine, threonine, and leucine and supplemented to a concentration of 4 mM with glutamine and 1.8 mM with $CaCl_2$ ($\frac{1}{20}$ medium) (15). In one patient with plasma cell leukemia, heparinized peripheral blood was obtained by venipuncture. After the red cells had sedimented at 37°C, the leukocyte-rich plasma was spun at 1300 rpm for 10 min and the pellet was resuspended in $\frac{1}{20}$ medium.

Cells collected from either source were washed once in cold $\frac{1}{20}$ medium. The red cells were lysed by gentle dispersion of the washed cell pellet in distilled water for 1 min. The cell suspensions were then brought to isotonicity with 10X spinner A salts (Microbiological Associates). The lysis procedure was repeated if large numbers of unlysed red cells remained. The remaining intact cells were then washed again with $\frac{1}{20}$ medium and resuspended in a small volume of this medium. The number of viable, nucleated cells was estimated by counting the cells which excluded erythrocin B dye (16).

Continuous labeling experiments were performed at cell concentrations between 10^6 and 10^7 cells/ml in $\frac{1}{20}$ medium supplemented with either 10 μCi each of ^{14}C-labeled valine, leucine, and threonine (VTLeu)[1] or with 25 μCi each of the same amino acids labeled with tritium. After exposure for 1 min to a gentle stream of 5% CO_2 gas, the incubation mixture was placed in a shaking water bath at 37°C for 3 hr. Labeled cytoplasmic material was prepared from cells after 45 to 60 min of incubation by first washing the cells in cold $\frac{1}{20}$ medium, lysing the red blood cells with distilled water as described above, and finally washing the residual cells with $\frac{1}{20}$ medium. To disrupt the cells, the pellet was suspended in 0.5 ml cold isotonic buffer pH 7.2 (8). Nonidet P-40 (Shell Chemical Co., New York, N.Y.), was added to a final concentration of 0.5%, the cells were allowed to stand in ice bath for 15 min, and then spun at 40,000 rpm (100,000 *g*) for 30 min to remove nuclei and ribosomes (17). In some experiments, the cells were disrupted either by freezing and thawing three times or by sonication. The cells

[1] *Abbreviations used in this paper:* MPC-11, Merwin plasma cell tumor-11; SDS, sodium dodecyl sulfate; VTLeu = valine, threonine, and leucine.

broken by the latter methods were suspended in 2% sodium dodecyl sulfate (SDS) and 0.03 M iodoacetamide and placed in a boiling water bath to solubilize the proteins. The medium from cell cultures incubated for 3 hr contained the labeled secreted material.

Pulse-chase experiments were carried out in the manner of Scharff et al. (9). Cells were prepared as described above, but medium totally depleted of VTLeu was used for the final cell wash and for suspension of the cells. The cells were incubated with 15 μCi each of ^{14}C-labeled VTLeu for 5 min, after which a large excess of unlabeled VTLeu was added in order to chase the labeled polypeptide chains off the polyribosomes. Equal aliquots were then removed at given times and placed in an ice bath to stop protein synthesis.

Preliminary studies revealed a significant degree of proteolysis in cytoplasmic samples. Marker proteins added to cytoplasmic preparations were often degraded to small polypeptides. The amount of proteolysis was reduced to a very low level by performing all manipulations at 4°C and by osmotically lysing the erythrocytes in the aspirate before and after incubation. In addition, immediate immunologic precipitation of aliquots of cytoplasm seemed to protect the precipitated proteins from degradative processes.

The cytoplasm and secreted material were subjected directly to polyacrylamide electrophoresis and the products of synthesis were identified by comparing their mobility to known marker proteins from the mouse MPC-11 tumor. Whenever possible, additional portions of cytoplasm and secreted material were precipitated with rabbit antisera to γG-globulin reactive with γ, κ, and λ chains (anti-7S), and additional antisera specific for the Fc fragment of γG (anti-Fc) and antisera to κ- or λ-Bence Jones proteins (anti-κ and -λ, respectively). Anti-7S serum was added to the cytoplasm or secreted material in the presence of 1 μl of "carrier" normal human serum. In most instances, a preliminary non-specific precipitation with albumin and anti-albumin was carried out before precipitation with anti-7S. With the other antisera, which appeared to form soluble antigen-antibody complexes, only a small fraction of trichloracetic acid precipitable radioactivity was recovered unless subsequent "indirect" precipitation with anti-rabbit γ-globulin was carried out. For indirect precipitation, either rabbit anti-Fc, anti-κ, or anti-λ was added to the labeled aliquot and incubated at 37°C for 1 hr; sheep anti-rabbit γ-globulin was then added and incubation at 37°C continued for another hour. All precipitation tubes were stored overnight at 4°C. The precipitates were washed twice with cold saline, solubilized in 7M urea, pH 5.9, and then subjected to electrophoresis.

Electrophoresis was performed in two different acrylamide systems. The first was modified after Reisfeld et al. (18). All solutions were made 7M in urea. Ethylene diacrylate was used as the gel polymerizing agent to facilitate gel solubilization after electrophoresis; 1 ml of ethylene diacrylate (Monomer-Polymer Laboratories, Borden, Inc., Philadelphia, Pa.) was used in place of 1 g of bisacrylamide. Unprecipitated cytoplasm or specific precipitates were dispersed in 7 M urea at pH 5.9 and applied to the gel. Tritium- or ^{14}C-labeled 7S γG, half molecules (HL), light chain dimers and monomers, synthesized in vitro by mouse plasma cell tumor MPC-11, were added as markers to each ^{14}C- or ^{3}H-labeled sample, respectively. After electrophoresis for 3.5 hr at 7 ma/gel, the gels were cut into 25 slices of 2 mm width and each slice was placed in a scintillation vial. After addition of 0.5 ml of 30% hydrogen peroxide to each vial, they were placed in a 60°C oven for 6 hr (19). 10 ml of a toluene-Biosolve III scintillation cocktail (containing: 17.1 g PPO, 0.428 g dimethyl POPOP, 3,420 ml toluene, and 380 ml Biosolve III (Beckman, Inc., Palo Alto, Calif.)) was added to each sample prior to counting in a Beckman LS-150 liquid scintillation counter.

The second electrophoretic system employed gels made in 0.1% sodium dodecyl sulfate (SDS) (20). SDS cell lysates were placed directly on these gels, while immune precipitates were dissolved in 2% SDS with 0.03 M iodoacetamide. After the addition of marker proteins, each sample was placed in a boiling water bath for 1 min and applied to the gel. These gels were fractionated by the gel grinding technique of Maizel (20). The ^{14}C-labeled fractions were ex-

truded onto planchettes, dried, and counted in a Nuclear Chicago gas flow counter. The ^{3}H-labeled fractions were extruded into scintillation vials and counted in Bray's modified scintillation cocktail (20).

The advantages of precipitating aliquots of cultured material with specific antisera to remove non-γ-globulin radioactivity and to immunologically identify the protein in each peak, are shown in Fig. 1. In this experiment, cytoplasmic material from a cultured tumor, which synthesized a γG κ-serum paraprotein and κ-Bence Jones protein, was electrophoresed before and after precipitation with anti-Fc, and anti-λ. In the sample which was unprecipitated, 7S, L chain monomer, and a small amount of an intermediate complex was present. When the same material was examined with anti-Fc, only protein migrating in the 7S peak was precipitated which indicates that H chain determinants were demonstrable only in 7S material and

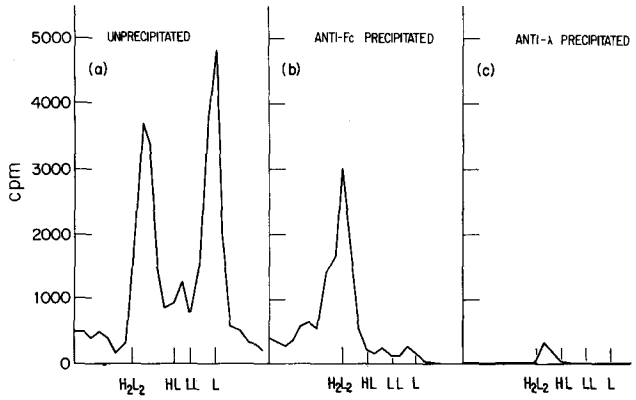

FIG. 1. Fractionation in urea-acid polyacrylamide gels of ^{3}H-labeled cytoplasmic material of marrow cells cultured from patient Ca. The cytoplasm was electrophoresed (*a*) without immunologic precipitation, (*b*) after precipitation with anti-Fc serum and (*c*) after precipitation with anti-λ serum. Each sample was run with ^{14}C-labeled γG (H_2L_2), half molecules (HL), light chain dimers (LL), and light chain monomers (L) synthesized by the mouse tumor MPC-11. The migration of these marker proteins is indicated on the abcissa. The anode is at the left and migration is towards the right.

not in proteins with light chain or intermediate migration. Precipitation of a very small amount of material with anti-λ antiserum may represent residual normal immunoglobulin production in the marrow. Thus, immunologic precipitation not only removes a significant amount of background radioactivity but also contributes to the identification of the constituents of each radioactive peak.

Patients' sera and urine were examined by paper strip electrophoresis. The nature of the proteins was determined by immunoelectrophoresis using antisera to γ-, κ-, and λ-chains, and antisera to the four subclasses of γ-chains. These antisera have been previously described (21). Some sera and urine were fractionated by gel filtration (Sephadex G-200) and studied by analytical ultracentrifugation.

RESULTS

Table I summarizes the results of the biosynthetic studies using the cells of 18 subjects with multiple myeloma. On the basis of the proteins produced in

vitro, the tumors have been divided into three groups. Plasma cells from two subjects with group I tumors produced light chains only; marrows from 12 individuals of group II produced an intact γG globulin and excess light chains; cells from four subjects in group III synthesized only a γG myeloma protein. The last two columns in Table I list the proteins found in the patients' sera and urine. It is apparent that the serum and urinary paraproteins were similar

TABLE I

Immunoglobulin Components in Cytoplasmic and Secreted Material from Malignant Human Plasmacytes

Group	Patient	Cytoplasmic material*				Secreted material				Paraprotein in serum	L chains in urine
		γG	Intermediate component	LL	L	γG	Intermediate component	LL	L		
I	Hu			×	×			×	×	λ	λ
	Sa			×				×		0	λ
II	Ga					×			×	γk	k
	Li					×		×		0	λ
	Pa					×			×	γ_1k	0
	Lo					×			×	γ_1k	k
	Ra					×			×	γ_1k	k
	Bo	×	×		×	×			×	γ_1k	0
	Mu	×	×		×	×			×	γ_4λ	N.D.
	Ca	×	×		×	×			×	γ_1k	k
	Kw	×	×		×	×			×	γ_1λ	N.D.
	Gob	×	×		×	×			×	γ_1λ	0
	Th					×		×	×	γ_1k	0
	Ru	×		×	×	×		×	×	γ_1k	k
III	Ni					×	×			γ_4k	0
	Le	×				×				γ_1λ	0
	Goa	×	×		×	×				γ_1λ	N.D.
	Ev	×				×				γ_1λ	0

N.D., not done.

*Horizontal lines (within field for groups II and III) signify experiments were not done.

to the products of cell culture with the exception of four subjects in group II whose cells produced light chains in tissue culture, while none were detected in the urine, and a fifth patient in group II whose serum showed no paraprotein but whose cells, in culture, produced a γGλ protein.

Fig. 2 illustrates the findings derived from cells of a group I tumor. Patient Hu with plasma cell leukemia excreted large amounts of λ light chains in the urine and had a small amount of light chain in the serum. 90% of the nucleated cells obtained from the peripheral blood of this patient were plasmacytes. While both monomers and dimers were present in the secreted material (Fig.

2) and in the cytoplasm (not shown in Fig. 2), only dimers were demonstrable in the urine. The small amount of material that migrated in the region of half molecules did not contain H chain determinants since precipitates of cytoplasmic material made with anti-Fc and anti-γA contained no labeled protein. Cytoplasm prepared from the cultured marrow of Hu gave results essentially

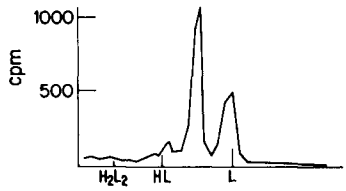

FIG. 2. Fractionation in SDS polyacrylamide gels of unprecipitated ^{14}C-labeled secreted material from the circulating plasmacytes of patient Hu. The region of migration of mouse immunoglobulin components run simultaneously on another gel is indicated on the abcissa. The anode is at the right and migration is towards the right.

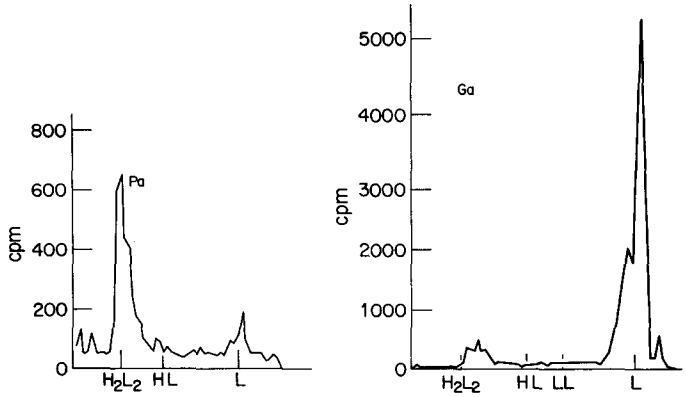

FIG. 3. Fractionation on SDS polyacrylamide gels of unprecipitated ^{14}C-labeled secreted material from marrow cells cultured from patient Pa and patient Ga. Both samples were run with ^{3}H-labeled immunoglobulins synthesized by MPC-11. The migration of these markers is indicated on the abcissa.

identical with those obtained with the peripheral cells. The presence of LL inside the cells indicates that L chain dimerization can occur intracellularly.

Additional information on the synthesis of light chains was provided by patient Ga in group II who synthesized and excreted large amounts of κ-chain monomers and had, in addition, a very small amount of monoclonal γG κ-protein in the serum. In culture, the patient's cells synthesized and secreted L chain monomers (Fig. 3) in contrast to the cells of Hu which secreted mostly dimers. The small amount (5%) of labeled protein migrating in the γG region may represent either the synthesis of the small amount of γG myeloma protein de-

tected in the serum or the synthesis of small amounts of normal γ-globulin in the marrow. Insufficient materials were available to prepare specific immunologic precipitates in this study.

A culture from patient Mu who had a γG myeloma protein in the serum and Bence Jones proteins in the urine (group II) contained significant amounts of

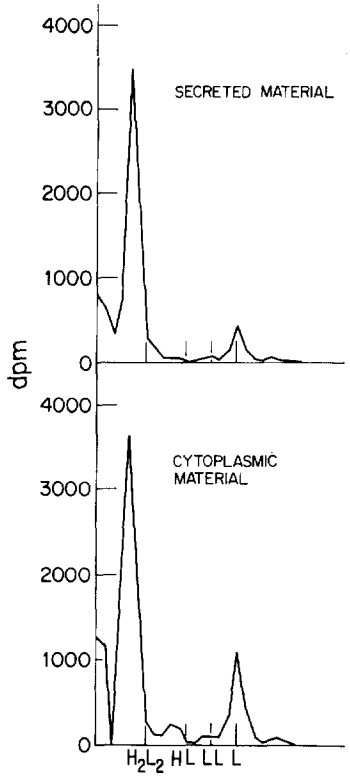

FIG. 4. Fractionation on urea-acid polyacrylamide gels of ^{3}H-labeled cytoplasm and ^{14}C-labeled secreted material from cells of patient Mu. Both samples were precipitated with anti-7S serum and electrophoresed with labeled mouse immunoglobulin components as markers. The migration of these markers is indicated on the abcissa.

a γG λ-myeloma protein and free light chains in the material secreted by the cells in culture (Fig. 4). The cytoplasm contained immunologically precipitable γG, an intermediate component, and free λ-chain monomers. The quantity of γG globulin exceeded the quantity of L chains in both the cytoplasmic and secreted materials.

Another pattern of synthesis and assembly in group II subjects was found in two cases where γG as well as L chain monomers and dimers were secreted.

In one of these (Ru) where both cytoplasm and secreted material were available for study, the ratio of L:LL was greater than one in the cytoplasm, but less than one in the secreted material; this provides another example of secretion of both monomers and dimers from the cell. The predominance of L chain dimers over monomers in the secreted material could represent either the preferential secretion of dimers or a continuing extracellular process of L chain dimerization.

The products from tumors of group II, which secreted γG myeloma proteins and free light chains, varied greatly; the relative proportions of γG and free light chains which were synthesized, the proportion of γG and light chains which were secreted, and the state of polymerization of the L chain components produced (Tables I and II) were different. For example, 76% of the material secreted from cultured cells of Ga was free, monomeric light chain while only

TABLE II

Nature of Light Chains

Patient	In urine	In secreted material from culture
Hu	Dimers	Dimers
Sa	Dimers	Monomers and dimers
Goa	Monomers	Monomers
Li	Dimers	Dimers
Lo	Monomers	Monomers
Ra	Monomers	Monomers
Pa	None	Monomers
Bo	None	Monomers
Gob	None	Monomers
Th	None	Monomers and dimers

5% of the secreted protein from cultured cells of Pa was light chain (Fig. 3). Other tumors in group II were intermediate between these two extremes. In the majority of instances L chain monomers, rather than dimers, were found in the cytoplasmic and secreted material. However, one tumor (Li) secreted only an L chain dimer in addition to whole γG, and two tumors (Ru and Th) secreted L chain monomers, L chain dimers, and whole γG (Table I).

In 4 out of 10 cases where both γG and free L chains were being secreted in culture, no Bence Jones proteins were found in the urine (Table I). Failure to find Bence Jones proteins in the urine of some patients whose cells synthesized and secreted them in vitro is not surprising since studies of the survival of autologous L chains in vivo have indicated that a large percentage of L chains is catabolized rather than secreted; clinical and animal studies suggest that the major site of L chain degradation is the kidney (22, 23). The presence or absence of L chains in the urine therefore is probably a function of the amount

synthesized, the state of renal function of the patient, and the susceptibility of the L chains to proteolytic degradation (24).

Four patients whose marrows were cultured and found to synthesize only γG myeloma proteins were assigned to group III. Each had a γ spike on serum electrophoresis, and in no instance were free light chains demonstrable in the urine. Polyacrylamide electrophoresis of the cytoplasm and secreted material from the cultured cells of one of these patients (Le) is shown in Fig. 5. Both aliquots contained labeled protein precipitable with anti-7S which migrated in the 7S region only. Cultured cells from two other patients also showed no demonstrable L chains in cytoplasmic or secreted material while a fourth culture (Goa) contained small amounts of L chain in the cytoplasmic but not in the secreted material. It would be difficult to rule out a small imbalance in

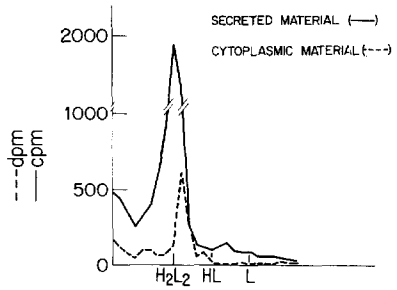

FIG. 5. Fractionation on urea-acid polyacrylamide gels of ^{3}H-labeled cytoplasm and ^{14}C labeled secreted material from cells of patient Le. Both samples were precipitated with anti-7S serum and electrophoresed with labeled mouse immunoglobulin components as markers. The migration of these markers is indicated on the abcissa.

synthesis in these and other patients even if there were more precipitable counts in the secreted material; in most experiments, free light chains containing less than 5% of the counts in the γG peak would go undetected and in some experiments where incorporation was less than optimal, such as the one illustrated in Fig. 5 for cytoplasm, labeled light chains in amounts up to about 10% of the counts in the γG peak could be present without being detected. While the absence of detectable free light chains from the cytoplasm and secreted material of these cultures suggests that the synthesis of H and L chains is balanced, the possibility that one chain is synthesized in excess and then rapidly degraded intracellularly cannot be excluded (6).

The assembly of γG globulin was studied in the cytoplasm of a tumor secreting γG and free L chains (Gob). The results of a pulse-chase experiment, in which the incorporated radioactivity was precipitated with anti-7S, are shown in Figs. 6 and 7. A half molecule intermediate was characterized by its migration in polyacrylamide in the region of the HL marker and by its precipitation

with anti-λ and anti-7S serum. The amount of L chains decreased during the first 10 min of the chase. The formation of HL intermediates and γG began immediately. The amount of intermediate component increased during the first 7 min of chase and then decreased in amount as the intact γG continued to accumulate. The results of this experiment suggest that newly synthesized L chains were incorporated into intact γG via an HL intermediate.

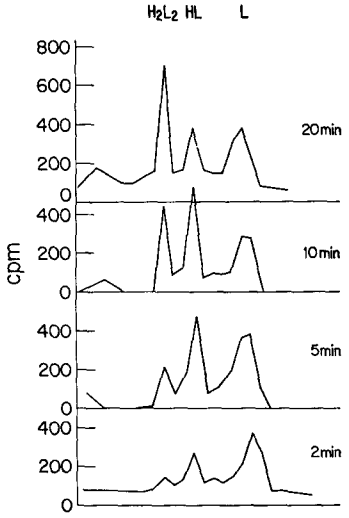

FIG. 6

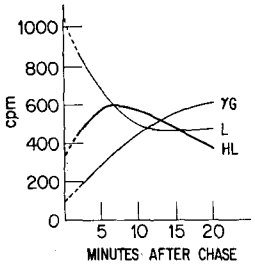

FIG. 7

FIG. 6. Fractionation in urea-acid polyacrylamide gels of ^{14}C-labeled cytoplasm from marrow cells cultured from patient Gob. The cytoplasms were prepared from aliquots of cells removed from culture after a 5 min pulse with ^{14}C-valine, threonine and leucine followed by either 2, 5, 10, or 20 min of chase with these same, unlabeled amino acids. Each cytoplasmic preparation was precipitated with anti-7S serum. Electrophoresis was carried out without internal markers; migration in each of the four gels was normalized with respect to migration of the H_2L_2 peak. The region of migration of mouse immunoglobulin components run simultaneously on another gel is indicated above the migration patterns.

FIG. 7. The kinetics of assembly of γG in the cytoplasm of cells cultured from patient Gob were computed from the pulse-chase experiment illustrated in Fig. 6. The counts in the three major peaks of each gel were combined and normalized; the number of normalized counts in each peak was plotted against the time after addition of cold amino acids to the incubation mixture.

DISCUSSION

Dysproteinemias have been classified according to the presence and nature of the serum paraproteins, urinary L chains, or both urinary L chain and the serum paraprotein. Since the amount of each protein present in serum and urine is dependent not only on the synthetic rate but also on the catabolic rate of the protein, it is obvious that serum and urine analyses can give only an in-

direct, and sometimes misleading picture of the actual synthetic processes in the neoplastic cells.

On the basis of studies of short-term in vitro cell cultures, malignant plasma cell tumors from humans were classified into three categories (Table I): those which display extreme unbalanced synthesis as evidenced by the production of light chains only; those which are characterized by unbalanced synthesis, where more light chains than heavy chains are produced; and those which exhibit balanced synthesis of H and L chains.

The results obtained from the study of tissues of 18 patients indicate that in the majority of cases, serum and urine studies correspond in a qualitative sense with the in vitro biosynthetic studies. However, there are at least 4 instances out of 14 where free light chains were produced and secreted from cultured cells but could not be detected in the serum or urine of the patients from which the cells were obtained; and in one case (Li) out of 16, a γG protein was present in the cultured, secreted material although no γG paraprotein was demonstrable in serum.

Within each of the three tumor groups defined by this study, quantitative and qualitative variations are apparent: light chains can be secreted at the cellular level as monomers, dimers, or as both (patients Ga, Sa, and Ru), and either L chains or intact γG can predominate in tumors characterized by secretion of both γG and free light chains (group II). For example, patient Li, secreted 17% γG and 83% L chains while Bo secreted 80% γG and 20% light chains. When viewed in light of the relative amounts of free light chains and intact γG produced by each tumor, a complete spectrum emerged which ranged from the production of only L chains to the production of only intact γG. This spectrum of protein production by cells from several tumors can best be observed by sequentially examining Fig. 2, 3 *b*, 3 *a*, and 5.

The designation of three types of synthetic patterns in plasma cell tumors is useful for the sake of discussion but may be an oversimplification since it depends on two assumptions: (*a*) that rapid intracellular degradation of either L or H chains does not occur and thus does not affect the observed molar ratio of L and H; and (*b*) that the immunoglobulin synthesizing cells are a homogeneous population, each producing the same components in the same relative amounts, rather than a mixture of cells, some of which produce an intact protein while others produce only L chains. The validity of the first assumption is supported by the results of the marrow studied by the pulse-chase technique (Fig. 6); radioactively labelled globulin subunits were chased into intact immunoglobulin, with no evidence of degradation. However, degradation has been reported in some mouse tumors (6, 7). No evidence is available on the degree of homogeneity of malignant human plasma cells but studies with certain mouse tumors have demonstrated a heterogeneity with respect to assembly and synthesis of immunoglobulin subunits (26, 27).

A comparison of the monomeric or dimeric nature of the light chains in cellular secretions and in the urine of 10 patients is shown in Table II. When light chains were secreted into the urine, they existed either as monomers, dimers or both and were generally found to be in the same state of polymerization in which they were secreted from the cell. When it occurred, covalent dimerization of light chains appeared to take place primarily intracellularly, although extracellular dimerization may occur. Whether light chains remain monomeric or dimeric probably depends on the chemical and physical properties of the chains rather than on quantitative aspects of synthesis or an active process (28). The presence of an intracellular disulfide forming enzyme cannot, however, be excluded.

The studies of malignant human plasma cells support and extend the findings obtained with mouse plasmacytomas (1–12). In the mouse, γG has been shown to be assembled through a variety of intermediates (9, 10); when present, these subunits often occur in small amounts and are difficult to detect. Intermediate components were noted in six of nine human tumors from which cytoplasm was available for study. These intermediate components migrated with the half molecule (HL) marker although in one case (Ca), antiserum to the Fc fragment of γG did not precipitate this component (Fig. 1). Since, however, an HL or HH dimer might not be precipitable by antiserum to Fc, the possibility cannot be excluded that this component was in fact an intermediate form containing H chains. The precise nature of the intermediates remains to be determined since the difference in mobility of HH, HL and light chain polymers in the acid-urea acrylamide system is not enough to distinguish these components, and sufficient material was not available to characterize the intermediates by immunologic methods. It is also possible that minor intermediates were not detected since the techniques used in the work reported here were not sensitive enough to pick up small amounts of rapidly turning over intermediates. It was possible to study the assembly of γG using the pulse-chase technique in only one subject whose marrow produced a γ_1 λ-protein. The data from that study indicate that the synthesized L chains were incorporated into an intermediate half molecule (HL), with subsequent dimerization of two HL half molecules to yield the intact γG. The whole process appears to have been completed in about 10 min.

Pathways of assembly may be related to γG subtype (29) since different subtypes vary in the number and position of intra-chain disulfide bonds (30). However, the number of proteins belonging to subclasses other than γ_1 was too small to permit any conclusions on this point. Sufficient numbers of tumors produceding κ- or λ-chains were studied to demonstrate that no correlation exists between the L chain class and the pattern of cellular secretion.

The variations in synthesis and assembly of γ-globulin in the human plasma cell tumors described above and the variations described previously in several

mouse plasmacytomas demonstrate that the biosynthetic process differs qualitatively and quantitatively in different tumors; it is therefore impossible to extrapolate from tumor to tumor. Similarly it is not possible to apply data obtained from studies of malignant plasmacytes to normal γ-globulin–producing cells. Further studies must be done to determine whether heterogeneity of assembly mechanisms exists among normal cell populations. Biosynthetic studies of clones derived from single cells are needed to determine if balanced or unbalanced synthesis is more characteristic of normal cells, and whether normal cells exist which produce only L chains, only intact γG, or both intact globulin and its subunits.

SUMMARY

Three basic patterns of γ-globulin synthesis are described in malignant human plasmacytes: extreme unbalanced synthesis where only L chains are synthesized; unbalanced synthesis in which intact γG globulin and an excess of free L chains are synthesized and secreted; and balanced synthesis where H and L chains appear to be synthesized in equimolar amounts. Studies of the cellular products appear to reflect the biosynthetic processes of the cells in a more reliable fashion than does analysis of serum or urinary proteins.

The absence of Bence Jones proteins from the urine does not necessarily indicate that free L chains are not being synthesized and secreted at the cellular level. Similarly, the completed globulin molecule secreted by malignant plasma cells may not be demonstrable by examination of serum.

Patterns of globulin synthesis in human myelomatous tissues vary as do patterns of globulin synthesis in mouse plasmacytomas.

Pulse-chase studies of the cells from one patient showed that a γG myeloma protein was assembled via an HL (half molecule) intermediate.

We wish to thank Mrs. Ellyn Fischberg for her excellent technical assistance and Mrs. Marlene Chavis for her valuable assistance in preparing this manuscript.

BIBLIOGRAPHY

1. Shapiro, A. L., M. D. Scharff, J. J. Maizel, and J. W. Uhr. 1966. Synthesis of Excess L chains of γ-globulin by rabbit lymph node cells. *Nature* (*London*). **211**:243.
2. Askonas, B. A. and A. R. Williamson. 1967. Balanced synthesis of L and H chains of γG. *Nature* (*London*). **216**:264.
3. Shapiro, A. L., M. D. Scharff, J. J. Maizel, and J. W. Uhr. 1966. Polyribosomal synthesis and assembly of the H and L chains of γ-globulin. *Proc. Nat. Acad. Sci.*, U.S.A. **56**:216.
4. Williamson, A. R. and B. A. Askonas. 1967. Biosynthesis of immunoglobulins: The separate classes of polyribosomes synthesizing H and L chains. *J. Mol. Biol.* **23**:201.

5. Askonas, B. A. and A. R. Williamson. 1966. Biosynthesis of immunoglobulins: Free L chains as an intermediate in the assembly of γG molecules. *Nature (London)*. **211:**369.
6. Schubert, D. 1968. Immunoglobulin assembly in a mouse myeloma. *Proc. Nat. Acad. Sci.*, U.S.A. **60:**683.
7. Schubert, D. and M. Cohn. 1968. Immunoglobulin biosynthesis. III. Blocks in defective synthesis. *J. Mol. Biol.* **38:**273.
8. Williamson, A. R. and B. A. Askonas. 1968. Time course of labeling of L and H chains of immunoglobulin and the turnover of the free L chain intermediates *Arch. Biochem. Biophys.* **125:**401.
9. Scharff, M. D., A. L. Shapiro, and B. Ginsberg. 1967. Synthesis, assembly and secretion of γ-globulin polypeptide chains by cells of a mouse plasma cell tumor, *Cold Spring Harbor Symp. Quant. Biol.* **32:**235.
10. Askonas, B. A. and A. R. Williamson. 1968. Interchain disulfide formation in the assembly of immunoglobulins. *Biochem. J.* **109:**637.
11. Melchers, F., and P. M. Knopf. 1967. Biosynthesis of the carbohydrate portion of immunoglobulin chains: possible relation to secretion. *Cold Spring Harbor Symp. Quant. Biol.* **32:**255.
12. Askonas, B. A. and A. R. Williamson. 1967. Biosynthesis and assembly of IgG. *Cold Spring Harbor Symp. Quant. Biol.* **32:**223.
13. Cioli, D. and C. Baglione. 1967. Studies on the synthesis of the peptide chains of human immunoglobulins. *Nobel Symp. No 3.* 401.
14. Mullinax, F., G. L. Mullinax, and B. Himrod. 1967. RNA metabolism and protein synthesis in plasma cell leukemia. *Amer. J. Med.* **42:**302.
15. Eagle, H. 1959. Amino acid metabolism in mammalian cell cultures. *Science (Washington)*. **130:**432.
16. Merchant, D. J., R. J. Kahn and W. Murphy. 1960. Handbook of Cell and Organ Culture. Burgess Publishing Co., Minneapolis.
17. Borun, T., M. D. Scharff and E. Robbin. 1967. The preparation of polyribosomes with the detergent Nonidet P-40, *Biochim. Biophys. Acta.* **149:**302.
18. Reisfeld, R. A., U. J. Lewis and D. E. Williams. 1962. Disc electrophoresis of basic proteins and peptides on polyacrylamide gels. Nature (London), **195:**118.
19. Tishler, P. V. and C. J. Epstein. 1968. A convenient method of preparing polyacrylamide gels for liquid scintillation spectrometry. *Anal. Biochem.* **22:**89.
20. Maizel, J. V., Jr. 1969. Acrylamide gel electrophoresis of proteins and nucleic acids. *In* Fundamental Techniques in Virology, K. Habel and N. Salzman, editors. Academic Press, New York.
21. Franklin, E. C. 1964. Structural studies of human 7S gamma globulin. *J. Exp. Med.* **120:**691.
22. Meyer, F. and F. Putnam. 1963. The fate of injected Bence Jones proteins. *J. Exp. Med.* **117:**573.
23. Wochner, R. D., W. Strober, T. A. Waldman. 1967. The role of the kidney in the catabolism of Bence Jones proteins and immunoglobulin fragments. *J. Exp. Med.* **126:**207.
24. Soloman, A., T. Waldman, J. Fahey, and A. McFarlane. 1964. Metabolism of Bence Jones proteins. *J. Clin. Invest.* **43:**103.

25. Cohn, M. 1967. Natural history of myeloma. *Cold Spring Harbor Symp. Quant. Biol.* **32:**211.
26. Laskov, R. and M. D. Scharff. 1970. Synthesis, assembly and secretion of γ-globulin by mouse myeloma cells. I. Adaptation of the Merwin plasma cell tumor-11 to culture, cloning, and characterization of γ-globulin subunits. *J. Exp. Med.* **131:**515.
27. Scharff, M. D. and R. Laskov. 1970. Synthesis and assembly of immunoglobulin polypeptide chains. *Progr. Allergy.* **29:**577.
28. Stevenson, G. T. and D. Straus. 1968. Specific dimerization of L chains of human immunoglobulins. *Biochem. J.*, **108:**375.
29. Baumal, R. and M. D. Scharff. 1970. Synthesis assembly and secretion of IgG_{2A} by the mouse plasma cell tumors MOPC-173 and MOPC-494. *Fed. Proc.* **29:**577.
30. Frangione, B. and C. Milstein. 1968. Variations in the disulfide bridges of immunoglobulin G: interchain disulfide bridges of γG3 myeloma proteins. *J. Mol. Biol.*, **33:**893.