IMMUNOGLOBULIN SYNTHESIS AND SECRETION

VI. Synthesis and Intracellular Transport of Immunoglobulin in Nonsecretory Lymphoma Cells*

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The initial steps in the synthesis and intracellular transport of immunoglobulin (Ig) secreted by plasma cells are partially understood. The concept has emerged that Ig chains are synthesized by membrane-bound polyribosomes (1–3), enter the cisternae of the rough endoplasmic reticulum (RER)[1] (4–15), and remain within membranes until their secretion from the cell (16).

It is well established, however, that lymphocytic cells which lack a well developed RER (17) are also capable of Ig synthesis (6, 13, 14, 18, 19). Thus, mechanisms of synthesis and intracellular transport other than those described for plasma cells may operate in these cells. To investigate this possibility, we studied a human lymphoma cell line which synthesizes IgM but lacks a well developed RER. This cell line (Daudi) has been shown not to secrete its IgM (20, 21).

The results indicate that many of the mechanisms of synthesis and intracellular transport utilized by plasma cells are operative in lymphoma cells. We suggest that the failure to release Ig molecules from the plasma membrane may represent the major difference between the myeloma and this type of lymphoma cell.

Materials and Methods

Cells and Labeling.—Daudi, an established line of Burkitt lymphoma cells synthesizing IgM, was kindly provided by Dr. Eva Klein. Cells were maintained in suspension culture in

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[1] *Abbreviations used in this paper*: B cells, bone marrow-derived cells; ER, endoplasmic reticulum; PBS, phosphate-buffered saline; RER, rough endoplasmic reticulum; SDS, sodium dodecylsulfate; T cells, thymus-derived cells.

Dulbecco's medium with 10% fetal calf serum and 1% antibiotic-antimycotic (all from Grand Island Biological Laboratories, Grand Island, N. Y.). The generation time was approximately 15 hr. P_3K, an established line of mouse myeloma cells that secretes IgG_1 was a gift of Dr. Kengo Horibata of the Salk Institute. P_3K cells were grown as above except that 10% horse serum (Grand Island Biological Laboratories) was substituted for fetal calf serum. P_3K has a generation time of 16–20 hr; approximately 20% of ^{3}H-leucine incorporated into macromolecules is in IgG.

For labeling with ^{3}H-L-leucine (50 Ci/mmole; New England Nuclear Corp., Boston, Mass.), cells were collected by centrifugation at 500 *g* for 10 min and were resuspended at 1×10^7/ml in Eagle's medium (22) containing $\frac{1}{100}$ the usual concentration of leucine. For labeling with ^{3}H-D-galactose (6.8 Ci/mmole), or ^{3}H-D-fucose (4.3 Ci/mmole) (New England

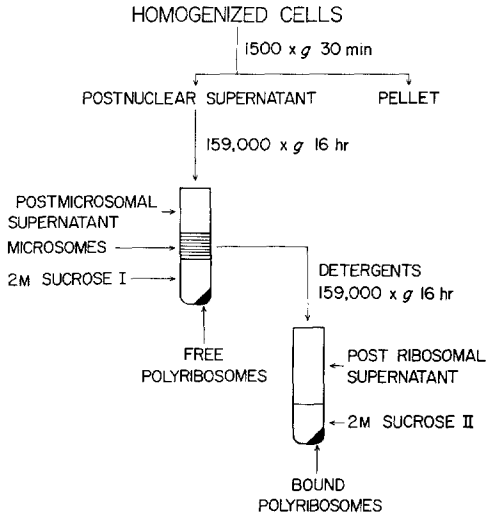

FIG. 1. Procedure for separation of free and membrane-bound polyribosomes.

Nuclear), cells were suspended at 1×10^7/ml in normal medium. All labeling was performed at 37°C using 10 μCi/ml ^{3}H-isotope. Incorporation was terminated by diluting suspensions into a fivefold excess of cold (4°C) medium containing ice.

Ig in Secretions and Lysates.—2×10^8 Daudi or P_3K cells were labeled with ^{3}H-leucine and incorporation was stopped in 4 ml samples. Cells were collected by centrifugation and the incorporation medium (secretion) was saved. Cells were resuspended in 2 ml 0.01 M phosphate buffer, pH 7.2–0.15 M sodium chloride (phosphate-buffered saline [PBS]) and were lysed with a 0.5% solution of the detergent Nonidet P40 (Shell Chemical Corp., New York). Nuclei were pelleted at 1500 *g* for 15 min, and the postnuclear supernatant fluids (lysates) were decanted. Lysates and secretions were then precipitated with antiserum against Ig as described below. Immune precipitates were dissolved, reprecipitated with trichloroacetic acid (TCA), and radioactivity was determined.

Isolation of Free and Bound Polyribosomes.—2×10^8 Daudi cells were labeled for 10 min with ^{3}H-leucine. Free and bound polyribosomes were obtained by a modified method (2) of Blobel and Potter (23) as summarized below (Fig. 1). Liver cell supernatants were used as "carrier" in order to visualize pellets and bands in gradients and to inhibit ribonuclease. Nascent chains were released from polyribosomes with RNAase (2) and precipitated with antiserum as described below.

Fractionation of Microsomes and Cell Sap.—2×10^8 Daudi cells were labeled with either ^{3}H-leucine or ^{3}H-sugars and 4 ml samples were removed during the labeling period for fractionation. After a 15 min leucine pulse, one fraction was "chased" for 75 min by the addition of a 100 X concentration of unlabeled leucine. Cells collected by centrifugation were resuspended in 2 ml 0.05 M tris(hydroxymethyl)aminomethane(Tris) Cl, pH 7.5 (20°C)–0.025 M KCl–0.005 M $MgCl_2$–0.25 M sucrose (RNAase-free, Mann Research Labs, Inc., New York), and were broken with 15 strokes of a tight-fitting Dounce homogenizer (Kontes Glass Co. Vineland, N.J.). Nuclei were sedimented at 1500 g_{max} for 20 min and the postnuclear supernatant fluids were spun for 2 hr at 100,000 g_{aver} in the 40-rotor of the Spinco Model L ultracentrifuge to pellet the microsomes. Postmicrosomal supernatant fluids (cell saps) were decanted, microsomal pellets were resuspended by Dounce homogenization, and the microsomal contents were released with a solution of 0.5% Triton X 100–0.5% sodium desoxycholate. The cell saps and microsomal contents were then precipitated with antiserum against Ig.

Identification of Incorporated ^{3}H-Sugars.—Hexoses from galactose-labeled specific precipitates were separated from an acid hydrolysate of the protein (100°C, 3.6 N HCl, 6 hr) on Dowex 50 (H+ form; Bio-Rad Laboratories, Richmond, Calif.) according to Boas (24), and were identified by thin layer chromatography (MN 300, normal cellulose, ascending; Analtech, Inc. Newark, Del.) with butanol:pyridine:water (6:4:3 v/v) as solvent. *N*-acetylglucosamine (Mann Research Labs.), ^{3}H-galactose, ^{3}H-fucose, and ^{14}C-mannose (45 μCi/mmole; New England Nuclear Corp.) were used as standards. *N*-acetylglucosamine was visualized with Partridge's reagent (25).

Immunological Precipitation and Electrophoresis.—Precipitation of P_3K lysates and secretions was carried out for 30 min at 37°C and for 2 hr at 4°C using goat antiserum to mouse Ig and 20 μg/ml carrier mouse Ig (Pentex Biochemical, Kankakee, Ill.). Precipitations of Daudi cell lysates, secretions, microsomal contents, cells saps, and polyribosomal nascent chains were performed by a "sandwich" procedure using 0.020 ml of rabbit antiserum to human IgM (30 min at 37°C) and excess goat antiserum to rabbit IgG (30 min at 37°C, 2 hr at 4°C). As a control for nonspecific coprecipitation, 0.020 ml of normal rabbit serum or of rabbit antiserum against an unrelated antigen (*Salmonella flagellin*) was substituted for rabbit anti-human IgM in the first step. In all cases, due to the small amount of IgM in Daudi cells, radioactivity in nonspecific precipitates was 20–50% of that in specific precipitates. Values obtained for nonspecific precipitations have been subtracted from values obtained for specific precipitations in calculating the data. To confirm the identity of immunologically precipitated Ig, some specific precipitates were dissolved in 8 M urea–1% sodium dodecylsulfate (SDS)–0.05 M Tris Cl, pH 8.4 (20°C), and reduced and alkylated as described previously (26). Electrophoresis in SDS-acrylamide gels was then performed (27).

Scintillation Counting.—Specific precipitates were washed four times in cold (4°C) PBS and were dissolved in 0.25 N acetic acid. Portions were reprecipitated with cold 5% TCA. Acid precipitates were collected on Millipore filters, washed twice with cold 5% TCA, and counted in 10 ml of scintillation cocktail (100 g naphthalene and 5 g 2,5-diphenyloxazole/liter of dioxane). Samples from subcellular fractions were precipitated with 5% TCA and were similarly counted. SDS-acrylamide gels, cellulose scraped from thin layer plates, and fractions from Dowex columns were counted in the same scintillation cocktail. All determinations of radioactivity were made to the ±5% error (2 sigma) in a Beckman LS-250 scintillation counter. Efficiencies were routinely 30% for ^{3}H except for acrylamide gels (10%) and 90% for ^{14}C.

RESULTS

Morphology of Daudi Cells.—Fig. 2 shows a representative electronmicrograph in which portions of the cytoplasm of four cells are shown. As can be seen, there is a paucity of rough endoplasmic reticulum in contrast to the appearance

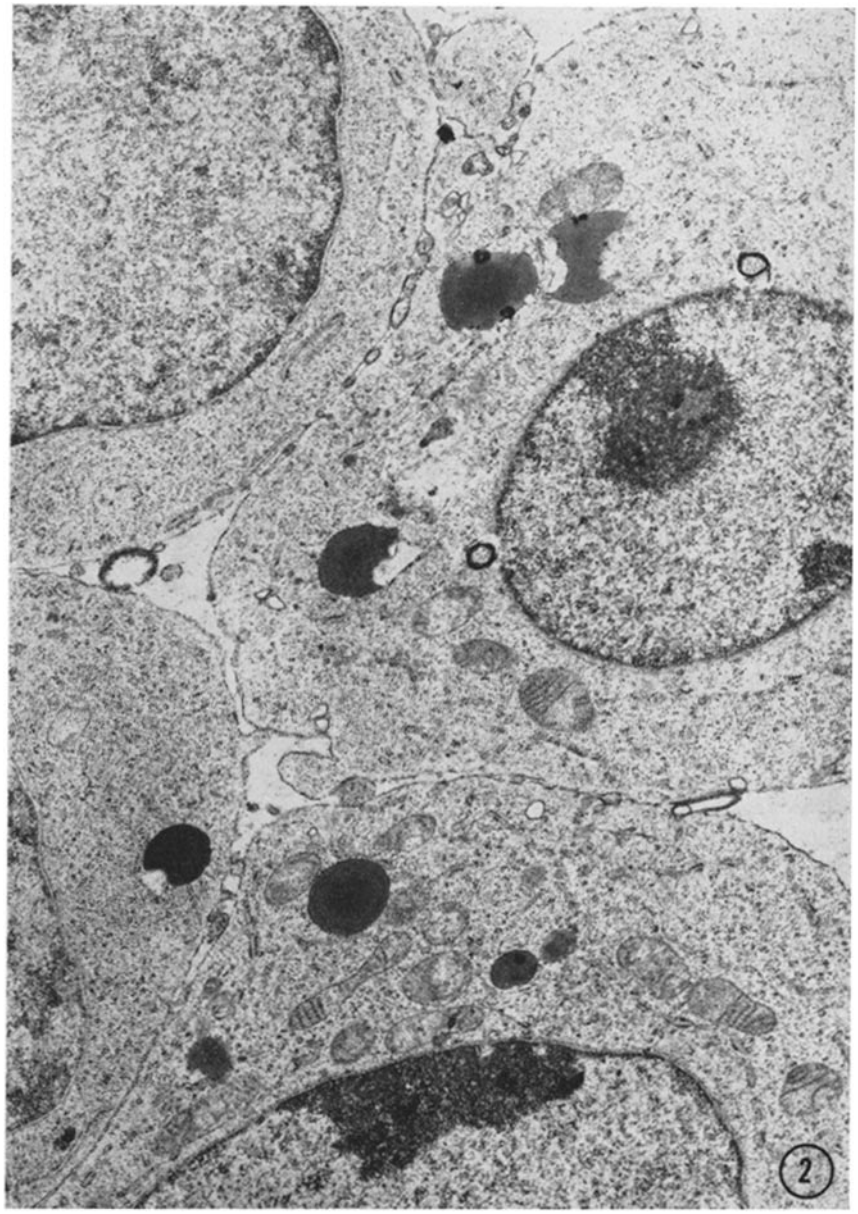

FIG. 2. Electronmicrograph of Daudi cells. × 9900.

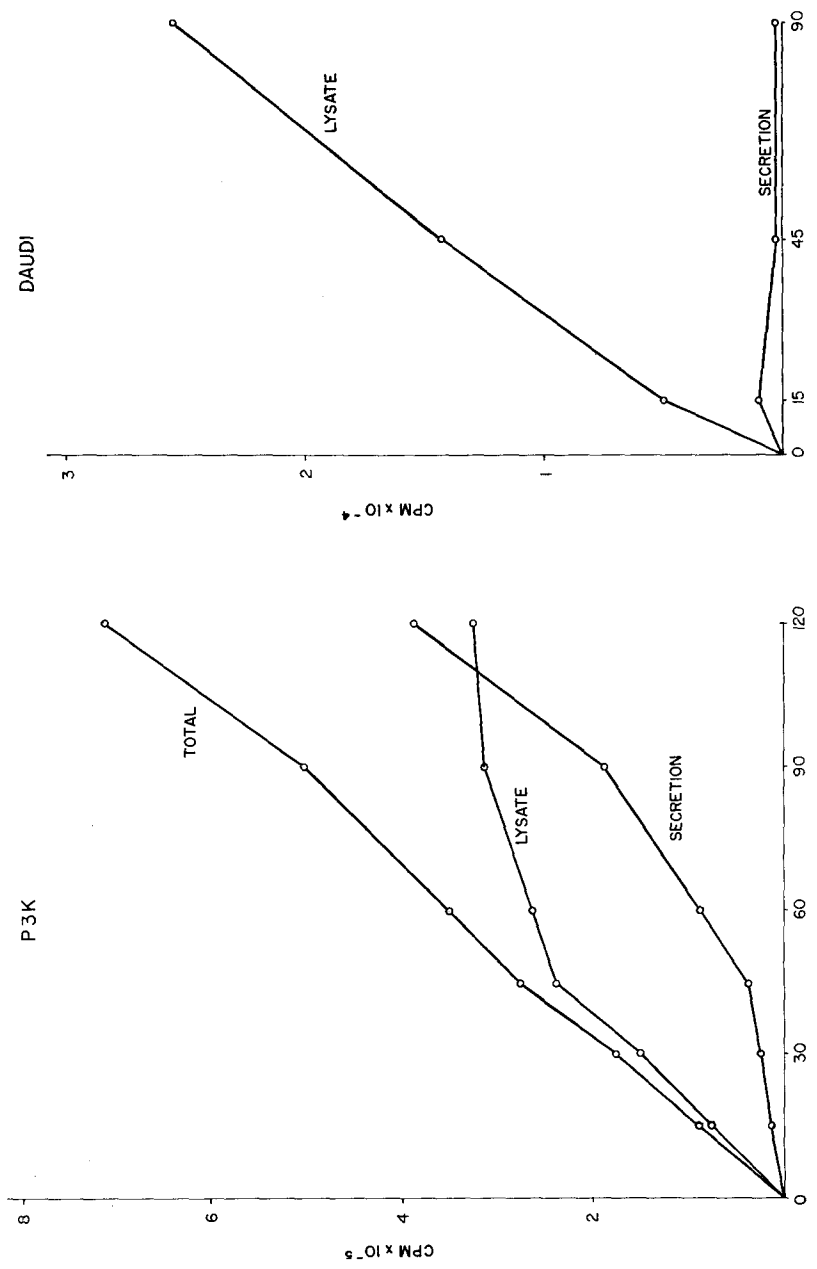

FIG. 3. Kinetics of incorporation of ^{3}H-leucine into Ig in mouse myeloma cells (P_3K) and Daudi cells. Cells were labeled for various times and Ig was immunologically precipitated from cell lysates and secretions.

of plasma cells (6, 13, 14, 19). The vast majority of polyribosomes are free in the cell sap. These results confirm an earlier description of the ultrastructure of Daudi cells (28).

Absence of Secretion by Daudi Cells.—It has previously been reported that no Ig was detected in the culture medium of Daudi cells even though these cells reacted strongly with fluorescein- (20, 21, 29, 30) and ferritin-labeled (28) anti-immunoglobulin sera. To further investigate whether Ig is secreted by Daudi cells, the kinetics of incorporation of ^{3}H-leucine into intracellular and secreted Ig by Daudi cells and the secreting plasmacytoma, P_3K, were compared.

As can be seen in Fig. 3, the pattern of incorporation observed with P_3K cells is similar to that observed with other cells that secrete Ig (31, 32). The incorporation of ^{3}H-leucine into total Ig (lysate + secretion) is linear throughout the labeling period. Incorporation into intracellular Ig (lysate) is also linear for approximately 60 min. After a short lag phase, Ig is secreted into the medium (secretion). A "steady state" in which the rate of incorporation into Ig equals the rate of secretion of radioactive Ig is reached in 60–120 min. In contrast to P_3K cells, Daudi cells incorporate ^{3}H-leucine into intracellular Ig, but no Ig is secreted into the medium (even when incorporation is continued for 6 hr). In addition, the percentage of newly synthesized protein which is Ig is significantly less in Daudi than in P_3K cells (1–2% vs. 20%).

Synthesis of Protein and Ig by Free and Membrane-Bound Polyribosomes.—It has previously been established in mouse myeloma (1, 2) and rabbit lymph node cells (3) that Ig is preferentially synthesized on polyribosomes attached to membranes (bound polyribosomes) rather than those in the cytoplasm (free polyribosomes). Since electronmicroscopic examination has indicated that Daudi cells have very few bound polyribosomes, it has been suggested that Ig synthesized by these cells is made on free polyribosomes (28). Synthesis of Ig on free polyribosomes could account for the lack of secretion.

To investigate this point, Daudi cells were labeled with ^{3}H-leucine and free and bound polyribosomes were separated (see Materials and Methods). The recovery of incorporated radioactivity in the various cell fractions together with our previously published results with mouse myeloma (2) and rabbit lymph node cells (3) are shown in Table I. Daudi cells had approximately threefold more radioactivity in the postmicrosomal supernatant than the other cell types. This fraction contains molecules which were originally present in the cell sap. In contrast, less radioactivity was found in the postribosomal supernatant, a heterogenous fraction containing the microsomal contents and additional molecules from the postmicrosomal supernatant which sedimented with the microsomes during the first gradient centrifugation (see Fig. 1). These findings suggest that relatively less radioactivity is incorporated into microsomes in Daudi cells, as would be expected from the relative lack of RER observed in electronmicrographs.

When nascent chains were released from polyribosomes and precipitated with specific antisera to Ig, 3.3 ± 1.3% of the incorporated radioactivity in the bound polyribosomes and 0.4 ± 0.5% of the incorporated radioactivity in the free polyribosomes were precipitated as Ig. These results indicate that Ig is preferentially made by bound polyribosomes.

When portions of the postribosomal supernatant were similarly precipitated, 2.5 ± 0.9% of the incorporated radioactivity was found in Ig. In contrast, only 0.5 ± 0.3% of the radioactivity incorporated into the postmicrosomal supernatant was found in Ig. These results indicate that newly synthesized Ig rapidly enters the microsomes and that only small amounts can be found in the cell sap.

TABLE I

Percentcge of Incorporated Radioactivity Recovered in Subcellular Fractions

Fraction	Daudi ceils	P3K cells (2)	Rabbit lymph node cells (3)
Postnuclear supernatant	100	100	100
Postmicrosomal supernatant	25.1 ± 7.6	7.4 ± 2.4	9.6 ± 3.2
2 M sucrose I	2.6 ± 0.7	5.1 ± 2.9	2.7 ± 0.7
Postribosomal supernatant	46.6 ± 5.1	62.5 ± 12.5	65.0 ± 2.1
2 M sucrose II	1.6 ± 0.3	2.8 ± 1.6	1.4 ± 0.5
Free polyribosomes	16.9 ± 3.7	16.5 ± 5.7	9.9 ± 1.0
Bound polyribosomes	2.9 ± 1.1	5.0 ± 2.1	4.7 ± 0.7
Per cent of total recovered	95.7	99.3	93.3

The small amounts of Ig in the cell sap could easily have arisen from microsomal vesicles which were disrupted during the fractionation.

^{3}H-Leucine Incorporation into Ig in Microsomes and Cell Sap.—Since the above experiments suggested that Ig synthesized on bound polyribosomes enters the microsomes, a simplified fractionation procedure was employed to study the fate of Ig within the microsomes during long periods of incorporation (see Materials and Methods). Thus, microsomes and cell sap were separated and specifically precipitated for Ig. A representative experiment is shown in Fig. 4.

The kinetics of incorporation into total acid-precipitable molecules in both the microsomes and cell sap is virtually linear throughout the labeling period. The level of incorporation after 15 min is virtually the same as that obtained after a 15 min pulse and 75 min chase indicating that the chase was effective.

When incorporation of radioactivity into Ig was studied, almost all Ig was found in the microsomes regardless of the duration of the labeling period. The leucine chase did not affect the distribution of radioactive Ig between the two

compartments indicating that Ig entering the microsomes does not exit into the cell sap in appreciable amounts.

To study the specificity of immune precipitation, precipitates taken at various times were reduced, alkylated, and electrophoresed in SDS-acrylamide

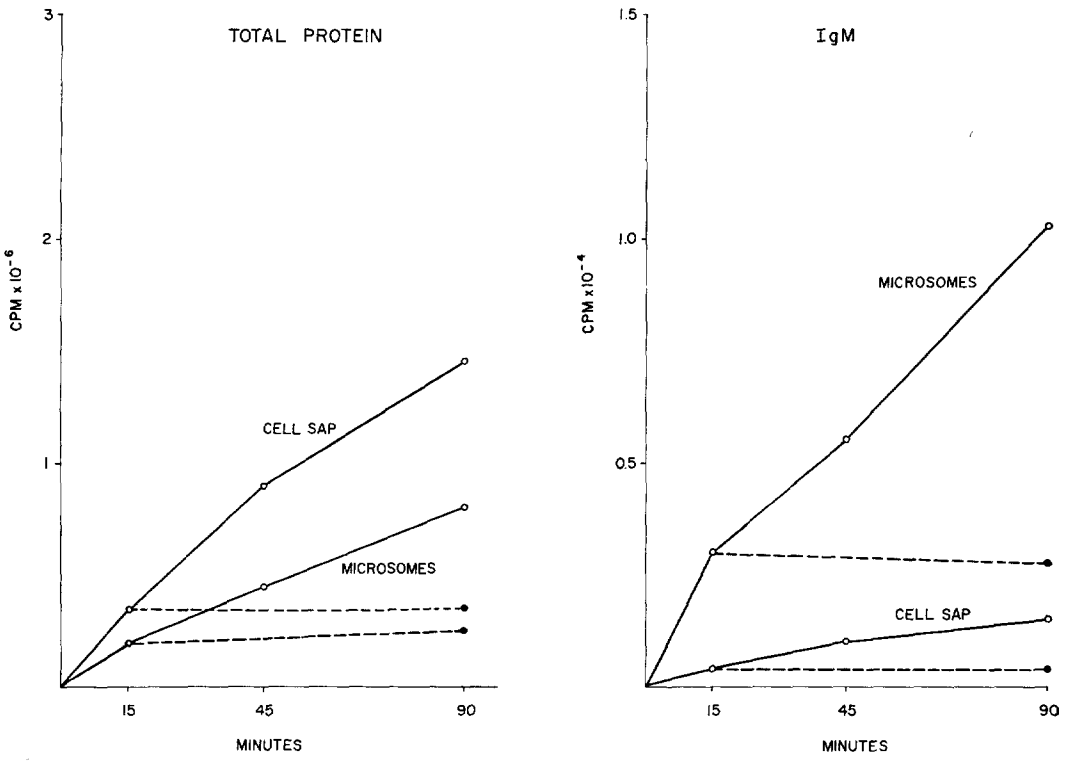

FIG. 4. Incorporation of ^{3}H-leucine into total protein and Ig in the microsomes and cell sap of Daudi cells. Cells were labeled for various times, microsomes and cell sap were separated, and radioactivity in acid precipitates and immunoprecipitates was determined. O——O continuous labeling, O----● 15 min labeling, 75 min chase.

gels. The 90-min samples (Fig. 5) show that more than half of the radioactivity recovered from these gels was contained in peaks having the mobility of heavy and light chains. This is consistent with the comparative values obtained from specific and control immunological precipitations (see Materials and Methods).

Data from this experiment and two additional ones are summarized in Table II. The results indicate that newly synthesized Ig accumulates within the microsomes for at least 90 min; very little Ig is found within the cell sap. The experiments also indicate that the amount of radioactive Ig remains constant

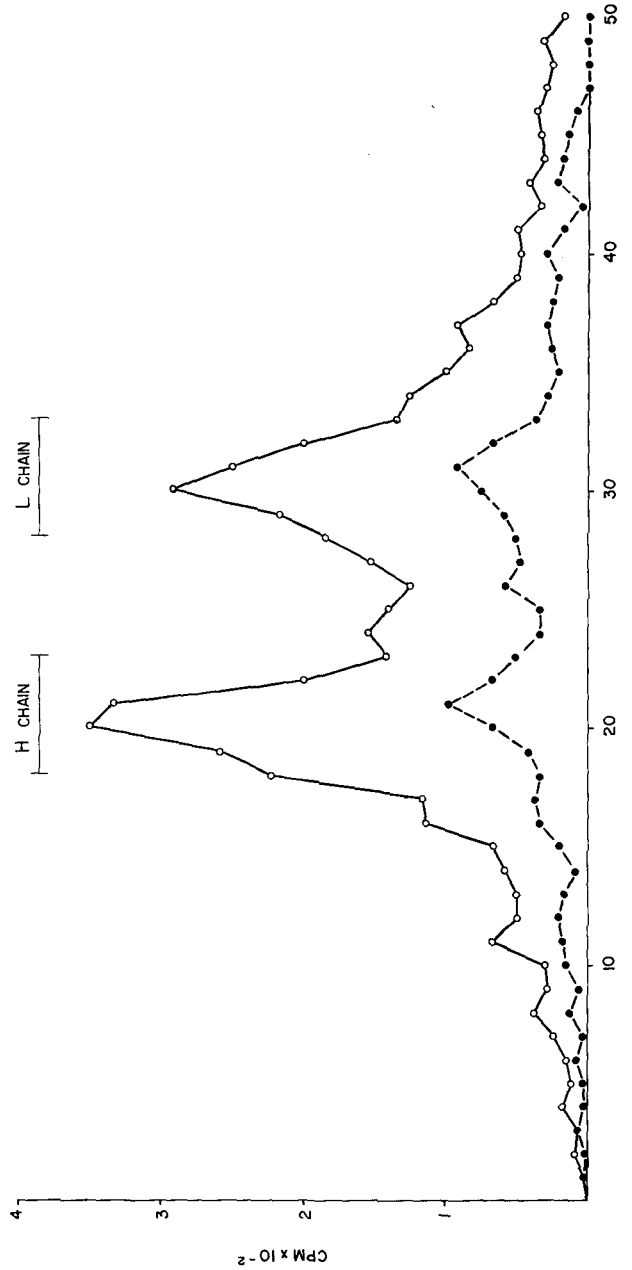

FIG. 5. Acrylamide gel electrophoresis of specific precipitates obtained by treating microsomal extracts and cell sap with anti-Ig. Daudi cells were labeled for 90 min with ^{3}H-leucine. Specific precipitates were reduced and alkylated before electrophoresis. O——O microsomes, ●----● cell sap.

during the chase; that is, there is no detectable degradation of Ig within the microsomes. Since the population of Daudi cells presumably maintains the average level of Ig per cell throughout many division cycles, either (*a*) Ig is degraded but the pool of intracellular Ig is sufficiently large so that degradation is not detected in 75 min of chase, or (*b*) Ig is not degraded, but the cell generation time equals the doubling time of intracellular Ig. There are reports of degradation of Ig chains in myeloma cells (33, 34).[2]

Incorporation of ^{3}H-Sugars into Ig in Microsomes and Cell Sap.—Electronmicroscopic (4, 5) and subcellular fractionation (12)[3] experiments have indicated that galactose and fucose addition occurs within the Golgi complex of

TABLE II

Percentage of Total Radioactivity in Subcellular Fractions Incorporated into Ig

Duration of labeling	Microsomal contents	Cell sap	Ratio: Microsomal contents / cell sap
(*min*)			
15	1.78 ± 0.61	0.165 ± 0.13	10.8
45	1.67 ± 0.96	0.214 ± 0.098	7.8
90	1.83 ± 1.3	0.143 ± 0.058	12.7
15 followed by 75 min chase	1.51 ± 0.62	0.162 ± 0.11	9.3

cells which secrete Ig. Preliminary results using electronmicroscopic radioautography have also suggested that galactose incorporation occurs principally within the Golgi complex of Daudi cells.[4] Thus, if Daudi cells could incorporate

[2] It has been suggested that molecules which enter the cell sap are synthesized on free polyribosomes while molecules which enter the microsomes are synthesized on bound polyribosomes (reviewed in references 1 and 2). Since electronmicrographs suggest that Daudi cells have few bound polyribosomes, we would expect that the proportion of newly formed protein found in the microsomal fraction would be small. Although more radioactive protein was found in the cell sap than in the microsomes regardless of the duration of labeling, as much as 30% of the radioactive protein was associated with the microsomes. Several possibilities should be considered for this unexpected finding: (*a*) Molecules synthesized on free polyribosomes enter into or become structural proteins of the microsomes. (*b*) Some molecules from the cell sap bind artifactually to microsomes during the cell fractionation. (*c*) Free polyribosomes are less active than bound polyribosomes in protein synthesis. The last possibility could be tested by determining the specific activities of free and bound polyribosomes (radioactivity in nascent chains per milligram of ribosomes). No such determination was possible in our experiments since liver cell ribosomes were used as carrier in the ribosomal fractionation procedure. However, the substantial percentage of radioactivity found in bound polyribosomes (Table I) suggests that this polyribosomal class may be more active than free polyribosomes in protein synthesis.

[3] Zagury, D., I. Schenkein, and J. W. Uhr. Manuscript in preparation.

[4] Zagury, D., and J. W. Uhr. Unpublished observations.

these sugars into Ig, this would suggest that Ig is transported to the Golgi complex in thse cells. We therefore repeated the previous experiments with ^{3}H-galactose or ^{3}H-fucose instead of ^{3}H-leucine.

Incorporation of ^{3}H-galactose into acid-precipitable molecules (Fig. 6) occurred entirely within the microsomes and presumably reflects the membranous location of the galactosyl transferase (35). Incorporation into Ig also

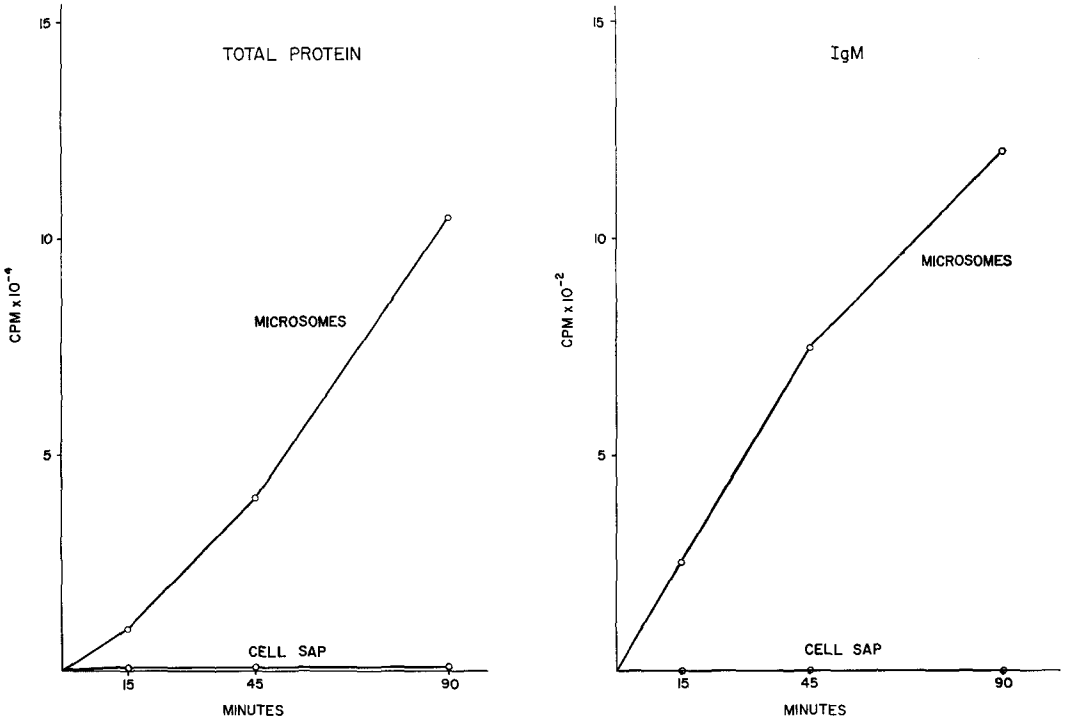

FIG. 6. Incorporation of ^{3}H-galactose into total protein and Ig in the microsomes and cell sap of Daudi cells. (See Fig. 4).

occurred entirely within the microsomes; no Ig was detected in the cell sap. This finding is consistent with galactose addition to Ig in the Golgi complex and with post-Golgi transport within vesicles.

Microsomal samples obtained after 90 min of labeling were specifically precipitated, reduced and alkylated, and electrophoresed on SDS-acrylamide gels. The results (Fig. 7) confirmed the specificity of immunoprecipitation and showed also that galactose is added to both heavy and light chains. The addition of carbohydrate to light chains produced by myeloma cells is well recognized (26, 36–38).

To prove that the ^{3}H-radioactivity was incorporated as galactose and not as a metabolically transformed product of the sugars, hydrolysates of specific precipitates obtained after 90 min of labeling were chromatographed on Dowex-50. No radioactive material bound to the resin indicating that deacetylated, ^{3}H-amino sugars were absent. Subsequent analysis by thin layer chromatography indicated that all of the radioactive material found in the Dowex-50 fractions cochromatographed with galactose but not with fucose, mannose, or *N*-acetyl-

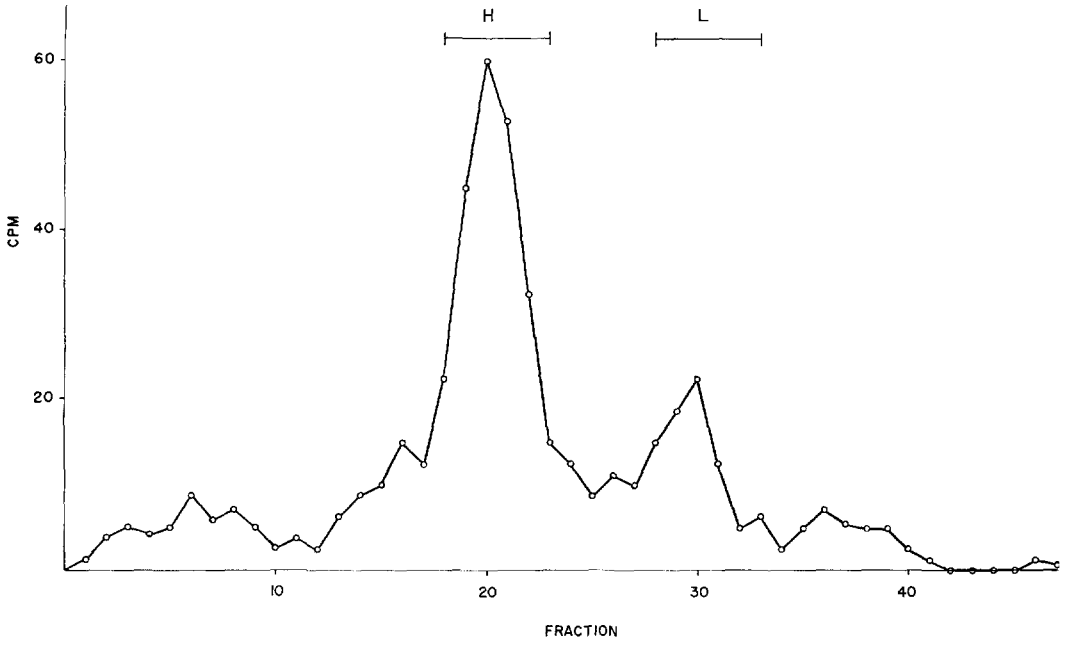

FIG. 7. Acrylamide gel electrophoresis of a specific precipitate of a microsomal extract of Daudi cells labeled for 90 min with ^{3}H-galactose. The specific precipitate was reduced and alkylated before electrophoresis.

glucosamine. More than 92% of the radioactivity in the initial acid hydrolysates was recovered as ^{3}H-galactose.

Further incorporation experiments performed with ^{3}H-fucose, a terminal sugar of the carbohydrate moiety of IgM (39), indicated that fucose is also incorporated into the heavy and light chains of the Ig synthesized by Daudi cells. These incorporation experiments with sugars suggest, therefore, that Ig synthesized by Daudi cells is transported to the Golgi complex.

The possibility that sugars play a role in secretion has been suggested by others (40), and it could be argued that the failure of Daudi cells to secrete Ig is caused by an inability to incorporate sugars. The above studies have shown that

Daudi cells incorporate both galactose and fucose into Ig. However, the possibility that the carbohydrate moiety is incomplete or abnormal cannot be excluded.

DISCUSSION

To elucidate subcellular events underlying Ig synthesis and secretion, we have previously studied mouse myeloma cells that have morphological characteristics of plasma cells and which secrete a homogeneous Ig. Several basic features emerged from such studies: (*a*) Light and heavy chains are synthesized on polyribosomes attached to the endoplasmic reticulum (ER) and rapidly enter the ER cisternae. Thus, segregation of Ig from nonsecretory proteins begins early in the intracellular life of the molecule (1–3, 7–12). (*b*) Ig is transported through the RER to the Golgi complex (4, 5, 12). The mechanism of post-Golgi transport has not been proven, but Ig is presumed to remain within vesicles until secretion (16). (*c*) Carbohydrate is added to the Ig molecule in the RER (2, 4, 12, 26, 36) and in the Golgi complex (4, 12, 36, and footnote 4) by membrane-bound glycosyl transferases (35).

The experiments reported here with the human Burkitt lymphoma line (Daudi) indicate that these mechanisms of Ig synthesis and intracellular transport are utilized in cells which have two characteristics strikingly different from plasma cells: a paucity of RER and a failure to secrete Ig. The experiments show that (*a*) Ig is synthesized by bound polyribosomes (indicating that the biosynthetic assay is more sensitive and informative than the morphologic), (*b*) Ig rapidly enters the microsomes, (*c*) Ig is presumably transported to the Golgi complex wherein galactose and fucose are added, and (*d*) Ig appears to remain associated with membranes throughout its intracellular life as judged by subcellular fractionation. These results, therefore, remove the impetus for suggesting novel mechanisms of synthesis and transport of Ig within lymphocytic cells and suggest that the above features may be general ones for cells synthesizing Ig.

At what point is intracellular transport of Ig terminated within Daudi cells? The biosynthetic studies described above imply that the block in intracellular transport does not occur at a pre-Golgi site. This conclusion together with the demonstration that Daudi cells can specifically bind fluorescein- (20, 21, 29, 30, 41), ferritin- (28), and peroxidase-labeled[4] anti-immunoglobulin to their cell surface strongly suggests that Ig molecules are transported to the plasma membrane but are not released from the cell.

The inability of Daudi cells to secrete Ig could result from a defect in the secretory mechanism or it might be a characteristic feature of a certain class of lymphoma cells. Ig synthesis by Burkitt lymphoma cells has been studied ex-

tensively. The results are summarized in Table III. Of the 27 lines examined, 10 secrete Ig as judged by the appearance of Ig in the culture medium, 5 lines synthesize but do not secrete Ig, 6 lines synthesize Ig but secretion has not been studied, and Ig synthesis has not been detected in 6 lines. Of the 21 lines which

TABLE III

Immunoglobulin Synthesis by Burkitt Lymphoma Cell Lines

Cell line	Ig synthesis and secretion	Type of Ig chains detected		References
		Cells	Culture fluid	
EB-1	Producers and	μ, κ	μ, κ, λ	42, 43, 49
EB-2	Secretors	γ, λ	γ, λ	42, 44, 49
EB-3		μ, λ	μ, λ	20, 29, 42, 44, 49
Ogun		μ	μ	41, 42, 44, 49
Jijoye		μ, κ	μ	20, 29, 41, 42, 49
AL-2		γ, κ	γ, κ	44, 49
AL-3		γ, λ	γ, λ, κ	43, 44
SL-1		μ	μ, γ, κ	41, 42, 45, 46, 49
OB-2		—	γ, κ	47
OB-6		—	α	47
Raji	Producers,	μ, κ	N.D.	41, 42, 43, 49
Kudi	Nonsecretors	μ	N.D.	41, 42, 49
AL-1		μ	N.D.	41, 44, 49
SS-1		γ, λ	N.D.	43, 49
Daudi		μ, κ	N.D.	20, 21, 29, 30, 41, 48
Gor	Producers,	λ	—	41
B35M	Secretors?	μ, γ, κ	—	41, 48
NK-9		μ, λ	—	20, 41
NK-15		μ, γ, κ	—	41
P-3J		μ, κ	—	45, 46
P-2K		μ	—	45
EB-4	Nonproducers	N.D.	—	20, 29
EB-5		N.D.	—	20, 29
NK-6		N.D.	—	20
NK-8		N.D.	—	20
NK-11		N.D.	—	20
OB-3		—	N.D.	47

N.D. = not detected; — = not determined.

synthesize Ig, 14 make μ chains. In evaluating these results, it should be noted that the kinetics of secretion were studied in only one report (47). The remaining incorporation studies usually involved incubation with radioactive precursors for 24–48 hr and then determination of radioactive Ig in the medium. It is possible, therefore, that in some of these studies, Ig was released into the culture medium by dead cells rather than through secretion. Thus, the substantial per-

centage of Burkitt lymphoma cells that synthesize Ig but are nonsecretory (5/15) may be an underestimate.

There is additional evidence to suggest that the absence of secretion may be a characteristic of some lymphomas. First, murine lymphomas induced experimentally are typically nonsecretory (50, 51), although intracellular Ig has been detected in some lines (51); in contrast, nonsecretory variants which arise from myeloma lines usually do not synthesize Ig (50–53). Second, proliferative disorders of lymphoid tissue are rarely associated with the appearance of homogeneous Ig in the serum or urine (54); however, the appearance of such proteins is considered pathognomonic for multiple myeloma (55).

These considerations and the high incidence of Ig-synthesizing, nonsecretory lymphoma lines suggest that these lines may have arisen from normal lymphocytes which do not secrete Ig. Klein *et al.* have previously suggested this possibility (20, 21) because of their findings that Ig could be detected on the surface of Daudi cells (20, 21, 29, 30). There are two major classes of such lymphocytes: (*a*) bone marrow-derived (B) cells which are the precursors of antibody-secreting plasma cells (56, 57), and (*b*) thymus-derived (T) cells that mediate delayed hypersensitivity and cooperate with B cells in the antibody response to certain antigens (58). Both T and B cells bind antigen (59, 60), and several classes of Ig have been detected on the surfaces of antigen-binding cells (59, 61–67). In particular, μ chains are reported to be the predominant heavy chain on lymphocytic, antigen-binding cells (62, 68) and the only heavy chain on T cells (69). Thus, Burkitt lymphoma lines which do not secrete Ig and which show a high incidence of μ chains (four of five) may be derived from either T or B cells.

Our previous studies with myeloma cells suggested a model for the synthesis and transport of Ig destined for secretion (16). The present studies with Daudi cells and the considerations discussed above suggest that this model can readily explain the genesis of the immunoglobulin which acts as antigen-specific receptor: (*a*) Ig is made on bound polyribosomes, enters the cisternae of the RER, and is transported to the Golgi complex; (*b*) Ig becomes bound by its Fc portion to the membrane of the Golgi vesicle; (*c*) a post-Golgi vesicle migrates to the plasma membrane and fuses with it; (*d*) by reverse pinocytosis, the Ig molecule becomes exteriorized with its Fab portions available for antigen binding and its Fc portion attached to the plasma membrane (formerly Golgi vesicle). This model is similar to the one proposed for secretory cells except that Ig is not released from the plasma membrane. The model can be tested by experiments designed to establish the intracellular life history of the Ig receptor and its relationship to the plasma membrane in normal lymphocytic cells.

SUMMARY

Cells from an established line of Burkitt's lymphoma (Daudi) and a mouse myeloma (P_3K) were pulse-labeled in vitro with ^3H-leucine, and immunoglobulin was immunologically precipitated from cell lysates and secretions. In con-

trast to P_3K cells, Daudi cells synthesize a small amount of Ig which is not secreted. Subcellular fractionation experiments indicated that Ig of Daudi cells is synthesized on membrane-bound polyribosomes and enters the cisternae of the microsomes. Ig in the microsomes could be labeled with either ^{3}H-galactose or ^{3}H-fucose suggesting that transport proceeds to the Golgi complex. Additional evidence indicates that Ig molecules are transported to the plasma membrane but are not cleaved from the cell surface. These results together with other studies of Burkitt lymphoma cells suggest that the Daudi line may represent a clone of neoplastic cells derived from normal lymphocytes which synthesize but do not secrete Ig. Similarities between lymphoma cells and antigen-binding cells are discussed.

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