CELL SURFACE IMMUNOGLOBULIN

III. Isolation and Characterization of Immunoglobulin from Nonsecretory Human Lymphoid Cells*

By CHARLES J. SHERR,‡ SONIA BAUR, INGE GRUNDKE, JOSEPH ZELIGS,‡ BARBARA ZELIGS, and JONATHAN W. UHR

(*From the Irvington House Institute and the Department of Medicine, New York University School of Medicine, New York 10016*)

(Received for publication 26 November 1971)

An established line of human lymphoma cells (Daudi) has been shown to synthesize a small amount of IgM which is not secreted from the cell (1–4). Both IgM (1–7) and a binding site for complement (C) (8) are present on the cell surface. These findings suggest that Daudi cells are neoplastic analogues of bone marrow–derived (B) lymphocytes and that the IgM on their surface is the counterpart of the antigen-specific receptor.

In the present study, we have attempted to characterize this surface IgM using a previously reported method for radioiodinating the surface of viable cells (9, 10). Our results indicate that there is an average of 10^5 molecules of monomeric IgM on the surface of each cell; in contrast, only "free" μ- and L chains are found intracellularly.

Materials and Methods

Cells and Labeling.—Daudi, an established line of human Burkitt lymphoma cells synthesizing IgM, was kindly provided by Dr. Eva Klein (Karolinska Institutet, Stockholm). Cells were maintained in suspension culture in Dulbecco's medium with 10% fetal calf serum and 1% antibiotic-antimycotic (all Grand Island Biological Company, Grand Island, N. Y.).

Enzymatic radioiodination of the surfaces of cells was performed by the method of Phillips and Morrison (9) as applied to lymphoid cells by Baur et al. (10). Lactoperoxidase was purified by the method of Morrison and Hultquist (12) or was purchased from Sigma Chemical Co., St. Louis, Mo. For labeling of cells with L-leucine-^{3}H (50 Ci/mmole) or L-tyrosine-^{3}H (25 Ci/mmole; both New England Nuclear Corporation, Boston, Mass.), cells were collected by centrifugation at 1000 *g* for 10 min and suspended at 5×10^5/ml in 100 ml of growth medium.

* This work was done under the sponsorship of the Commission on Immunization of the Armed Forces Epidemiological Board and was supported in part by the US Army Medical Research Development Command, Department of the Army, under research contract DADA 17-69-C 9177, in part by US Public Health Service grant No. AI-0834, and by the National Science Foundation grant No. GB-7473-X.

‡ Recipient of Medical Scientist Fellowship from US Public Health Service (grant 5TO-5-GMO-1668-08).

Cultures were labeled for 24 hr with 0.5 mCi of either isotope and cells were washed twice with 0.15 M sodium chloride–0.05 M phosphate buffer, pH 7.1 (PBS).[1]

Washed cells were resuspended in 2 ml of PBS containing 0.50 molar recrystallized iodoacetamide (pH 7.1). Cells were lysed by adding Nonidet P-40 (NP-40, Shell Chemical Corp., New York) to a final concentration of 0.5%, and nuclei were removed by sedimentation at 2000 *g* for 20 min. Lysates were then dialyzed against 500 volumes of PBS at 4°C. In some experiments, lysates similarly prepared from unlabeled cells were radioiodinated and again dialyzed against PBS to remove unincorporated ^{125}I.

Immunoprecipitation and Electrophoresis.—Precipitation of cell lysates was performed by a "sandwich" technique using 0.03 ml of rabbit antiserum to human IgM (30 min at 37°C) and excess goat antiserum to rabbit Ig (30 min at 37°C; 2 hr at 4°C). As a control for nonspecific precipitation, 0.03 ml of normal rabbit serum was substituted for rabbit anti-human IgM in the first step. Specific precipitates were washed six times with PBS and dissolved at 37°C in 8 M urea–1% sodium dodecyl sulfate (SDS)–0.05 M tris(hydroxymethyl)aminomethane (Tris) buffer, pH 8.4 (20°C). For reduction and alkylation, 2-mercaptoethanol was added to a final concentration of 0.2 M and incubation was continued at 37°C for 30 min. A 0.5 M solution of iodoacetamide adjusted with solid Tris to pH 8.4 was added to a final concentration of 0.25 M, and incubation was continued for another 30 min.

Dissolved specific precipitates were dialyzed overnight against 1% SDS–8 M urea–0.01M phosphate buffer, pH 7.0, and were electrophoresed on 5% acrylamide gels (13) for 3½ hr at 15 ma per tube. Urea was absent from the running buffer but was included in the gels. In some experiments, dissolved specific precipitates were dialyzed overnight against 2% SDS–0.01 M phosphate buffer, pH 7.0, and were electrophoresed on agarose–2.5% acrylamide gels using a modified method of Dingman and Peacock (14). 2% SDS was included in both the running buffer and gels, and electrophoresis was for 90 min at 7.5 ma per tube. In both electrophoretic systems, 19S IgM, IgG, and reduced and alkylated IgG were used as "markers" in a companion gel. Gels were fractionated in the Savant gel extruder (Savant Instruments, Hicksville, N.Y.) and were counted in a Beckman LS-250 liquid scintillation counter (Beckman Instruments Inc., Fullerton, Calif.) (^{3}H) using 5 g of 2,5-diphenyloxazole and 100 g of naphthalene/liter dioxane or in a Packard Model 3001 crystal scintillation counter (Packard Instrument Co., Downers Grove, Ill.) (^{125}I). Counting efficiencies were approximately 30% and all determinations were made to the ±5% error.

Subcellular Fractionation.—2×10^7 Daudi cells labeled with ^{125}I were mixed with 2×10^9 unlabeled cells and were suspended in 50 ml 0.1 M Tris buffer, pH 7.4, containing 1 mmolar $MgCl_2$ (TMg). All steps were carried out at 4°C. Cells were broken with 20 strokes of a tight-fitting Dounce homogenizer and nuclei were sedimented at 1500 *g* for 20 min. The postnuclear supernatant was sedimented at 12,000 g_{max} for 10 min and the pellet (mitochondrial fraction) was saved. The postmitochondrial supernatant was then respun at 100,000 g_{max} for 90 min and both the pellet (microsomes) and postmicrosomal supernatant were stored at 4°C.

Nuclear and mitochondrial fractions were suspended together in TMg buffer with a loose Dounce homogenizer and 70% sucrose (w/w)–TMg was added to give a final concentration of 55% sucrose. 8-ml portions of the suspension were loaded into polyallomer tubes (SW-25.1, Spinco model L [Beckman Instruments Inc.]) and were overlayed with 8 ml each of 45, 35, and 25% sucrose–TMg solutions. Tubes were centrifuged for 20 hr at 24,000 rpm and at the completion of the run, gradients were sampled with a Pasteur pipette. Two major bands and a pellet were obtained. The top band (crude plasma membrane, P_{av} 1.17) and the bottom band (mixed

[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; NP-40, Nonidet P-40 (Shell Chemical Corp.); PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; TMg, Tris buffer containing $MgCl_2$.

fraction, P_{av} 1.24) were diluted with 3 volumes of TMg and were resedimented at 75,000 g_{max} for 3 hr. All pellets were either taken for electron microscope examination or were resuspended in 5–10 ml of buffer for chemical determinations. Protein was determined by the method of Lowry et al. (15), RNA by the method of Fleck and Munro (16), 5′ nucleotidase by the method of Touster et al. (17), and inorganic phosphate by the method of Lowry and Lopez (18). For determination of incorporated ^{125}I, fractions were dialyzed overnight against TMg and portions were precipitated with 5% trichloroacetic acid (TCA), collected on Millipore filters (type HA, white; Millipore Corp., Bedford, Mass.), washed twice with 5% TCA, and counted in the Packard crystal scintillation counter.

Radioiodination of Soluble Proteins.—Purified human IgM was obtained from Melpar Laboratories, Falls Church, Va., or was a gift of Dr. E. C. Franklin, New York University School of Medicine, and Dr. H. G. Kunkel, Rockefeller University. Mouse IgM was prepared from secretions of myeloma MOPC-104E (11). Mouse IgG and bovine serum albumin (BSA) were obtained from Pentex Biochemical, Kankakee, Ill. Proteins were iodinated in solution using the same conditions employed for cells. Iodoacetamide was added to a final concentration of 0.5 M to solutions containing IgM; NP-40 was then added to a final concentration of 0.5%. Solutions were dialyzed, precipitated with antiserum against IgM, dissolved, and electrophoresed on acrylamide gels as above.

Electron Microscopy.—Pellets to be processed for electron microscopy were fixed for 1 hr in 2% osmium tetroxide buffered to pH 7.4 with 0.1 M sodium phosphate (19). Fixed, undisrupted pellets were impregnated with 4% agar (20) to facilitate their orientation for complete cross sectioning. Impregnated pellets were dehydrated with ethanol and propylene oxide, embedded in Epon 812 (21), thin sectioned, counterstained with uranyl acetate and lead citrate (22–24), and examined with a Zeiss EM9a electron microscope (Carl Zeiss, New York).

RESULTS

Isolation of μ- and L Chains from the Cell Surface.—To study the nature of the Ig bound to the surface of Daudi cells, cells were enzymatically radiolabeled with ^{125}I. Previous experiments have shown that only molecules on the cell surface are radioiodinated by this method (10, 11, 25) thereby permitting labeled cell surface Ig to be recovered and studied independently of unlabeled intracellular Ig molecules. After completion of the reaction, labeled cells were washed, lysed with detergent, and after sedimentation of nuclei, iodinated molecules derived from the cell surface were specifically precipitated with antiserum directed against Ig. As a control for nonspecific precipitation, normal rabbit serum was substituted for rabbit anti-Ig in the sandwich precipitation. Immune precipitates were then dissolved, reduced and alkylated, and subjected to electrophoresis on SDS–5% acrylamide gels.

As can be seen in Fig. 1, two peaks, one with μ- and one with L chain mobility, were identified by electrophoresis on acrylamide gels. No peaks were observed as a result of the control immunoprecipitation. The results indicate that Ig chains can be isolated from the cell surface of Daudi cells.

Subcellular Fractionation of Surface-Labeled Cells.—If only the cell surface is radiolabeled, subcellular fractionation of labeled cells should reveal concentration of radioactivity in fractions rich in plasma membrane and no radioactivity in fractions free of plasma membrane such as the cell cytoplasm. Accordingly, surface-labeled cells were fractionated and the fractions were examined by elec-

tron microscopy and evaluated for their content of protein, RNA, 5′ nucleotidase, and acid-precipitable ^{125}I.

As shown in Table I, a crude plasma membrane fraction contained the highest specific activity of 5′ nucleotidase (an enzyme generally associated with plasma membranes of other cells [26]) and also of ^{125}I-labeled proteins. Significant activities of these markers, however, also appeared in other membrane fractions suggesting that they were contaminated with plasma membrane or with unbroken cells. In contrast, soluble proteins in the cell sap (postmicrosomal supernatant) although representing the majority of total cellular proteins contained almost no radioactive proteins. This finding provides further evidence that

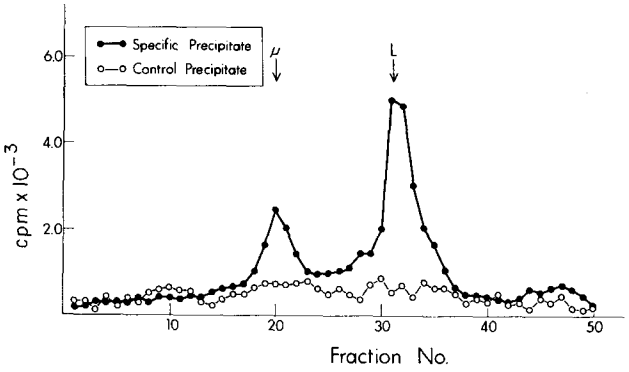

FIG. 1. Electrophoresis of anti-Ig (specific) and control precipitates obtained from surface-labeled cells on SDS–5% acrylamide gels. The precipitates were reduced and alkylated before electrophoresis. The positions of μ- and L chains electrophoresed on companion gels are noted.

intracellular proteins are not iodinated. The results also suggest that surface radioiodination may be useful in procedures used for the purification of plasma membrane.

Additional experiments were performed to show that ^{125}I-labeled Ig could be recovered from the plasma membrane fraction. This fraction was solubilized with detergents, dialyzed, and precipitated with antisera directed against Ig. SDS–5% acrylamide gel electrophoresis of reduced and alkylated precipitates showed an analogous result to that illustrated in Fig. 1.

Molecular Weight of Cell Surface Ig Molecules.—This was studied by radioiodinating cells and lysing them into PBS containing iodoacetamide which was included to alkylate free sulfhydril groups and thereby inhibit self-assembly of Ig chains (27). After sedimentation of nuclei, Ig from the lysate was specifically precipitated, dissolved, and electrophoresed without reduction and alkylation on SDS–agarose–2.5% acrylamide gels. Previous experiments had established that such gels would include molecules of molecular weights up to approximately

10^7 daltons and that the electrophoretic mobility was a function of the log of the molecular weight.

The results of a representative experiment are shown in Fig. 2 *a*. A single radioactive peak with an electrophoretic mobility slightly less than IgG was detected suggesting that monomeric IgM is present on the cell surface of Daudi cells.

Because agarose–acrylamide gels do not permit very precise estimation of molecular weights due to their large pore size, unreduced specific precipitates

TABLE I

Subcellular Fractionation of Daudi Cells after Surface Radiolabeling

Fraction	Morphology	Per cent of total				Relative specific activity*		
		Protein	RNA	^{125}I	5′NTDase‡	RNA	^{125}I	5′NTDase
Crude plasma membrane	Predominantly smooth surfaced vesicles; some mitochondria, dense bodies, lysosomes	10.3	7.2	37.2	49.2	0.7	3.6	4.8
"Mixed" fraction	Unbroken cells; many mitochondria, dense bodies, lysosomes; occasional nuclei	8.0	11.0	26.1	21.9	1.4	3.3	2.7
Nuclei	Many nuclei; some unbroken cells and smooth surfaced vesicles	15.7	35.6	31.2	10.0	2.3	2.0	0.6
Microsomes	Smooth surfaced vesicles; some RER; few mitochondria, lysosomes and dense bodies; many "free" ribosomes	3.8	16.0	4.5	10.0	4.2	1.2	2.6
Postmicrosomal supernatant	—	61.4	30.8	1.8	4.0	0.5	<0.1	<0.1
Total		99.2	100.6	100.8	95.1			

* Per cent of activity/per cent of protein in fraction.

‡ 5′ nucleotidase.

were also electrophoresed on SDS–5% acrylamide gels. Previous experiments indicated that the resolution of such precipitates was improved by incorporating 8 M urea in the gels. The results of this experiment (Fig. 2 *b*) showed that one radioactive peak with the mobility of IgM monomer (11) was found as expected. No radioactivity was excluded from the gels confirming the absence of IgM polymer.[2] These results are in agreement with those obtained with agarose–2.5% acrylamide gels cited above.

The possibility was considered that the monomeric form of IgM results from

[2] When 0.1 M iodoacetamide was used during cell lysis, some radioactivity was found in the region of gels corresponding to 19S IgM and smaller polymers. Since these molecular species were absent when 0.5 M iodoacetamide was used, we interpret these results as indicating that self-assembly and/or disulfide exchange had occurred with lower concentrations of this reagent.

depolymerization of polymeric IgM due to the conditions of iodination and/or extraction. This possibility was investigated by iodination of human IgM from three sources and mouse IgM ("secretions" of myeloma MOPC-104E). Analysis on gels as above showed no evidence of depolymerization.

Molecular Weight of Total Cellular Ig Molecules.—To determine the molecular forms of total cellular Ig (i. e. intracellular Ig and cell surface Ig), cells were

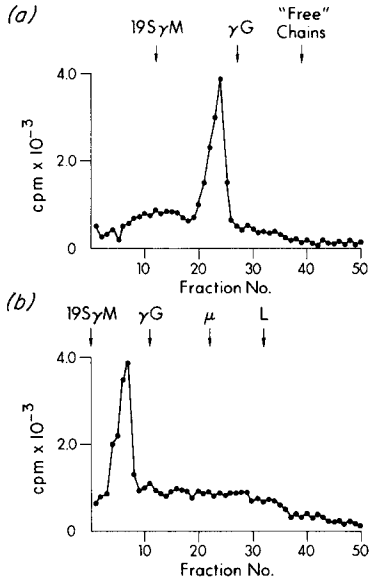

FIG. 2. Electrophoresis on SDS-agarose-2.5% acrylamide (*a*) and SDS-5% acrylamide (*b*) gels of specific precipitates obtained from surface-labeled cells. The positions of several Ig "markers" of different molecular weights are noted.

lysed into buffer containing iodoacetamide, nuclei were sedimented, and the dialyzed lysate was then radioiodinated using lactoperoxidase. Radiolabeled Ig was specifically precipitated and electrophoresed on agarose–2.5% acrylamide gels (Fig. 3 *a*) or SDS–5% acrylamide gels containing 8 M urea (Fig. 3 *b*). One major peak was detected on agarose–acrylamide gels in the region of free μ- and L chains. As expected, two major peaks were detected on SDS–5% acrylamide gels, one with μ- and one with L chain mobility. In addition, a minor peak was found in the region of IgM monomer. The results indicate that the vast majority of intracellular μ- and L chains in Daudi cells is not covalently assembled. A small percentage of the chains is assembled into monomeric IgM and is most likely derived from the cell surface (see previous section).

To confirm this result, growing cell cultures were labeled for 24 hr (approximately equal to one "doubling-time") with leucine-^{3}H. Cells were lysed, nuclei were sedimented, and Ig was precipitated from the cell lysates. Electrophoresis

of such Ig on SDS–5% acrylamide gels containing 8 M urea showed an analogous result to that illustrated in Fig. 3 *b*.

These experiments demonstrate several important points: (*a*) Since the extent of assembly of Ig molecules is different for cell surface and total Ig, these experiments provide additional evidence that intracellular Ig is not labeled when whole cells are subjected to radioiodination. Furthermore, the results argue against self-assembly of Ig chains. (*b*) Since the percentage of total Ig which is assembled is small, and since most Ig on the cell surface is assembled,

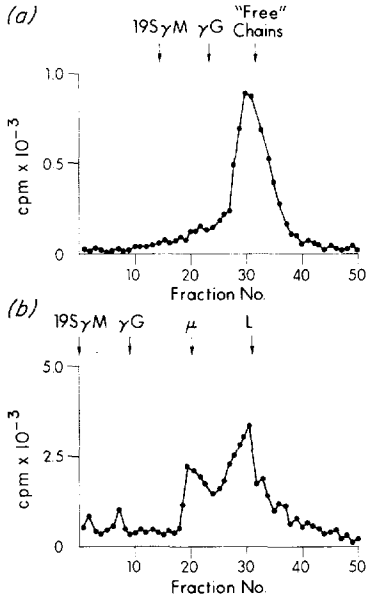

FIG. 3. Electrophoresis on SDS–agarose–2.5% acrylamide (*a*) and SDS–5% acrylamide (*b*) gels of specific precipitates obtained from ^{125}I-labeled cell lysates. See Fig. 2.

the percentage of total Ig which is on the cell surface must be small. These results also suggest that covalent assembly of Ig occurs at or near the time that Ig reaches the cell surface. (*c*) Since the μ/L chain ratio of ^{125}I radioactivity is similar for chains derived from the cell surface (Fig. 1) and those derived from iodinated lysates (Fig. 3 *b*), it is likely that attachment of Ig to the cell membrane does not prevent portions of the Ig chains from being iodinated by the lactoperoxidase. This result agrees with the results of previous experiments with normal spleen cells (11).

Iodination of μ- Versus L Chains.—Electrophoresis of radioiodinated specific precipitates indicates that more radioactivity is found in the L chain peak than in the μ-chain peak (Figs. 1, 3 *b*). Since lactoperoxidase iodinates tyrosine residues of proteins primarily (28), the distribution of tyrosine residues between μ- and L chain was investigated. Cells were labeled for 48 hr with tyrosine-^{3}H, and reduced and alkylated specific precipitates prepared from the cell lysates were electrophoresed on SDS–5% acrylamide gels. The results (Fig. 4) show that

more ^{3}H-label is found in μ-chains than in L chains. Since the ^{3}H-label reflects the relative tyrosine content of μ- and L chains, the results suggest that lactoperoxidase preferentially iodinates human L chains as compared with human μ-chains. Thus, the μ/L ratio of ^{125}I radioactivity may not reflect the molar ratio of these chains in Daudi cells. These results are in contrast to those obtained with mouse IgM in which the μ/L chain ratio of radioactivity was similar for tyrosine-^{3}H and ^{125}I-labeled chains (11).

Quantitation of Cell Surface Ig Molecules.—Experiments designed to estimate the average number of Ig molecules on the cell surface of Daudi cells are out-

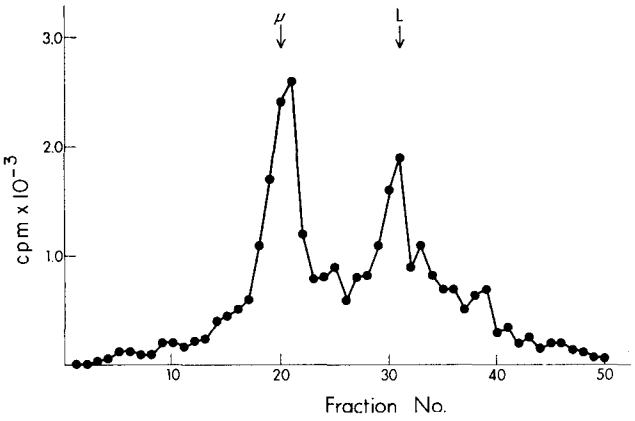

FIG. 4. Electrophoresis on SDS–5% acrylamide gel of a specific precipitate obtained from a lysate of cells that had been labeled with tyrosine-^{3}H. The specific precipitate was reduced and alkylated before electrophoresis. See Fig. 1.

lined below: (*a*) Protein in cell lysates was estimated to be 3.65 ± 1.83 mg/5 × 10^{7} cells. (*b*) A cell lysate was radioiodinated and portions were precipitated with TCA or with antiserum against Ig. The per cent of the total acid-precitable radioactivity which was precipitated as Ig was 2.7 ± 1.3%. (*c*) A known number of cells was radioiodinated and Ig from the cell surface was obtained by immune precipitation. A cell lysate prepared from the same number of cells was similarly iodinated and precipitated with specific anti-Ig serum. The radioactivity in cell surface versus total Ig was calculated to be 1.7 ± 0.9%.[3] (*d*) The total number of monomeric IgM equivalents was calculated from the following equation:

[3] The validity of this calculation relies on the assumption that Ig on the surface of intact cells can be radioiodinated with equal efficiency to Ig in lysates prepared with detergent. There is no definitive method of testing this assumption. It was possible to determine whether the detergent NP-40 affected the efficiency of radioiodination. Thus, several proteins (IgM, BSA) were treated with NP-40, dialyzed against PBS, and radioiodinated. The same proteins were radioiodinated without prior exposure to detergent. It was shown that detergent pretreatment followed by dialysis did not affect the efficiency of radioiodination.

$$\left(\frac{\text{Protein}}{\text{cells}}\right)\left(\begin{matrix}\%\text{ of total}\\ \text{protein}\\ \text{which is Ig}\end{matrix}\right)\left(\begin{matrix}\%\text{ of Ig which}\\ \text{is on the}\\ \text{surface}\end{matrix}\right)\left(\frac{\text{mole IgM}}{\text{monomer}}\right)\cdot\left(\begin{matrix}\text{Avogadro's}\\ \text{No.}\end{matrix}\right) = \text{No. of cell surface IgM monomer equivalents/cell}$$

$$\left(\frac{3.65 \times 10^{-3}\text{ g}}{5 \times 10^{7}\text{ cells}}\right)\left(0.027\right)\left(0.017\right)\left(\frac{1\text{ mole}}{180{,}000\text{ g}^{4}}\right)\left(\frac{6.23 \times 10^{23}}{\text{mole}}\right)$$

$$= 116{,}000\text{ molecules/cell}$$

DISCUSSION

Immunoglobulin on the cell surface of Daudi cells has been isolated, characterized, and quantified using a method of enzymatic radioiodination of viable cells (10). Subcellular fractionation of radiolabeled cells confirms our previous findings that only the cell surface is labeled since radioactivity was not found in the cell cytoplasm but was concentrated in fractions rich in plasma membrane. Radioactive Ig is found exclusively as monomeric (8S) IgM,[5] and there is an average of 10^5 Ig molecules per cell in general agreement with determinations of others using Daudi cells (30) leukemic lymphocytes (30, 31), a diploid lymphocyte line (32), and normal lymphocytes (33).

IgM recovered from the cell surface is not an artifact of self-assembly since iodoacetamide included in the lysis buffer would alkylate free sulfhydril groups and prohibit disulfide bond formation or exchange (27). In addition, the results of labeling of total cellular Ig which indicated that the majority of radioactivity was in the form of free μ- and L chains argue strongly against such self-assembly. Conversely, degradation of assembled IgM molecules under the conditions of iodination and extraction employed was not supported by control experiments performed with both human and mouse 19S IgM.

Since Ig molecules could be released from the cell surface by detergent lysis under nonreducing conditions and could be completely converted to free chains after reduction of specific precipitates, attachment of Ig to the cell surface presumably occurs via noncovalent interactions. It is also of interest that the ratio of μ/L chain radioactivity in surface Ig is similar to that of total cellular Ig radiolabeled in solution. If attachment of Ig to the cell surface is mediated by the Fc portion of the molecule, then our data argue against a model in which a large portion of the Fc fragment is buried within the plasma membrane. Similar conclusions were reached from studies of μ/L radioactivity

[4] Estimated by SDS–acrylamide gel electrophoresis (11).

[5] The sedimentation coefficient indicated above is the generally accepted value for monomeric IgM (29).

of surface Ig from mouse splenic lymphocytes (11) and from Fab/Fc radioactivity of surface IgG from a murine myeloma line (25).

In contrast to the results with cell surface Ig, when total cellular proteins in cell lysates were radioiodinated, Ig recovered by specific precipitation was found to be predominantly in the form of free μ- and L chains. This result was confirmed by labeling growing cultures with leucine-^{3}H. After labeling for 24 hr (approximately equal to one doubling-time), almost all newly synthesized Ig was recovered as free chains. These experiments indicate that only a small percentage of the total Ig of Daudi cells is bound to the cell surface and, in addition, suggest that covalent assembly of Ig occurs at or near the time that the molecule becomes part of the cell membrane.

The finding that most Ig in Daudi cells is in the form of free μ- and L chains is unexpected. In cells secreting Ig, covalent assembly of H and L chains proceeds rapidly. Thus, formation of interchain disulfide bonds can begin on polyribosomes (34–36), and assembly of the tetramer is virtually complete within 60 min after synthesis of Ig chains (27). Since the methods we have employed rely on the use of denaturants, only covalent assembly of Ig chains is detected. Therefore, it is possible that in Daudi cells, free μ- and L chains are assembled through noncovalent interactions.

The above considerations and our previously published data on biosynthesis of Ig by Daudi cells (3, 4) raise questions concerning the relationship of cell surface Ig to Ig in other cellular compartments. Biosynthetic data suggest that Ig is synthesized on membrane-bound polyribosomes, enters the cisternae of the rough endoplasmic reticulum, is transported to the Golgi complex and, presumably, in post-Golgi vesicles to the plasma membrane. Since neither secretion nor degradation of Ig has been detected, this concept in its simplest form implies that all Ig molecules eventually appear on the cell surface after an ordered sequence of transport steps.

Since most cell surface Ig is covalently assembled in contrast to intracellular Ig, it should be possible to estimate the rate of transport of Ig to the plasma membrane by studying the kinetics of Ig assembly. Preliminary results of "pulse-chase" experiments with leucine-^{3}H indicate that a large portion of intracellular Ig is in the form of free μ- and L chains many days after their synthesis. These experiments suggest that if "ordered transport" occurs, then transport of Ig to the plasma membrane is extremely slow.[6] In contrast, Ig destined to be secreted by plasma cells usually completes its intracellular transport within 1–2 hr of its synthesis (37, 38).

Another major possibility is that cell surface Ig may be synthesized in a

[6] This conclusion is also suggested by indirect evidence: If the average amount of Ig per cell remains constant throughout many generation cycles, then the doubling time of Ig molecules corresponds to the generation time of the cells. Our present data indicate that the amount of intracellular Ig is considerably greater than that of cell surface Ig. These considerations imply that if ordered transport occurs, transport to the cell surface would be slow.

separate compartment from the majority of intracellular Ig (e.g., in the rough endoplasmic reticulum adjacent to the plasma membrane).[7] Only a small percentage of the intracellular Ig would reach the cell surface. This portion may be transported rapidly but would not be detected in pulse-chase experiments due to the high background of total Ig synthesis. Such a concept proposes that the synthesis of cell surface and intracellular Ig might be independently regulated by the cell. Lerner et al. have previously suggested this possibility based on the different half-lives of cell surface Ig as compared with total Ig in a secretory human cell line (32). We have no data at the present time to exclude either of these possible models for synthesis of cell surface Ig.

It has recently been demonstrated that murine small splenic lymphocytes possess predominantly monomeric IgM on their cell surface (11). The cells bearing Ig are the bone marrow–derived (B) lymphocytes (8).[8] Similarities between cell surface Ig on normal splenic lymphocytes and on Daudi cells suggest that the latter is a neoplasm of the B cell type.

SUMMARY

Cells from an established line of Burkitt lymphoma (Daudi) were enzymatically radioiodinated, and labeled Ig from the cell surface was isolated and studied. Subcellular fractionation of labeled cells confirmed that intracellular proteins from the cytoplasm are not iodinated by this method. Radioactive Ig was identified as monomeric (8S) IgM, and an average of 10^5 Ig molecules was found per cell. Ig molecules could be released from the plasma membrane by detergent lysis under nonreducing conditions indicating that attachment of Ig to the plasma membrane occurs via noncovalent interactions. The ratio of μ/L radioactivity in surface Ig was the same as that of total cellular Ig radioiodinated in solution suggesting that a large portion of the Fc fragment is not buried within the membrane. In contrast to the results obtained with cell surface Ig, most intracellular Ig was found as "free" μ- and L chains regardless of whether lysates were labeled with ^{125}I or cells were labeled with leucine-^{3}H. The results indicate that only a small percentage of the total Ig of Daudi cells is associated with the cell surface and suggest that covalent assembly of Ig occurs at or near the time that the molecule becomes part of the plasma membrane. Similarities between cell surface Ig on normal splenic lymphocytes and Daudi cells suggest that the latter is a neoplasm of bone marrow–derived lymphocytes.

[7] Electron microscope studies of tonsillar lymphocytes which form rosettes in the presence of hybrid antibody (anti-Ig; anti-erythrocytes) have demonstrated the close proximity of rough endoplasmic reticulum and plasma membrane in such cells (D. Zucker-Franklin, personal communication).

[8] Vitetta, E. S., C. Bianco, V. Nussenzweig, and J. W. Uhr. 1972. Cell surface immunoglobulin. IV. Distribution among thymocytes, bone marrow cells and their derived populations. *J. Exp. Med.* In press.

Note Added in Proof.—After submission of this paper, Eskeland and Klein reported that mu and kappa "structures" were liberated by homogenization or by freezing and thawing from the surface of Daudi cells and could be detected by inhibition of passive hemagglutination (Eskeland, T., and E. Klein. 1971. Isolation of 7S IgM and kappa chains from the surface membrane of tissue culture cells derived from a Burkitt lymphoma. *J. Immunol.* **107**:1368). From these studies, it was concluded that both 7S IgM and free kappa chains were present on the surface of Daudi cells and that there was little, if any, intracellular Ig. Their results with regard to 7S IgM are in complete agreement with ours. However, our results differ in that (*a*) there is a substantial amount of intracellular Ig which exists as free chains, and (*b*) there are no free chains on the cell surface. The discrepancies between these results probably depend on the inability to rupture microsomes by freezing and thawing or by homogenization. We have previously demonstrated (3, 4) that intracellular Ig is membrane bound and can be solubilized with detergents but not by Dounce homogenization.

We would like to thank Dr. Isaac Schenkein for preparing the lactoperoxidase used in some of these studies. We gratefully acknowledge the excellent technical assistance of Mr. W. Dolan, Mr. Y. Chinn, and Mrs. M. Blanco.

REFERENCES

1. Klein, E., G. Klein, J. S. Nadkarni, J. J. Nadkarni, H. Wigzell, and P. Clifford. 1968. Surface IgM-kappa specificity in a Burkitt lymphoma cell *in vivo* and in derived culture lines. *Cancer Res.* **28**:1300.
2. Nadkarni, J. S., J. J. Nadkarni, P. Clifford, G. Manolov, E. M. Fengo, and E. Klein. 1969. Characteristics of new cell lines derived from Burkitt lymphomas. *Cancer.* **23**:64.
3. Sherr, C. J., and J. W. Uhr. 1971. Immunoglobulin synthesis and secretion. VI. Synthesis and intracellular transport of immunoglobulin in nonsecretory lymphoma cells. *J. Exp. Med.* **133**:901.
4. Sherr, C. J., I. Schenkein, and J. W. Uhr. 1971. Synthesis and intracellular transport of immunoglobulin in secretory and nonsecretory cells. *Ann. N. Y. Acad. Sci.* **190**:250.
5. Hammond, E. 1970. Ultrastructural characteristics of surface IgM reactive malignant lymphoid cells. *Exp. Cell Res.* **59**:359.
6. Klein, E., J. S. Nadkarni, G. Klein, J. J. Nadkarni, H. Wigzell, and P. Clifford. 1967. Surface IgM specificity in cells derived from a Burkitt's lymphoma. *Lancet.* **2**:1068.
7. Takahashi, M., Y. Yagi, G. E. Moore, and D. Pressman. 1969. Pattern of immunoglobulin production in individual cells of human hematopoietic origin in established culture. *J. Immunol.* **102**:1274.
8. Nussenzweig, V., C. Bianco, P. Dukor, and A. Eden. 1971. Receptors for C3 on B lymphocytes: possible role in the immune response. *In* Progress in Immunology. B. Amos, editor. Academic Press, Inc., New York. 78.
9. Phillips, D. R., and M. Morrison. 1970. The arrangement of proteins in the human erythrocyte membrane. *Biochem. Biophys. Res. Com.* **40**:284.
10. Baur, S., E. S. Vitetta, C. J. Sherr, I. Schenkein, and J. W. Uhr. 1971. Isolation of heavy and light chains of immunoglobulin from the surfaces of lymphoid cells. *J. Immunol.* **106**:1133.

11. Vitetta, E. S., S. Baur, and J. W. Uhr. 1971. Cell surface immunoglobulin. II. Isolation and characterization of immunoglobulin from mouse splenic lymphocytes. *J. Exp. Med.* **134:**242.
12. Morrison, M., and D. E. Hultquist. 1963. Lactoperoxidase. II. Isolation. *J. Biol. Chem.* **238:**2847.
13. Shapiro, A. L., E. Vinuela, and J. V. Maizel. 1967. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-acrylamide gels. *Biochem. Biophys. Res. Com.* **28:**815.
14. Dingman, C. W., and A. C. Peacock. 1968. Analytical studies on nuclear ribonucleic acid using polyacrylamide gel electrophoresis. *Biochemistry.* **7:**659.
15. Lowry, O. H., N. J. Rosebrough, R. J. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193:**265.
16. Fleck, A., and H. N. Munro. 1962. The precision of ultraviolet absorption measurements in the Schmidt-Thannhauser procedure for nucleic acid estimation. *Biochim. Biophys. Acta.* **55:**571.
17. Touster, O., N. N. Aronson, J. J. Dulaney, and H. Hendrickson. 1970. Isolation of rat liver plasma membranes. Use of nucleotide pyrophosphatase and phosphodiesterase I as marker enzymes. *J. Cell Biol.* **47:**604.
18. Lowry, O. H., and J. A. Lopez. 1946. The determination of inorganic phosphate in the presence of labile phosphate esters. *J. Biol. Chem.* **162:**421.
19. Milonig, G. 1961. Advantages of a phosphate buffer for osmium tetroxide solutions in fixation. *J. Appl. Physiol.* **32:**1637.
20. Felix, M. D., and A. J. Dalton. 1956. A comparison of mesothelial cells and macrophages in mice after the intraperitoneal inoculation of melanin granules. *J. Biophys. Biochem. Cytol.* **2** (Suppl.):109.
21. Luft, J. H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* **9:**409.
22. Stempak, J. C., and R. T. Ward. 1964. An improved method for electron microscopy. *J. Cell Biol.* **22:**697.
23. Venable, J. H., and R. Coggeshall. 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.* **25:**407.
24. Rowden, G. 1969. A method for obtaining stained electronmicroscope specimens free from contamination. *J. Microsc.* **89:**229.
25. Baur, S., I. Schenkein, and J. W. Uhr. 1972. Cell surface immunoglobulin. I. Isolation and characterization of immunoglobulin from murine myeloma cells. *J. Immunol.* **103:**748.
26. Benedetti, E. L., and P. Emmelot. 1968. Structure and function of plasma membranes isolated from liver. *In* The Membranes. A. J. Dalton and F. Huguenau, editors. Academic Press, Inc., New York. 33.
27. Buxbaum, J., S. Zolla, M. D. Scharff, and E. C. Franklin. 1971. Synthesis and assembly of immunoglobulins by malignant human plasmacytes and lymphocytes. II. Heterogeneity of assembly in cells producing IgM proteins. *J. Exp. Med.* **133:**1118.
28. Marchalonis, J. J. 1969. An enzymatic method for the trace iodination of immunoglobulins and other proteins. *Biochem. J.* **113:**299.
29. Suzuki, T., and H. F. Deutsch. 1967. Dissociation, reaggregation, and subunit structure studies of some human γM globulins. *J. Biol. Chem.* **242:**2725.

30. Klein, E., T. Eskeland, M. Inoue, R. Strom, and B. Johansson. 1970. Surface immunoglobulin moieties on lymphoid cells. *Exp. Cell Res.* **62:**133.
31. Eskeland, T., E. Klein, M. Inoue, and B. Johansson. 1970. Characterization of immunoglobulin structures from the surface of chronic lymphocytic leukemia cells. *J. Exp. Med.* **134:**265.
32. Lerner, R. A., P. McConahey, I. Jansen, and F. J. Dixon. 1972. Synthesis of plasma membrane–associated and secretory immunoglobulin in diploid lymphocytes. *J. Exp. Med.* **135:**136.
33. Rabellino, E., S. Colon, H. M. Grey, and E. R. Unanue. 1971. Immunoglobulins on the surface of lymhphocytes. I. Distribution and quantitation. *J. Exp. Med.* **133:**156.
34. Shapiro, A. L., M. D. Scharff, J. V. Maizel, Jr., and J. W. Uhr. 1966. Polyribosomal synthesis and assembly of the H and L chains of gamma globulin. *Proc. Nat. Acad. Sci. U.S.A.* **56:**216.
35. Schubert, D. 1968. Immunoglobulin assembly in a mouse myeloma. *Proc. Nat. Acad. Sci. U.S.A.* **60:**683.
36. Lisowska-Bernstein, B., M. E. Lamm, and P. Vassalli. 1971. Cell-free synthesis of rat immunoglobulin. III. Analysis of the cell-free made chains and of their mode of assembly. *J. Mol. Biol.* **56:**1.
37. Helmreich, E., M. Kern, and H. N. Eisen. 1961. The secretion of antibody by isolated lymph node cells. *J. Biol. Chem.* **236:**464.
38. Helmreich, E., M. Kern, and H. N. Eisen. 1962. Observations on the mechanism of secretion of γ-globulins by isolated lymph node cells. *J. Biol. Chem.* **237:**1925.