Immunoglobulin of T Lymphoma Cells. Biosynthesis, Surface Representation, and Partial Characterization†

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ABSTRACT: Four cloned continuously cultured mouse T lymphoma cell lines, WEHI-22.1, WEHI-7.1, S49.1, and EL-4.1, were examined for immunoglobulin biosynthesis and the presence of immunoglobulin on the cell surface. Incorporation of [^{3}H]leucine into cellular proteins followed by serological analysis showed that immunoglobulin constituted between 0.1 and 1.1% of protein synthesized by the different cell lines during a 6-hr period. Under the same conditions cultured cells of nonlymphoid origin, the mastocytoma P-815 X-2.1, did not synthesize any detectable immunoglobulin. Lactoperoxidase-catalyzed radioiodination was used to label proteins on the surface of viable lymphoma and mastocytoma cells. Although the lymphoma lines lacked immunoglobulin as assessed by fluorescent antibody staining, immunoglobulin was detected in surface proteins of all four lymphoma lines. Estimates of the number of immunoglobulin molecules on the cell surface were 1.1 × 10^4/cell for S49.1 and EL-4.1, 1.7 × 10^4 for WEHI-7.1, and 4.3 × 10^4 for WEHI-22.1. Electrophoretic mobilities in sodium dodecyl sulfate polyacrylamide gel indicated that intact cell surface immunoglobulin was slightly larger than IgG, and on disulfide bond reduction to dissociate into two components, one with the mobility of serum immunoglobulin light chain, the other with a mobility similar to that of μ heavy chain. The heavy chain from the T lymphoma cells possessed an apparent molecular weight of about 65,000 compared with 70,000 for μ chain, although both chains shared antigenic determinants characteristic of μ chains. These findings are interpreted as support for other reports that T lymphocytes carry immunoglobulin on their surface and as direct evidence that thymus-derived lymphoid cells synthesize an immunoglobulin resembling the 7S subunit of IgM.

Various indirect and direct approaches (Greaves et al., 1973; Warner, 1974; Marchalonis, 1974a) provide evidence that immunoglobulin, predominantly 7S IgM (Marchalonis and Cone, 1973; Vitetta and Uhr, 1973), is the receptor molecule for antigen on B lymphocytes. The identification of the antigen receptor on T lymphocytes has been more difficult. Surface immunoglobulin has not been readily demonstrated on T lymphocytes by binding of anti-immunoglobulin (Rabellino et al., 1971; Lamelin et al., 1972) although other workers using similar techniques have reported finding such immunoglobulin (Hämmerling and Rajewsky, 1971; Bankhurst et al., 1971; Nossal et al., 1972; Pernis et al., 1974; Santana et al., 1974). Likewise, some workers have reported failure to find immunoglobulin in extracted radioiodinated surface proteins of thymus and T lymphocytes (Grey et al., 1973; Liskowska-Bernstein et al., 1973; Vitetta and Uhr, 1973) whereas others have detected immunoglobulin using similar approaches (Marchalonis and Cone, 1973; Marchalonis et al., 1972b; Moroz and Hahn, 1973; Moroz and Lahat, 1973; Cone and Marchalonis, 1974; Ladoulis et al., 1974; Boylston and Mowbray, 1974). However, even direct surface radioiodination approaches do not establish the source of the immunoglobulin detected. Studies of biosynthesis of immunoglobulin by T lymphocytes are complicated because observed immunoglobulin synthesis might result from contamination of the populations with small numbers of B lymphocytes or plasma cells (Moroz and Hahn, 1973; Moroz and Lahat, 1974; Vitetta et al., 1973; Roelants et al., 1974; Loor and Roelants, 1974). In contrast to lymphocyte populations taken from animals, θ antigen-bearing T lymphoma cells can be obtained as homogeneous cell populations growing in continuous cell culture. Any mouse protein detected on cloned, cultured T lymphoma cells must be a product of those cells. Furthermore, if T lymphoma cells carry immunoglobulin on their surface, they would serve as a useful source of such molecules for study of their structural and functional properties.

For this purpose, murine T lymphoma cells of the lines WEHI-22, WEHI-7, S49, and EL-4 were investigated. All four lymphoma cells exhibit the θ alloantigen (Shevach et al., 1971; Hyman, 1972; Harris et al., 1973) which is characteristic of thymus-derived lymphocytes (Raff, 1971). Cells of the lines WEHI-22 and WEHI-7 originated as thymic lymphomas (Harris et al., 1973). In this publication we report the results of studies designed to determine whether immunoglobulin is synthesized by T lymphoma cells and whether immunoglobulin is exhibited on the surface of these cells. In addition immunoglobulin isolated from the surface of the T lymphoma cells was characterized by polyacrylamide gel electrophoresis.

Materials and Methods

Cells. The origins of the cloned cultured mouse lymphoma lines S49.1 (Horibata and Harris, 1970), WEHI-22.1 (Harris et al., 1973) and the mastocytoma line P-815 X-2.1 (Dunn and Potter, 1957; Green and Day, 1960; Harris et

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Table I: Characteristics of Cell Lines.

Cultured Cell Line		Mouse Strain of Origin	T_D[a] (hr)	Chromosome No.	θ (Thy 1.2) Antigen
WEHI-22.1	X-Ray-induced thymoma	BALB/c	11	42	+
WEHI-7.1	X-Ray-induced thymoma	BALB/c	12	40	+
S49.1	Mineral oil induced lymphoma	BALB/c	16	40	+
EL-4.1	Dimethylbenzathracene-induced leukemia	C57BL	14	39	+
P-815 X-2.1	Mastocytoma	DBA/2	10	40	−

[a] T_D, mean cultured cell population doubling time.

al., 1973) have been described previously. The leukemia line EL-4 (Gorer, 1950) was obtained in 1970 from Dr. J. C. Cerottini (Lausanne, Switz.) and cloned in culture in this laboratory to yield a cell line designated EL-4.1. The thymoma, WEHI-7 (Harris et al., 1973), was established in culture and cloned by single cell isolation to yield WEHI-7.1. Table I lists some of the characteristics of these lines.

Culture Conditions. Lymphoma cells for experiments were grown in 150-mm diameter plastic Petri dishes (Falcon Plastics, Oxnard, Calif.) containing Dulbecco's modified Eagle's medium (Cat. H-16, Grand Island Biological Co., Grand Island, N.Y.) with 3.4 g/l. of $NaHCO_3$ and 10% heat-inactivated fetal calf serum (Commonwealth Serum Laboratories, Parkville, Australia) in an atmosphere of 10% CO_2 in air at 37–38°. Cells were counted with a Coulter Counter Model F_N (Coulter Electronics Ltd., Dunstable, England) after dilution in phosphate buffer.

Immunoglobulin Biosynthesis of T Lymphoma Cells. Lymphoma cells were washed once with DMEM.[1] Then 10^8 cells were suspended in 10 ml of leucine-free DMEM (obtained from Biocult Laboratories Ltd., Paisley, Scotland) containing 10% FCS, 50 μCi of L-[4,5-^{3}H]leucine/ml (specific activity 46 Ci/mmol; radiochemical purity: 98%; The Radiochemical Centre, Amersham, England), and carrier leucine of a final concentration of 5×10^{-5} *M*. The cell suspension was placed in a plastic Petri dish and incubated for 6 hr in an atmosphere of 10% CO_2 in air at 37°. After incubation the cell suspension was centrifuged at 380*g* for 6 min at 4°, the supernatant was removed, and the cells were washed with DMEM and dissolved in 1% NP40–6 *M* urea in phosphate buffer (7 ml/10^8 cells; incubation of cell pellet in NP40–urea, 30 min at room temperature). The cell lysate and culture fluid were dialyzed exhaustively at 4° against phosphate buffer. After dialysis the amount of ^{3}H-labeled total macromolecular protein in the cell lysate and culture period was determined by precipitation with Cl_3CCOOH (final concentration, 20% w/v). The precipitates were washed three times with 10% Cl_3CCOOH. The amounts of ^{3}H-labeled immunoglobulin in the dialyzed cell lysate and culture fluid were estimated by specific serological precipitation (see Immunological Coprecipitation). Cl_3CCOOH precipitates and coprecipitates were dissolved in 0.5 ml of Soluene (Soluene T.M. -100, Packard Instrument Company, Downers Grove, Ill.). The solubilized material was transferred into scintillation counting vials, mixed with 10 ml of scintillation mixture (1 l. of toluene A.R.; 3 g of 2,5-diphenyloxozole; 0.3 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene; Packard Instrument Company). Samples were counted in a Packard TriCarb liquid scintillation spectrometer.

Radioiodination of Cell Surface Proteins of T Lymphoma Cells. The procedure used is a modification (Haustein, 1975) of the lactoperoxidase-catalyzed radioiodination procedure originally devised to surface label lymphocytes (Marchalonis et al., 1971). In brief the procedure was as follows: T lymphoma cells obtained from exponentially growing cultures were washed twice with phosphate buffer. Aliquots of 10^7 washed cells (viability measured by eosin dye exclusion was more than 95%) in 10-ml capacity polystyrene centrifuge tubes were resuspended in a mixture of 20 μl of phosphate buffer, 10 μl of a solution of lactoperoxidase (0.25 mg/ml), 5 μl of carrier-free [^{125}I]iodide (100 Ci/l., The Radiochemical Centre), and 5 μl of [^{127}I]iodide (0.15 m*M*). The reaction was initiated by addition of 10 μl of 0.03% H_2O_2 which was diluted from a 30% stock solution (British Drug Houses, Ltd., Melbourne, Australia) immediately before use. The cell suspensions were vigorously mixed and incubated at 30° for 4 min. After incubation, another 10 μl of lactoperoxidase (0.25 mg/ml) and 10 μl of H_2O_2 (0.03%) were added to reinitiate the reaction, and the suspension was again mixed and incubated at 30° for 4 min. The latter step was repeated once with 10 μl of lactoperoxidase and 10 μl of H_2O_2 and then with 10 μl of H_2O_2 only (a total of three additions of 10 μl of lactoperoxidase and four additions of H_2O_2 to 10^7 cells were made). The iodinated cells were washed twice with 2 ml of phosphate buffer per 10^7 cells at 4° and the radioactivity incorporated by the cells counted using a Packard scintillation spectrometer. About 35% of the added radioactive iodide was retained by the washed cells without any significant decrease in either cell viability, as judged by eosin dye exclusion, or total cell number.

Solubilization of Radioiodinated Cell Surface Proteins. Two methods were used. (a) Extraction with 1% NP40–6 *M* urea in phosphate buffer: after washing of the radioiodinated cells NP40–urea was added to the cell pellet (0.7 ml NP40–urea/10^7 cells). Then the cell pellet was disrupted by pipetting the suspension up and down. The disrupted cells were incubated in NP40–urea for 30 min at room temperature. The lysate was centrifuged at 380 *g* for 15 min. The supernatant was dialyzed exhaustively at 4° against PBS and then centrifuged at 12,000 *g* for 20 min. (b) Metabolic release: radioiodinated cells (usually 5×10^7) were suspended in DMEM (without FCS; 1 ml of DMEM/10^7 cells); the cell suspension was placed in a 55-mm plastic Petri dish and incubated for 6 hr in an atmosphere of 10% CO_2 in air at 37°. After incubation the cell suspension was centrifuged at 380*g* for 5 min and the supernatant dialyzed and centrifuged as above.

[1] Abbreviations used are: DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; phosphate buffer, phosphate-buffered saline, pH 7.3, containing 0.05 *M* sodium phosphate–0.15 *M* NaCl; NP40, Nonidet P40; MIgM, mouse immunoglobulin M; MIgG, mouse immunoglobulin G; FIgG, fowl immunoglobulin G; SDS, sodium dodecyl sulfate.

Table II: Immunoglobulin Biosynthesis by T Lymphoma Cells.

Cell line	Radioactivity[a] Cl_3CCOOH precipitable (cpm × 10^{-3}) A	Radioactivity[a] Precipitated by [b] MIgM + anti-MIgM (cpm × 10^{-3}) B	Radioactivity[a] Precipitated by [b] FIgG + anti-FIgG (cpm × 10^{-3}) C	Radioactivity[a] specific precipitated (cpm × 10^{-3}) B − C	Percent of Cl_3CCOOH-Precipitable Material specific precipitated [(B − C)100/A]
		Cell Associated			
WEHI-22	7735	155.0 ± 8.1	69.3 ± 3.2	85.7	1.1
WEHI-7	8820	51.8 ± 3.2	18.6 +± 2.8	33.2	0.4
S49	6930	8.8 ± 1.2	2.2 ± 0.5	6.6	0.1
EL-4	6760	38.0 ± 0.5	11.2 ± 0.6	26.8	0.4
P-815	8260	16.1 ± 1.4	17.2 ± 1.8	n.s.[c]	0.0
		Released into Medium			
WEHI-22	36	0.42 ± 0.05	0.39 ± 0.04	n.s.	0.1
WEHI-7	294	4.20 ± 0.50	0.91 ± 0.07	3.29	1.1
S49	178	0.74 ± 0.10	0.84 ± 0.10	n.s.	0.0
EL-4	29	0.80 ± 0.04	0.80 ± 0.04	n.s.	0.0
P-815	14	0.53 ± 0.04	0.63 ± 0.04	n.s.	0.0

[a] Radioactivity (^3H cpm) is normalized for 10^7 cells. [b] Results represent the arithmetic mean ±SE of 5 replicates. [c] n.s. not significant.

Purified Immunoglobulins and Antisera. Mouse IgG and mouse IgM were prepared from normal mouse serum by zone electrophoresis on starch (Kunkel, 1954) followed by gel filtration on Sephadex G-200. Fowl IgG was isolated from normal chicken serum as described by Benedict (Benedict, 1967). These immunoglobulins were free of contaminants as judged by immunoelectrophoresis and polyacrylamide gel electrophoresis. Rabbit antisera to MIgG, MIgM, and FIgG were made by immunizing rabbits with MIgG, MIgM, and FIgG purified as described above. The immunization procedure was the following: 2 mg of immunoglobulin in complete Freund's adjuvant was injected subcutaneously into each rabbit. The injection was repeated after 4 weeks using 2 mg of immunoglobulin in incomplete Freund's adjuvant and blood taken after further 4–8 weeks. Rabbit antiserum to FIgG was absorbed with ammonium sulfate precipitated globulins of normal mouse serum (50% saturated ammonium sulfate) coupled to Sepharose 4B. The specificities of the anti-IgG and anti-IgM sera in a radioimmunoassay were determined by the inhibition by IgG and IgM of binding of [^{125}I]MIgG to anti-MIgG and by the inhibition by IgG and IgM of binding of [^{125}I]MIgM to anti-MIgM. For the anti-IgG 50% inhibition of the binding of [^{125}I]MIgG to anti-MIgG required 2.5 ng of MIgG or 25 ng of MIgM. For the anti-IgM 50% inhibition of the binding of [^{125}I]MIgM to anti-MIgM required 30 ng of MIgM, but 2500 ng of MIgG inhibited only 10% of the binding. This indicates that the anti-MIgG serum possessed specificity for γ chain, but was mainly specific for light chain, whereas the predominant antibody activity in the anti-MIgM serum was against μ chain.

Immunological Coprecipitation. Preparations of ^{125}I-labeled cell surface proteins or ^3H-labeled proteins were centrifuged at 12,000*g* for 20 min in order to remove microaggregates prior to coprecipitation. Radioiodinated cell surface immunoglobulin and ^3H-labeled immunoglobulin were isolated by specific coprecipitation with MIgG and anti-MIgG or with MIgM and anti-MIgM. Controls for nonspecific precipitation of ^{125}I-labeled cell surface proteins or ^3H-labeled proteins consisted of precipitation of FIgG with anti-FIgG. Conditions for coprecipitation were determined such that more than 80% of the carrier was precipitated. The conditions were determined with trace amounts of [^{125}I]iodide labeled MIgG, MIgM, or FIgG with various dilutions of carrier and antiserum. 200 μl of labeled test solution were mixed with 100 μl of carrier dissolved in phosphate buffer (concentration usually 0.1 mg/ml). To this mixture were added 100 μl of diluted antiserum (usually diluted 1 in 2). The precipitation was carried out in 5 × 0.5 cm round-bottom plastic tubes which had been coated previously with 1% normal rabbit serum (Marchalonis et al., 1974). The coprecipitation mixtures were incubated for 2 hr at 37° and then overnight at 4°. After incubation the mixtures were centrifuged at 12,000*g* for 20 min at 4°, the supernatants were discarded, and the radioactivity in the precipitates was determined. The precipitates were then resuspended in 300–400 μl of phosphate buffer, transferred to fresh uncoated plastic tubes, using a separate pasteur pipette for each transfer, and centrifuged. The precipitates were washed until the radioactivity of controls for nonspecific precipitation was 20–40% of the radioactivity of the specific precipitates (usually after four washes). At least five replicates for specific and nonspecific precipitation were carried out.

Disc Electrophoresis in Polyacrylamide Gel. Precipitated proteins were analyzed by polyacrylamide gel electrophoresis in a discontinuous buffer system according to the method of Laemmli (Laemmli, 1970). Samples were prepared for electrophoresis by dissolving precipitates in 200–300 μl of a buffer containing 10% glycerol, 5% mercaptoethanol, 3% SDS, and 6 *M* urea in 0.125 *M* Tris-HCl (pH 6.8). Samples to be subjected to electrophoresis without reduction were dissolved in similar sample buffer minus the mercaptoethanol. The samples (reduced and unreduced) were heated for 5 min at 100°. The gels were sliced into 65–70 fractions using a Canalco slicer (Canalco, Inc., Rockville, Md.) and slices were counted in plastic tubes using a Packard autogamma spectrometer. Mobilities are expressed as distance migrated relative to that of a Bromophenol Blue dye marker.

Results

Immunoglobulin Biosynthesis by T Lymphoma Cells. Biosynthesis of immunoglobulin by the cultured lymphoma cells was demonstrated by the detection of ^3H-labeled immunoglobulin in extracts of [^3H]leucine-labeled cells as de-

Table III: Surface Immunogloblin of T Lymphoma Cells Extracted with 1% NP40–6 *M* Urea.

Cell Line	Radioactivity[a] Nondialyzable (cpm × 10^{-3}) A	Radioactivity[a] Precipitated by [b] MIgM + anti-MIgM (cpm × 10^{-3}) B	MIgG + anti-MIgG (cpm × 10^{-3}) C	FIgG + anti-FIgG (cpm × 10^{-3}) D	Percent of Nondialyzable Material Specific Precipitated by the IgM System (cpm × 10^{-3}) (B − D)100/A = E	by the IgG System (cpm × 10^{-3}) (C − D)·100/A	Estimated No. of Ig Molecules/Cell Surface[c] (E)(3.67 × 10^6)
WEHI-22	7910	106.1 ± 7.0	42.0 ± 4.2	10.5 ± 3.5	1.2	0.4	4.3×10^4
WEHI-7	9310	58.8 ± 5.3	24.4 ± 2.5	12.3 ± 2.5	0.5	0.1	1.7×10^4
S49	9744	44.5 ± 7.4	15.4 ± 1.4	14.4 ± 2.1	0.3	0.0	1.1×10^4
EL-4	9677	37.8 ± 4.2	29.1 ± 3.2	8.8 ± 2.5	0.3	0.2	1.1×10^4
P-815	8431	17.9 ± 3.5	17.2 ± 2.8	16.9 ± 3.2	n.s.	n.s.	n.s.

[a] Radioactivity (^{125}I cpm) is normalized for 10^7 cells. [b] Results represent the arithmetic mean ±SE of 5 replicates. [c] These values are calculated as follows: Ig molecules/cell = E(membrane protein/cell)(Avogadro's number/7S IgM molecular weight); = $E \times 1.1 \times 10^{-12}$ g/cell × (6×10^{23} molecules/mol/1.8×10^5 g/mol); = $E \times 3.67 \times 10^6$ molecules/cell.

scribed under Materials and Methods. As shown in Table II WEHI-22, WEHI-7, S49, and EL-4 incorporated similar amounts of [^{3}H]leucine into proteins during 6-hr incubation (column A). Only small amounts of synthesized proteins were released into the medium. WEHI-7 and S49 released larger amounts of labeled proteins than the other lymphoma cells. Mastocytoma cells of the line P-815 which were included as a control nonlymphoid cell incorporated equivalent amounts of [^{3}H]leucine into protein. As shown by column B of Table II highly significant amounts of the cell associated radioactivity of the T lymphoma cells were precipitated by the specific coprecipitation system compared with the control system (column C). Radioactivity specifically coprecipitated (counts of the specific system minus counts of the control system) is given in column B-C. WEHI-7 released significant amounts of labeled immunoglobulin into the medium, the other lymphoma cells did not. No difference between specific and control precipitation systems was observed for the mastocytoma cells P-815 whether cell associated or released radioactive material was analyzed.

Isolation of Cell Surface Immunoglobulin of T Lymphoma Cells. Surface proteins of the cultured lymphoma cells of the lines WEHI-22, WEHI-7, S49, and EL-4 were radioiodinated as described under Materials and Methods. Under these conditions approximately 35% of the [^{125}I]iodide in the reaction mixture became associated with the cells. The same result was obtained when the P-815 mastocytoma cells were radioiodinated. In order to solubilize ^{125}I-labeled cell surface proteins two procedures were used: (a) extraction with 1% NP40-6 *M* urea followed by dialysis and centrifugation of the soluble material or (b) incubation of the cells in culture medium at 37° in which cells shed labeled surface material by metabolic release and subsequent dialysis and centrifugation of the culture medium. Immunoglobulin was then isolated from the high molecular weight ^{125}I-labeled cell surface proteins using the two specific coprecipitation systems and the control precipitation system described under Materials and Methods.

The coprecipitation data obtained for the T lymphoma cells WEHI-22, WEHI-7, S49, and EL-4 and the mastocytoma cell P-815, which were surface radioiodinated and lysed with 1% NP40-6 *M* urea, are summarized in Table III. The results given were obtained after four washes of the precipitates. Using MIgM + anti-MIgM as the specific precipitation system, highly significant amounts of immunoglobulin (see difference between MIgM + anti-MIgM system and FIgG + anti-FIgG system) were detectable in the cell lysates from all four lymphoma lines (between 0.3 and 1.2% of nondialyzable ^{125}I-labeled cell surface material), whereas no immunoglobulin was observed in the cell lysate of the mastocytoma cells.

In the same experiments soluble extracts of radioiodinated cells were analyzed by coprecipitation using the MIgG + anti-MIgG system as the specific precipitation system. In contrast to the data described above different results were obtained using this system. In general the amounts of immunoglobulin detectable by the MIgG + anti-MIgG system (see difference between MIgG + anti-MIgG system and FIgG + anti-FIgG in Table III) in the cell lysates of the lymphoma cells were markedly lower than the amounts precipitated with the MIgM system. In fact, in the cell lysate of the lymphoma cell S49 no immunoglobulin at all was detectable with the MIgG system.

We have made an attempt to calculate the number of surface immunoglobulin molecules per lymphoma cell. On the basis of the data of Allan and Crumpton (1971) that lymphocytes contain 1.1×10^{-12} g of membrane protein/cell, and the finding that all surface immunoglobulin of T cells and T lymphoma cells (see below) has the molecular weight of the 7S subunit of IgM immunoglobulin (180,000) it is possible to obtain an estimate of the approximate number of molecules of surface immunoglobulin on the lymphoma cells under investigation. The calculation assumes that the counts precipitated as immunoglobulins represent a true measure of mass contribution of this protein relative to other components of the cell membrane. It should be pointed out that T lymphoma cells may not have the same quantity of membrane protein as normal lymphocytes and this might also influence the accuracy of the calculations. The number of molecules of immunoglobulin computed on the surface of the T lymphoma cells ranged from 11,000 to 43,000 molecules per lymphoma cell (Table III, last column). These numbers are calculated on the basis of the precipitation data obtained with the MIgM + anti-MIgM system (radioactivity bound to the nonspecific precipitation system FIgG + anti-FIgG was subtracted).

Immunoglobulin was isolated both from radioiodinated cell surface material solubilized by extraction with 1% NP40-6 *M* urea and from cell surface material released into medium. The NP40-urea treatment solubilized 90-

Table IV: Surface Immunoglobulin Metabolically Released from T Lymphoma Cells.

Cell Line	Radioactivity[a] Nondialyzable (cpm × 10^{-3}) A	Radioactivity[a] Precipitated by [b] MIgM + anti-MIgM (cpm × 10^{-3}) B	FIgG + anti-FIgG (cpm × 10^{-3}) C	Percent of Nondialyzable Material Specific Precipitated [(B − C)100/A]
WEHI-22	1080	26.8 ± 0.2	7.2 ± 0.4	1.8
WEHI-7	1040	21.0 ± 1.0	2.2 ± 0.2	1.8
S49	1200	26.0 ± 0.2	7.4 ± 0.2	1.6
EL-4	1020	34.8 ± 2.6	11.6 ± 1.0	2.3
P-815	996	2.4 ± 0.2	2.2 ± 0.2	n.s.

[a] Radioactivity (^{125}I cpm) is normalized for 10^7 cells. [b] Results represent the arithmetic mean ±SE of 5 replicates.

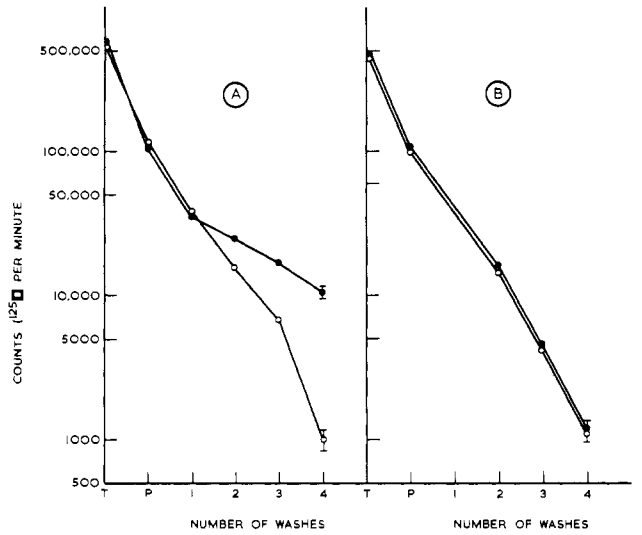

FIGURE 1: Precipitation analysis of ^{125}I-labeled cell surface proteins of WEHI-7 lymphoma (A) and P-815 mastocytoma cells (B). Radioiodinated cell surface proteins obtained in solution by metabolic release were dialyzed and the soluble proteins coprecipitated with MIgM + anti-MIgM (specific coprecipitation system, ●—●) and with FIgG + anti-FIgG (nonspecific coprecipitation system, O—O). Counts at T represent the total radioactivity of high molecular weight cell surface proteins isolated from 5 × 10^6 cells by metabolic release. Counts at P represent the radioactivity in the unwashed precipitates. Subsequent counts represent those associated with immunological precipitates undergoing sequential washes. Vertical bars denote standard errors of arithmetic mean of five replicates.

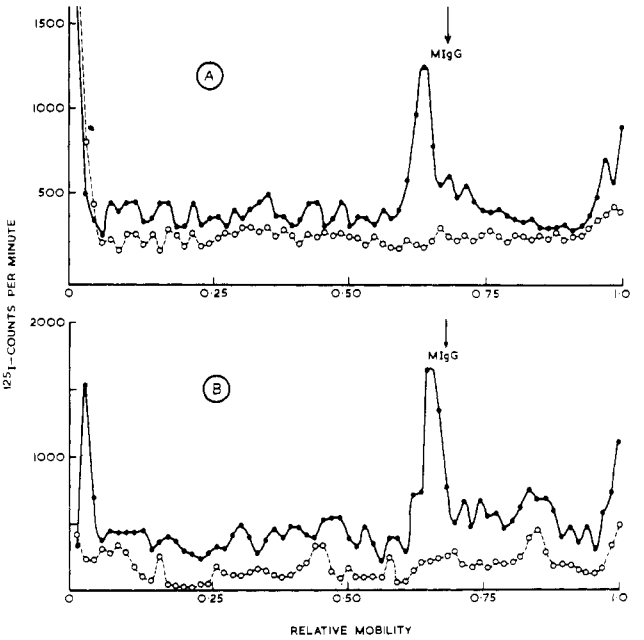

FIGURE 2: Polyacrylamide gel electrophoresis (5% acrylamide) in SDS of ^{125}I-labeled intact surface immunoglobulin of thymoma cells of the cell lines WEHI-22 (A) and WEHI-7 (B). Immunoglobulin was isolated by specific coprecipitation from ^{125}I-labeled cell surface proteins which were solubilized by metabolic release. (●) MIgM + anti-MIgM; (O) FIgG + anti-FIgG; MIgG refers to the position of intact mouse IgG standard.

95% of ^{125}I counts whereas 45–55% were obtained by metabolic release. As shown in Table IV, significant amounts of immunoglobulin were detectable in cell surface material released by the T lymphoma cells (between 1.6 and 2.3% of nondialyzable ^{125}I-labeled cell surface material), whereas no immunoglobulin was coprecipitated from material released by mastocytoma cells. The precipitation data given in Table IV were obtained after four washes of the precipitates. Figure 1 presents the "wash loss" curve of the precipitation data obtained for ^{125}I-labeled surface proteins of WEHI-7 lymphoma cells and P-815 mastocytoma cells. In the case of the lymphoma cell the presence of specifically precipitated material was detectable after two washes. The ratio of specifically precipitated radioactivity to radioactivity bound to the control precipitates was improved by further washes. The "wash loss" curves obtained for WEHI-22, S49, and EL-4 were similar to the curve for WEHI-7 given in Figure 1. In contrast to the lymphoma results, no significant difference between the experimental and control radioactivity of ^{125}I-labeled cell surface material of the mastocytoma cells P-815 was observed during four washes of the precipitates. The counts associated with the specific precipitates showed a rapid exponential loss as did counts associated with the control precipitates.

The possibility that proteins from the FCS-containing medium might interfere with immunological precipitations was excluded by results of two further experiments: (a) coprecipitation studies performed with ^{125}I-labeled FCS failed to reveal any component resembling μ chain on polyacrylamide gel electrophoresis, (b) an experiment in which thymoma cells were grown for over seven generations in medium containing ^{125}I-labeled FCS. After standard washing of these cells, less than 0.01% of the radioactivity was associated with the cells.

Characterization of Surface Immunoglobulins of T Lymphoma Cells. Specifically coprecipitated material was analyzed by polyacrylamide gel electrophoresis under dissociating conditions in order to obtain information regarding its molecular properties. Electrophoretic patterns of material isolated by precipitation from NP40–urea extracts of surface radioiodinated lymphoma cells showed that the isolated immunoglobulin was partially degraded. Experiments

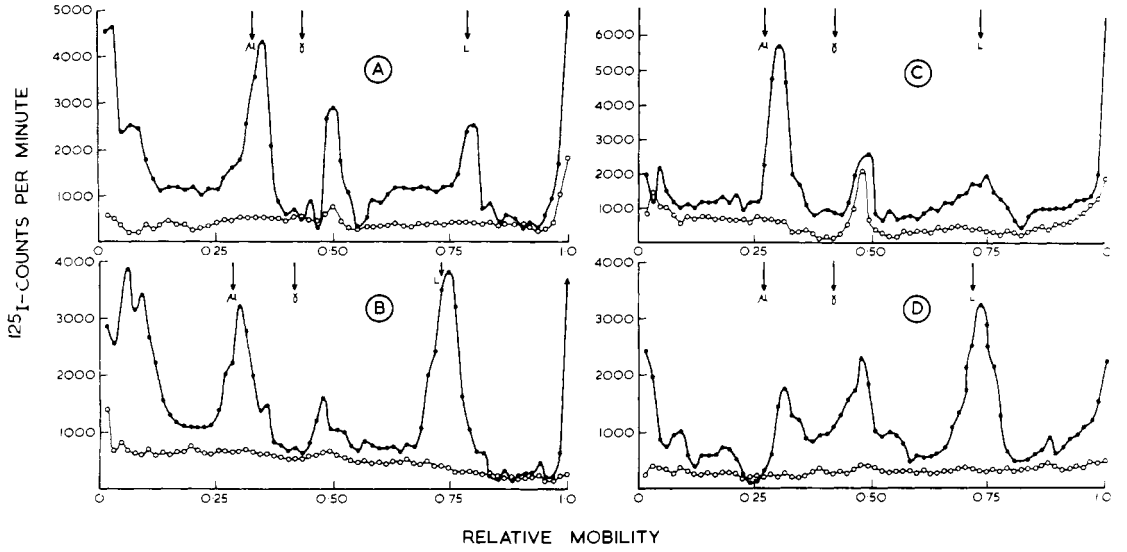

FIGURE 3: Polyacrylamide gel electrophoresis (10% acrylamide) in SDS of ^{125}I-labeled polypeptide chains of immunoglobulin of thymoma cells of the lines WEHI-22 (A), WEHI-7 (B), S49 (C), and EL-4 (D). Immunoglobulin was isolated by specific coprecipitation from ^{125}I-labeled cell surface proteins which were solubilized by metabolic release and then reduced with mercaptoethanol. (●) MIgM + anti-MIgM; (○) FIgG + anti-FIgG. μ, γ, and L refer to the positions of standard mouse immunoglobulin chains.

employing inhibitors of lysosomal proteases indicated that the degradation process can partially be inhibited by adding inhibitor to the extraction fluid and dialysis buffer.

Another effective way to circumvent degradation was to isolate immunoglobulin from metabolically released cell surface material (see above) and to analyze this material by polyacrylamide gel electrophoresis. Figure 2 depicts the electrophoretic pattern obtained for intact surface immunoglobulin of lymphoma cells of the lines WEHI-22 and WEHI-7. Similar patterns were obtained from S49 and EL-4. Under the conditions employed (5% gel), 19S IgM, molecular weight 900,000, would remain at the origin whereas 7S subunits would penetrate the gel matrix. All patterns showed a peak which was slightly retarded relative to the mouse IgG standard (mol wt 150,000) and which usually represented between 60 and 80% of the total radioactivity loaded on the gel. Radioactivity not represented by this peak remained at the origin (see Figure 2). Analysis of the coprecipitates by gel filtration on Sephadex G-200 in 1% SDS–4 *M* urea in phosphate buffer using a calibrated column has shown that this material does not have a distinct molecular weight and does not migrate with the 19S IgM standard. Therefore, it was concluded that the material which remained at the origin of the acrylamide gel was caused by aggregation or rather by incomplete dissociation of the coprecipitates. The fact that the bulk of the coprecipitated material migrated as a single component which was slightly retarded relative to the IgG standard suggested that surface immunoglobulin of T lymphoma cells existed as a molecule of somewhat higher molecular weight than IgG. The migration position of T lymphoma Ig was similar to that of cell surface 7S IgM which has previously been reported (Marchalonis et al., 1972b) to have a mass of approximately 180,000 daltons.

^{125}I-labeled surface immunoglobulin isolated from WEHI-22, WEHI-7, S49, and EL-4 cells by metabolic release was reduced to cleave interchain disulfide bonds and analyzed by polyacrylamide gel electrophoresis under dissociating conditions in a 10% gel where molecules ranging approximately in mass from 10,000 to 150,000 daltons were resolved. The results obtained are presented in Figure 3. The patterns of reduced immunoglobulin of every T lymphoma cell showed one peak which migrated slightly ahead of the μ chain of reduced mouse IgM standard and one peak which corresponded to the light chain standard. In addition, a third component with a relative mobility of 0.45–0.50 was observed. The mobility of this component was consistent with a molecular weight of 40,000–45,000. We would stress that this component was isolated from ^{125}I-labeled surface material of S49 cells equally by the nonspecific precipitation system FIgG + anti-FIgG, the amount of this component bound by the nonspecific precipitation system being similar to that isolated by the specific coprecipitation system MIgM + anti-MIgM (see Figure 3C). As shown in pattern A of Figure 3 only a very small amount of the material with a relative mobility of 0.5 was isolated by the nonspecific precipitation system from ^{125}I-labeled surface material of WEHI-22 cells compared with the specific system. In contrast to results obtained with S49 and WEHI-22, no detectable component of the ^{125}I-labeled surface proteins of WEHI-7 and EL-4 was bound by the FIgG + FIgG system. As shown mainly in patterns A and B of Figure 3, material with mobilities greater than those corresponding to a molecular weight of 10^5 was occasionally observed. Since the amount and distribution of this material were variable, its nature was not investigated further.

Discussion

T lymphocytes carry immunoglobulin on their surface, but it has been difficult to establish that such cells synthesize this immunoglobulin. The results obtained in the present work show that θ antigen bearing lymphoid cells synthesize and express on their surface an immunoglobulin similar to the 7S subunit of IgM. The study employed cultured monoclonal T lymphoma cells to exclude the possibility of contamination of the cell populations with B cells, plasma cells, or mouse serum proteins.

Immunoglobulin biosynthesis by the lymphoma cells was demonstrated directly by incorporation of [^{3}H]leucine into material specifically precipitable by anti-immunoglobulin antiserum. The amount of immunoglobulin synthesized as a percentage of total radioactivity incorporated varied from

cell line to cell line, covering a range between 0.1% for S49 and 1.1% for WEHI-22. These amounts were low relative to those reported for plasma cells and activated B-cells (Melchers and Andersson, 1973), but were comparable to those observed for chronic lymphocytic leukemia cells (Marchalonis et al., 1974a). The rate of secretion of immunoglobulin from the cells was particularly low, with WEHI-7 being the only cell line which showed detectable secretion during the time interval studied. It has been difficult to characterize [^{3}H]leucine-labeled immunoglobulin synthesized by these T lymphoma cells because of problems of degradation following extraction. Studies designed to circumvent these problems are being carried out with the eventual goal of obtaining detailed information on the structure of immunoglobulin produced by T lymphoma cells.

Study of the properties of surface immunoglobulins of these lymphoma cells was facilitated by surface labeling of the intact cells with [^{125}I]iodide in a reaction catalyzed by lactoperoxidase. It is now well established that lactoperoxidase catalyzed radioiodination conditions must be adapted specifically for the cell type under consideration for optimal amounts of isotope to be covalently bound to membrane proteins (Cone and Marchalonis, 1974; Haustein, 1975; Marchalonis et al., 1971; Tsai et al., 1973; Phillips and Morrison, 1970; Shin and Carraway, 1973). We would stress that the radioiodination conditions used in this study were designed to optimize surface labeling of T lymphoma cells (Haustein, 1975). Although previous studies indicated that WEHI-22 and WEHI-7 cell lines possessed surface IgM-like immunoglobulin (Marchalonis and Cone, 1973; Marchalonis et al., 1972b; Haustein et al., 1974) insufficient radioactivity was incorporated by lactoperoxidase-catalyzed radioiodination into the molecules to allow detailed analysis. The need for efficient uptake of [^{125}I]iodide is critical and accounts in part for failure of some workers to find surface immunoglobulin in T lymphocytes (Grey et al., 1973; Liskowska et al., 1973; Vitetta et al., 1972) and some T lymphoma cells (Vitetta and Uhr, 1973). Another crucial factor illustrated in the present study is that application of different solubilization procedures could alter the results appreciably. For example, using the solvent 1% NP40–6 *M* urea, 9.3×10^6 cpm were found in macromolecular protein isolated from 10^7 WEHI-7 cells; whereas only 2.6×10^5 macromolecular cpm were obtained from an equal number of these identically labeled cells by metabolic release. However, Ig comprised 1.8% of macromolecular counts (IgM system) in the latter case but only 0.5% in the former. Parallel results were obtained for the other T lymphoma lines studied. Since the NP40-urea extractions allowed greater than 95% solubilization of ^{125}I-radioactivity, we believed that this procedure was more reliable for calculating numbers of molecules. The metabolic release conditions enriched for immunoglobulin as a percentage of total macromolecular material recovered. It has been shown elsewhere that acid–urea extraction, likewise, enriches the fraction of immunoglobulin obtained from T lymphoma cells (Haustein, 1975); and normal T lymphocytes (Cone and Marchalonis, 1974; Marchalonis et al., 1974b). In contrast, ^{125}I-labeled cells of the line WEHI-22 yielded very little IgM (0.07% Ig; Haustein, 1975) when extracted with NP-40 under conditions successfully used with B cells (Vitetta et al., 1972).

Consideration of differences in precipitability of the immunoglobulins of the various lymphomas by either the anti-IgM system or the anti-IgG system raises another point which warrants comment. Immunoglobulins of lines WEHI-22, WEHI-7, and EL-4 are precipitated by both systems, although the anti-IgM reaction is more effective. In contrast, significant immunoglobulin of S49 cells was precipitated only by the IgM system. Since the anti-IgG precipitating system used here was directed largely against κ light chains these results suggest that S49 might possess λ chains while the other three monoclonal lymphomas express κ chains. Such a situation is possible because individual antigen-binding T cells express either κ or λ chains (Hogg and Greaves, 1972) and T lymphomas might arise from cells restricted to the production of either light chain class.

Although surface immunoglobulin of T lymphocytes apparently contains μ chain antigenic determinants (Greaves et al., 1973; Warner, 1974; Marchalonis, 1974a; Marchalonis and Cone, 1973; Ladoulis et al., 1974; Santana et al., 1974; Hogg and Greaves, 1972; Lawrence et al., 1973; Dwyer et al., 1972; Hämmerling and McDevitt, 1974; Hämmerling and Rajewsky, 1971), functional properties indicate that this molecule is not identical with the 7S IgM observed on B lymphocyte surfaces (Cone et al., 1974; Feldmann and Nossal, 1973; Dennert, 1973). The possibility has, therefore, been raised that the μ-like chains of T and B lymphocytes might be encoded by distinct cistrons which diverged from a common ancestral cistron (Marchalonis and Cone, 1973). The present study confirmed the similarity of T lymphocyte immunoglobulin to IgM because the anti-IgM system (directed predominantly against μ chain) was generally more effective than the anti-IgG system (directed predominantly against κ chain) in precipitating T lymphoma immunoglobulin. However, analysis of the heavy chains of the isolated immunoglobulins by polyacrylamide gel electrophoresis disclosed that these chains differed slightly, but reproducibly, from standard μ chains in mobility. T lymphoma immunoglobulin heavy chains migrated somewhat faster than μ chain and possessed an estimated molecular weight of 65,000 rather than 70,000. A similar observation was previously made for IgM-like immunoglobulin isolated from the surface of human thymus lymphocytes (Marchalonis et al., 1972a) and was recently found for IgM-like immunoglobulin of the human T lymphocyte line MOLT-4 (Hunt, S., Marchalonis, J. J. and Morris, P. J., unpublished observations). This difference in mobility on SDS gels might reflect differences in carbohydrate content or actual mass differences in the polypeptide chain. Studies to ascertain the structural basis of these differences are in progress. By contrast, B cell surface immunoglobulin possesses two heavy chain types (Abney and Parkhouse, 1974; Melcher et al., 1974; Hunt and Marchalonis, 1974), one of which is identical in mobility to serum μ chain, the other migrates more rapidly than does the T lymphocyte heavy chain (Haustein, D. and Goding, J. W., submitted).

An additional finding of the present study was that all of the T lymphoma lines analyzed possessed a protein of estimated mass 40,000–45,000 daltons which was precipitated along with mouse IgM immunoglobulin. Either the molecule is noncovalently associated with Ig on the lymphoma cell surface or it binds to antigen–antibody complexes in free solution. Direct evidence for the latter alternative was obtained for cell line S49 where this component occurred in equal amounts in the experimental system and in the heterologous precipitation control. This property suggests similarity to the cell surface receptor (Basten et al., 1972; Cline et al., 1972) which binds to the F_c portion of IgG-antibody

in antigen–antibody complexes. S49 cells possess a readily detectable F_c receptor when assayed using erythrocytes coated with IgG (Harris, A. W., unpublished observations). A small amount of a similar component was found in the control precipitate of WEHI-22, but none was observed in controls for WEHI-7 and EL-4. WEHI-22 has been reported to possess an F_c receptor, whereas the other two cell lines lack such a component when analyzed by formation of IgG rosettes (Shevach et al., 1971; Harris, A. W., unpublished observations). Although previous cell surface labeling studies (Marchalonis and Cone, 1973; Marchalonis, 1974b) with normal B lymphocytes and T lymphocytes did not reveal a component of this nature, preliminary analyses using the radioiodination conditions employed here disclosed the presence of a similar molecule on both cell types (Haustein, D., unpublished observations). Further work is in progress to obtain detailed information on the structural and biological properties of this molecule.

All four T lymphoma cell lines investigated here possessed surface immunoglobulin although there was variability from cell line to cell line in the estimated numbers of surface molecules and in rates of synthesis. Two additional T lymphoma lines, WEHI-112 and RIL-Q, have been analyzed (Harris, A. W. and Marchalonis, J. J., unpublished observations) and, likewise, found to produce immunoglobulin. Thus, this seems to be a general property of T lymphoma cells. In this context it is interesting that all four cell lines analyzed here were negative for binding of fluorescent antiglobulins when assayed using conditions under which B lymphocytes label heavily but normal T lymphocytes do not bind the reagent (Kincade, P. W., personal communication). Thus, these T lymphoma cells resemble normal T lymphocytes in this property as well as the presence of θ antigen. Surface immunoglobulin was detected on WEHI-22, however, when high concentrations of labeled reagents were used.

The number of surface IgM-like molecules ranged from ca. 10,000/cell for S49 and EL-4 to 43,000/cell for WEHI-22. These numbers are appreciably lower than those extracted from B cells which generally are reported to contain 100,000 or more (Greaves et al., 1973; Warner, 1974; Marchalonis, 1974a; Marchalonis and Cone, 1973; Vitetta and Uhr, 1973) surface 7S IgM molecules/cell. The present estimates are in accordance with those obtained for EL-4 cells (30,000 κ chains/cell; Boylston, 1973) and normal thymus lymphocytes (15,000–20,000 molecules κ chain/cell) using ^{125}I-labeled antiserum to κ chain (Boylston, 1973) and radioimmunoassay (Grey et al., 1973). The range of numbers of surface molecules exhibited by monoclonal T lymphoma lines might reflect the heterogeneity of a normal population of T lymphocytes. The relatively low rates of immunoglobulin synthesis and secretion, likewise, were consistent with the identification of these cells as T lymphomas. Comparing the data obtained by surface radioiodination with those of biosynthesis, it is interesting that the bulk of synthesized immunoglobulin was neither secreted nor associated with the plasma membrane. Similar results were found for chronic lymphocytic leukemia cells (Marchalonis et al., 1974a). This may therefore be a general property of neoplastic, and possibly, normal lymphoid cells.

Recent studies showed that IgM-like immunoglobulin isolated from T lymphomas possess properties similar to those of immunoglobulin from collaborative T cells. For example, WEHI-22 (Stocker et al., 1974) and EL-4 (Feldman, M., Boylston, A. W., and Hogg, N. M., submitted) immunoglobulins were able to suppress T-cell-dependent immune responses in vitro, presumably by binding to macrophages via their F_c regions and competing with antigen-specific 7S IgM (T) from specifically activated T cells. The results of this study in conjunction with the above suggest that monoclonal T lymphoma cells may prove as valuable in the analysis of the structure and function of T-cell IgM as myeloma cells were in the elucidation of the structure of serum antibodies.

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