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Cell-Surface Immunoglobulin of Human Thymus Cells and Its Biosynthesis *In Vitro*

(T cells/surface immunoglobulin/immunoglobulin degradation/Trasylol)

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ABSTRACT **Human thymus cells synthesize immunoglobulin in short-term culture, the nascent immunoglobulin appearing in both cytoplasmic and membrane fractions. Surface immunoglobulin was demonstrated by lactoperoxidase radioiodination of the cells. The demonstration of intracellular and surface immunoglobulin required procedures that minimize proteolytic degradation. Noncovalently linked, monomeric μ chains and light chains were found in the cytoplasm and on the surface of the cells by means of acrylamide gel electrophoresis and by specific immunoprecipitation of the isolated chains.**

Mammalian bone marrow-derived lymphocytes (B cells) have membrane immunoglobulins (Ig) which are considered to be receptors for antigens and are indicative of the capacity of these cells to synthesize Igs (1–4). Thymus-derived lymphocytes (T cells) facilitate antibody production by B cells and are involved in cell-mediated immune reactions by means of specific antigen-binding receptors (5). Efforts to demonstrate membrane Ig on T cells have led to conflicting conclusions.

In the present communication we report in evidence short-term tissue culture for synthesis by human thymus cells of IgM in the surface of these cells, and describe the immunochemical nature of the nascent cytoplasmic and surface immunoglobulin. Our findings emphasize the necessity for minimizing proteolytic degradation during the preparation of cellular and membrane fractions and suggest that variable proteolysis during solubilization of membrane components may account for conflicting reports on the presence or absence of surface Igs in T-cell membranes (6–11).

MATERIALS AND METHODS

In Vitro Biosynthesis. Thymuses were obtained from 5- to 9-year-old children undergoing cardiac surgery. Cells were teased from the thymus, filtered through a stainless steel screen, and washed three times and resuspended in Eagle's medium containing 1:100 the standard amount of amino acids. Cell suspensions (5×10^7 cells per ml) were preincubated 60 min at 37°. Then 5 μCi of [^{14}C]aminoacid mixture (uniformly labeled, 45 mCi/millatom of carbon, Amersham, England) was added to each ml of cell suspension. The suspensions were then incubated at 37° for 180 min before they were chilled to 4°. The cells were spun at 1000 rpm for 6 min and the pellet was washed twice with cold Eagle's medium or phosphate-buffered saline (pH 7.2) (PBS).

Preparation of Iodinated Thymus Cells. Human thymus cells were obtained as described above and were iodinated according to Marchalonis *et al.* (12). The cells were treated with 0.01 M iodoacetamide in PBS for 60 min at 4° before iodination. Cells were then washed three times with PBS, and suspended to a concentration of 10^7 cells per ml in PBS (90% viable cells based on exclusion of trypan blue). 0.25 ml of the cell suspension was incubated with 0.5 mCi of ^{125}I (5 μl, carrier-free Na^{125}I, Amersham, England), 10 μl of lactoperoxidase (Sigma) (0.3 mg/ml), and 30 μl of H_2O_2 (0.03%) for 10 min at 30° with vigorous shaking. Iodination was terminated by the addition of 40 volumes of cold PBS, and the enzyme was removed by two washes with PBS.

Preparation of Cell Lysates. The labeled cells were lysed by one of three procedures. (1) Cells were resuspended to twice the original cell concentration in TKM buffer [0.05 M Tris·HCl– 0.025 M KCl–0.05 M $MgCl_2$ (pH 7.5)] containing 0.25 M sucrose (STKM). Since degradation of radioactive proteins was evident in the initial experiments (see Fig. 3, below), 50,000 K.I.U. of Trasylol (Bayer, 5 ml, 250,000 K.I.U., Kalikrein inactivation units) per liter and 0.2 M iodoacetamide were added to the STKM buffer. The cells were disrupted by homogenization at 4° and fractionated into "cytoplasmic fraction" and "membrane fraction" (13). (*2*) Cells were resuspended at 4° for 15 min in 9 M urea in PBS containing Trasylol and iodoacetamide as above. Debris was removed by centrifugation at 15,000 rpm (27,000 × *g*) for 15 min at 4° and the supernatant ("cytoplasmic fraction") was collected. (*3*) Cells were resuspended in 0.5% Nonidet P_{40} (NP_{40}, Shell Chemical Co.) in PBS–Trasylol–0.2 M iodoacetamide for 15 min at 4°. Debris was removed by centrifugation at 15,000 rpm for 15 min at 4° and the supernatant ("cytoplasmic fraction") was collected. The amount of labeled amino acids incorporated into proteins was determined after precipitation with 5% trichloroacetic acid at 4°. All cell fractions were kept at −20° until used.

Immunoprecipitation and Electrophoresis. Antibody against human Bence–Jones lambda, antibody against human Bence–Jones kappa, and antibody against human IgM (μ-chain specific) (all prepared in goat) were obtained from Melpar, USA. Antibody against human IgG (γ-chain specific) pre-

Abbreviations: Ig, immunoglobulin; T cells, thymus-derived lymphocytes; PBS, phosphate-buffered saline (pH 7.2).

pared in goat was obtained from Hyland Laboratories. The potency and specificity of the antisera were determined by hemagglutination and hemagglutination inhibition tests. Antiserum against μ chain (titer 51,200) was not inhibited by 5 mg/ml of IgG, κ-chain, or λ-chain proteins but was inhibited 8-fold by 10 μg/ml of IgM. Antiserum against κ chain (titer 12,800) was not inhibited by 5 mg/ml of λ chain but was inhibited 4-fold by 10 μg/ml of κ chain. Antiserum against λ chain (titer 3200) was not inhibited by 5 mg/ml of κ chain but was inhibited 2-fold by 10 μg/ml of λ chain.

Radioactive immunoglobulins were isolated from cell lysates by coprecipitation with nonlabeled human myeloma proteins added to the cell cytoplasm and the monospecific antisera described above. Cytoplasmic preparations containing 9 M urea were dialyzed extensively against PBS–Trasylol at 4° before immunoprecipitation. All immunoprecipitations were done at 4° for 16 hr without prior incubation at 37° to minimize proteolysis. Each of the precipitates was collected by centrifugation at 10,000 rpm (12,000 × *g*) for 15 min at 4°, washed five times with cold PBS–Trasylol, suspended in PBS–Trasylol containing 1% Na dodecyl sulfate and 0.5 M urea, and placed in a boiling-water bath for 1 min. As control for nonspecific coprecipitation, an equal amount of precipitate was formed with equine Ig and antibody against equine gammaglobulin prepared in goat (Hyland). Radioactivity in the nonspecific precipitate amounted to 10–30% of that in the specific precipitates. The values reported are corrected for the nonspecific contribution. The dissolved precipitates were characterized by acrylamide gel electrophoresis according to Shapiro *et al.* (14). Before electrophoresis the samples were dialyzed for 2 hr at 4° against 4000 volumes of 0.01 M sodium phosphate buffer (pH 7.1) containing 0.1% Na dodecyl sulfate, 0.5 M urea, and Trasylol. Reduction, when performed, was done with 0.2 M mercaptoethanol for 2 hr at 37°, followed by alkylation with a 10% excess of iodoacetamide at pH 8.0 for 1 hr at room temperature. Electrophoresis was run at 15 mA per gel for 2.5 hr, and the gel was fractionated on a Savant autogel divider (15); fractions were counted in a Packard model 3380 Tricarb spectrophotometer.

RESULTS

Ig Synthesis in Thymus Cells. Thymus cells from two children were studied separately. Since similar findings were obtained, only one set of results is shown.

Electrophoresis on acrylamide gel of the cytoplasmic fraction (Fig. 1*A*) exhibited two main radioactive peaks with the mobility of monomeric subunits of immunoglobulin heavy and light chains and strikingly little of other radioactive protein. The heavier chain corresponded to a standard μ chain. The monospecific antisera precipitated either radioactive μ chains, or κ or λ chains (Table 1). The amount of radioactivity precipitated as immunoglobulin subunits amounted to 29.5% of the total radioactivity incorporated into the cytoplasmic proteins. High proportions of Ig synthesized probably reflects the low absolute level of total protein synthesis in these cells under these conditions. This result is consistent with the pattern in acrylamide gel electrophoresis in Na dodecyl sulfate (Fig. 1*B–D*). The nascent chains in the immunoprecipitates had less electrophoretic mobility than those in the cytoplasm. The nascent μ chains precipitated with the antiserum against μ moved in two peaks, a minor one similar to the IgM monomer (7 S) and a major peak similar to a μ-chain dimer; the latter was evident from their reversion to the monomeric form after reduction and alkylation. The electrophoretic mobility of the λ chains and κ chains in the immunoprecipitates also exhibited major peaks in the location of a light-chain dimer (which coincides with a

TABLE 1. *[^{14}C]Aminoacids incorporated into immunoglobulin subunits in the cytoplasm of human thymus cells obtained by homogenization*

Fraction	Radioactivity (cpm)	Radioactivity (%)
"Cytoplasmic proteins"	21,000	
μ chain	2,100	10.0
κ chain	2,250	10.7
λ chain	1,860	8.8
Total immunoglobulin ($\mu + \kappa + \lambda$)	6,210	29.5

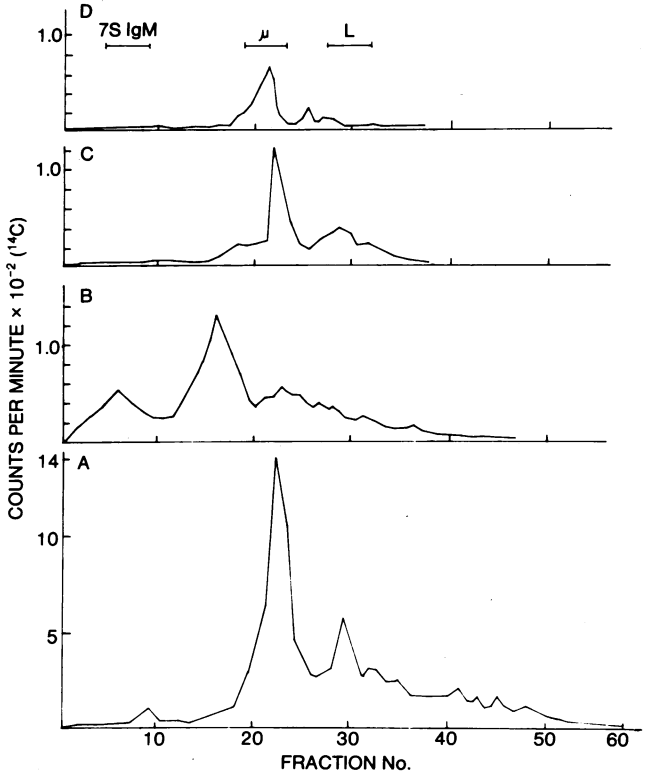

FIG. 1. Acrylamide gel electrophoresis of cytoplasmic protein synthesized by human thymus cells in culture. The cells were labeled with [^{14}C]aminoacids for 180 min and solubilized by homogenization in STKM buffer-Trasylol at 4°. For electrophoresis, solubilized proteins were treated with 1% Na dodecyl sulfate for 1 min at 100° and dialyzed against 0.1% Na dodecyl sulfate in 0.1 M sodium phosphate buffer (pH 7.1), and Trasylol for 120 min at 4°. Immunoprecipitates were dissolved in 1% Na dodecyl sulfate–0.01 M sodium phosphate buffer (pH 7.1)–Trasylol for 1 min at 100° and dialyzed as above. (*A*) "Cytoplasmic fractions;" (*B*) immunoprecipitate obtained by reacting the cytoplasmic fraction with antiserum against μ; (*C*) with antiserum against κ; (*D*) with antiserum against λ. The *bars* indicate the positions of 7S IgM, monomer μ chains, and monomer light chains that were used as standards. Numbers on ordinate have been multiplied by 10^{-2}.

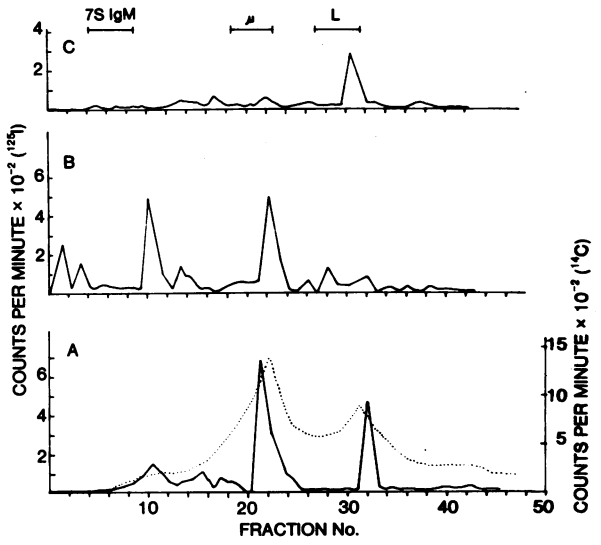

FIG. 2. Acrylamide gel electrophoresis of labeled proteins from thymus cells. Cells were solubilized by NP_{40}-Trasylol-0.2 M iodoacetamide at 4°. Soluble proteins and immunoprecipitates were prepared for electrophoresis as in Fig. 1. (*A*) (*dotted line*) cytoplasmic proteins synthesized by thymus cells in tissue culture using [^{14}C]aminoacids for 180 min; (*solid line*) radioiodinated surface proteins; (*B*) radioiodinated surface Ig obtained by reaction of soluble proteins with antiserum against μ; (*C*) with antiserum against κ.

standard monomeric heavy chain), which also resolved to monomeric form after reduction. It follows that native monomeric μ chains and light chains in the cytoplasmic fraction of the thymocytes were covalently dimerized in the course of the preparation of the immune precipitate.

Igs on the Surface of Thymus Cells. The presence and nature of the Igs on the surface of thymus cells obtained from a third child were studied by a modification of the enzymatic radioiodination procedure. Since the above experiment suggested that the cytoplasmic immunoglobulin chains were not disulfide-linked, it was considered necessary to preclude formation of such linkages, which might result from the use of H_2O_2 in the labeling procedure; the thymus cells were therefore treated with iodoacetamide before iodination.

By acrylamide gel electrophoresis the iodinated proteins were resolved into two peaks, corresponding to the two nascent protein peaks synthesized in short-term tissue culture by cells from the same thymus (Fig. 2*A*). Furthermore, the presence of iodinated μ and light chains was established with appropriate antisera. Addition of antiserum against μ to the soluble fraction yielded peaks corresponding to μ-chain monomers and heavier components, with no peak corresponding to monomeric light chains (Fig. 2*B*), while precipitation with antiserum against κ yielded a single major peak corresponding to light-chain monomer, with no indication of the presence of μ chains in the specific precipitate (Fig. 2*C*). The amount of radioactivity in the precipitated iodinated immunoglobulin chains amounted to about 25% of the total iodinated surface proteins. The ratio of the μ:L chain radioactivity in immunoprecipitates was 0.8:1 (Table 2).

Susceptibility of Ig Chains to Degradation. Preliminary observations suggested that in the absence of the special precaution used, various degrees of proteolysis occurs leading to the formation of peptides smaller than light chains. This is illustrated by the results (Fig. 3) obtained by the use of commonly used procedures for the solubilization of membrane proteins (15). Such procedures involve lactoperoxidase iodination followed by extraction with NP_{40} and overnight dialysis against 0.1% Na dodecyl sulfate, both at room temperature. Extensive degradation of the labeled surface proteins, which is apparent under these conditions, contrasts with the pattern obtained when the presently reported precautions were taken to avoid degradation (Fig. 2*A*). Because of the crucial significance of the type of procedure used for the detection of surface or cytoplasmic immunoglobulin in T cells, various conditions were used to minimize protein degradation, including low temperature, short time for the entire preparative process, and the use of Trasylol as a general inhibitor for proteases (16).

However, with all these precautions we could not entirely eliminate fragmentation in the extracts prepared in 9 M urea and then dialyzed to remove the urea before immunoprecipitation. The two-peak pattern of the urea extract, corresponding to μ and light chains, is altered quantitatively after dialysis (Fig. 4*A*). The degradation of protein, which is evident in this experiment, undoubtedly includes extensive fragmentation of Ig chains. The breakdown of μ chain is suggested by the electrophoretic pattern obtained with the protein precipitated with antiserum against μ (Fig. 4*B*). In addition to a radioactive peak at the location of a μ-chain monomer, another radioactive peak at the location of the light chains was demonstrated, possibly corresponding to Fc fragment of μ chain. It is unlikely that this peak is primarily due to light-chain complexes with μ chains, because antiserum against light chains does not exhibit a peak corresponding to a μ-chain monomer (Fig. 4*C* and *D*). The nature of the slow-moving peaks, which arise only after immunoprecipitation, is not clear; they could be incomplete dissociated immune complexes, due to brevity of the exposure to detergent.

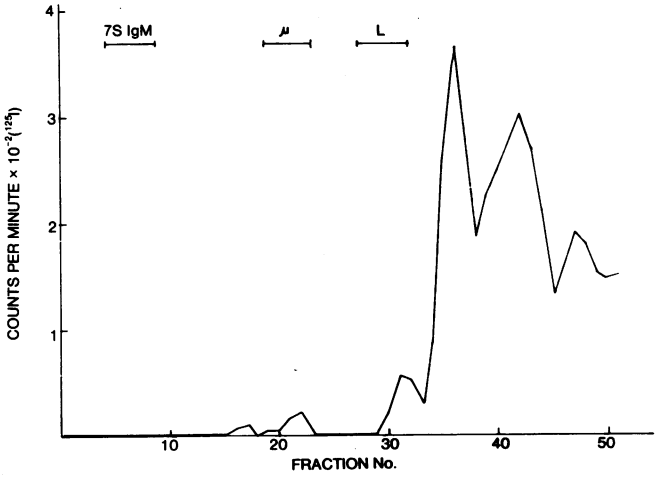

FIG. 3. Acrylamide gel electrophoresis of ^{125}I-labeled surface proteins from thymus cells. Iodinated cells were solubilized with NP_{40} at room temperature for 10 min. For electrophoresis, solubilized proteins were treated with 1% Na dodecyl sulfate for 1 min at 100° and dialyzed against 0.1% Na dodecyl sulfate in 0.01 M phosphate buffer (pH 7.1) at room temperature for 16 hr.

DISCUSSION

The present study suggests an explanation for the divergent results reported by other investigators concerning T-cell surface Ig (10, 11). Thus, sensitivity to proteolysis during conventional isolation procedures (NP_{40}, 9 M urea, or homogenization) could account for their inconsistent detection (11). Indeed, the most effective demonstration of T-cell Ig was provided by Cone *et al.*, who omitted cell breakdown by denaturing agents and examined the material shed by intact cells (10).

The detection of noncovalently linked monomeric chains was made possible in the present study by an isolation procedure that minimized formation of interchain disulfide bridges. With this procedure the bulk of newly synthesized cytoplasmic protein in T cells was Ig; this is in sharp contrast to what has been seen in B cells or plasma cells (13, 14). μ chain was the only class of heavy chain detected; the antiserum against μ used for this purpose was shown by hemagglutination inhibition assays to lack antibodies that precipitate γ, κ, or λ chains.

With the same precautions, the surface Ig detected by enzymic iodination of the cells was also found to be noncovalently bound μ and light chains. The possibility that the surface Ig was passively absorbed to the cell is unlikely since the active synthesis of Ig in these cells was demonstrated. Furthermore, IgM originating from plasma cells or B cells would be in a covalently linked structure (17). The possibility that the chains on T cells are also covalently assembled in the form of an IgM monomer appears unlikely since the antiserum against μ precipitated little or no radioactive light chains. Whether the T-cell chains are associated in some noncovalent structure is uncertain. It is also uncertain whether the noncovalent linkage of Ig chains is related to the enhancing effect of sulfhydryl-containing reagents on the mixed lymphocyte reaction (18) and on the response of mouse spleen cells to immunization *in vitro* with sheep erythrocytes (19). There is an apparent discrepancy between our evidence for nondisulfide-linked structures and other evidence for a stable IgM monomer solubilized from the cell surface after iodination by the lactoperoxidase procedure (12). It should be noted, however, that the latter procedure involves the presence of H_2O_2, and the sulfhydryl groups of the surface Ig would thus be expected to undergo oxidation, resulting in formation of interchain disulfide bridges. It is perhaps significant in this connection that Marchalonis *et al.* (9) report the occasional appearance of light-chain dimers in their Ig preparations from thymus lymphocytes.

TABLE 2. *^{125}I incorporated into immunoglobulin subunits on the surface of human thymus cells solubilized with NP_{40}*

Fraction	Radioactivity (cpm × 10^{-4})*	Radioactivity (%)
Total proteins	31.5	
μ chain	3.32	10.5
κ chain	2.65	8.4
λ chain	1.5	4.8
Total immunoglobulin ($\mu + \kappa + \lambda$)	7.47	23.7

* Numbers in column have been multiplied by 10^{-4}.

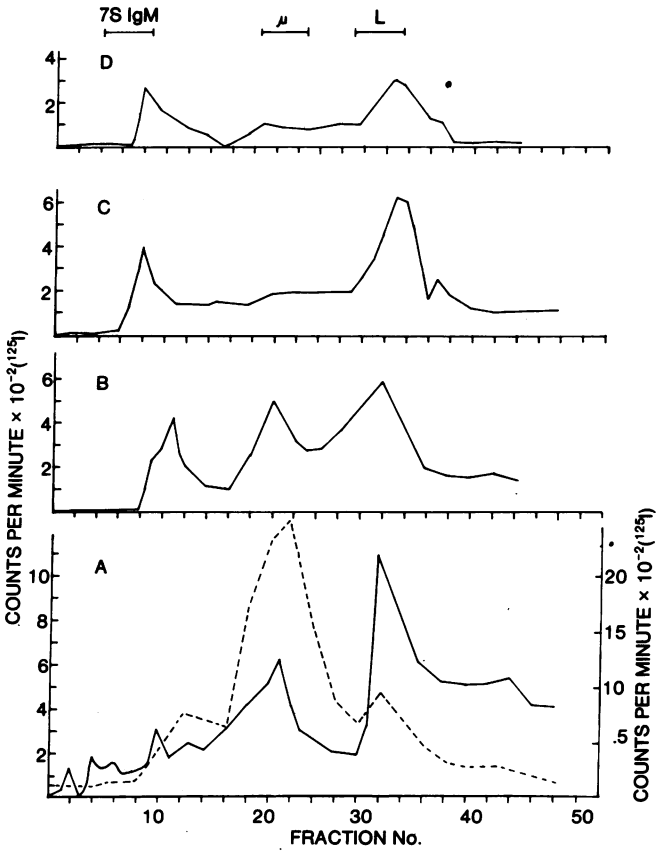

FIG. 4. Acrylamide gel electrophoresis of ^{125}I-labeled surface proteins from thymus cells. Iodinated cells were solubilized with 9 M urea–Trasylol–0.2 M iodoacetamide at 4° followed by exhaustive dialysis against PBS–Trasylol for 16 hr at 4°. The labeled proteins and precipitated Ig were treated for electrophoresis as in Fig. 1. (*A*) (*dashed line*) labeled proteins before exhaustive dialysis against PBS–Trasylol; (*solid line*) dialyzed labeled surface proteins; (*B*) labeled surface Ig obtained by reaction of dialyzed soluble protein with antiserum against μ; (*C*) antiserum against κ; (*D*) antiserum against λ.

Our demonstration of the reduced state of surface Ig was based on the use of iodoacetamide before iodination. Since the Ig obtained from the surface as well as from cytoplasm of the thymus cells were present in substantial amounts (25–30% of all newly synthesized protein), and the heavy and light chains were not covalently linked, the isolated Ig is unlikely to have arisen from a small contamination of thymus cells with B cells which, according to Vitteta *et al.* (11), could contribute approximately 0.7–2% of iodinated surface protein as covalently linked 19S IgM, 8S IgM, and IgG.

We conclude that T cells possess surface Ig of a distinctive structure, consisting of noncovalently linked μ and light chains. The significance of this structure for the specific function of the T cells needs further investigation.

NOTE ADDED IN PROOF

The methods utilized in the above paper when applied to thymus cells revealed the synthesis of Ig by the cells and their presence in the surface membrane. Different Ig's are present in the thymus cells of different mouse strains, IgA in the cells of BALB/c, IgM in those of C57Bl.

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