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Research Article

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ABSTRACT Human α_1-microglobulin was isolated from the urine of patients with tubular proteinuria, and its molecular weight was established by sodium dodecyl sulfate-polyacrylamide gel electrophoresis at 33,000 daltons. The carbohydrate content was 21.7%. Anti-α_1-microglobulin serum was prepared and observed to react monospecifically in gel diffusion to purified α_1-microglobulin, as well as to normal human serum and urine. Sera from the domestic chicken, mouse, rat, rabbit, dog, calf, cow, goat, sheep, and horse, however, did not react to anti-α_1-microglobulin serum in immunodiffusion. The lymphocyte culture supernate was found to contain α_1-microglobulin. Both thymus-derived(T)- and bone marrow-derived(B)-lymphocyte culture media clearly displayed a specific precipitin line against anti-α_1-microglobulin serum when tested with the Ouchterlony immunodiffusion method. The tissue distribution of α_1-microglobulin was studied under immunofluorescence, and a positive staining was recognized on the lymphocyte surface. Identical staining patterns were noted on both T and B lymphocytes, though B lymphocytes took a more intense stain. It would thus seem quite possible that lymphocytes are the primary source of α_1-microglobulin and that this is filtered through the glomerular basement membrane and partly reabsorbed by the renal tubules. This, then, would suggest the possibility that α_1-microglobulin shares some immunological role in vivo with lymphocytes and(or) is one of the membrane proteins of lymphocytes.

INTRODUCTION

Recently, a new, low molecular weight protein was isolated from the urine of patients with tubular proteinuria, and its physicochemical properties were reported (1–3). Ekström et al. designated this protein as α_1-microglobulin (1) because it migrated at the α_1-region on agarose gel electrophoresis. This protein has subsequently been detected in normal human serum, urine, and cerebrospinal fluid; and its serum concentration has been reported to increase in patients with renal insufficiency (1–3).

In the present experiments, a low molecular weight glycoprotein was isolated from the urine of patients with tubular proteinuria because of chronic cadmium poisoning and Fanconi's syndrome. This isolated glycoprotein closely corresponds to α_1-microglobulin in terms of electrophoretic mobility, amino acid and carbohydrate composition, and molecular weight. Its monospecific antisera, moreover, were prepared successfully, and its tissue distribution in lymphocytes, human lymphoid cell lines, and autopsy materials were studied by the immunofluorescence technique.

METHODS

Preparation of urine. Pooled urine from three patients with marked tubular proteinuria was concentrated ≅200 times by ultrafiltration in a Membrane G-05T, 76 m/m ϕ, with a normal retention limit of ≅5,000 daltons (Bioengineering Co., Tokyo).

Purification of α_1-microglobulin. Following the method of Svensson and Ravnskov (2), concentrated urine was analyzed with affinity chromatography using a column (2.5 × 40 cm) packed with Con-A-Sepharose (Pharmacia, Inc., Uppsala, Sweden) equlibrated with a 0.1 M acetate buffer at pH 6.0 containing 1 M NaCl and 10 mM $MnCl_2$, $MgCl_2$, and $CaCl_2$. Elution was conducted using 0.3 M α-methyl-D-mannoside (Sigma Chemical Co. St. Louis, Mo.) added to the starting buffer. The flow rate was 24 ml/h, and fractions of 4 ml each were collected. The material obtained after Con-A-Sepharose chromatography was gel-filtered on a Sephadex G-75 (Pharmacia, Inc.) column (2.5 × 90 cm), equilibrated with a 0.01 M Tris-HCl buffer at pH 7.3 and containing 0.05 M NaCl. The flow rate of the 7-ml fractions was 14 ml/h. For the pooled material obtained through Sephadex G-75 gel filtration, ion-exchange chromatography was conducted in a 2.5 × 40-cm column packed with Whatman DE 52 (Whatman Chemicals, Div. W & R Balston, Maidstone, Kent, England), equilibrated in a 0.01 M Tris-HCl buffer at pH 7.8, and eluted with a linear NaCl gradient from 0.05 M to 0.2 M NaCl with a total volume of 800 ml. The collected material, after ion-exchange chromatography, was run through a Sephadex G-25 column for final desalting.

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Estimation of molecular weight. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, using 7.5% gels, was carried out following the method of Weber and Osborn (4) for estimation of molecular weight. Reference proteins included trypsin inhibitor (Sigma Chemical Co.), bovine serum albumin (Seikagakukogyo Co., Tokyo), RNA-polymerase (Boehringer Mannheim GmbH, West Germany), myoglobin (Sigma Chemical Co.), and cytochrome *c* (Seikagakukogyo Co.).

Amino acid and carbohydrate analyses. Amino acids were analyzed with a model JLC 6AH Automatic Amino Acid Analyzer (Japan Electron Optics Laboratories, Ltd.) following the method of Spackman et al (5). Samples were hydrolyzed in vacuo in 6 N HCl at 110°C for 24 and 72 h, and carbohydrate analysis was performed as follows. Hexose was determined according to the method of Dubois et al. (6), and sialic acid by the method of Warren (7) after hydrolization with 0.1 M H_2SO_4 at 80°C for 1 h in a sealed tube. Gardell's method (8) was used in the analysis of hexosamine after the addition of 4 N HCl, and hydrolization at 100°C for 6 h and vacuum drying.

Preparation of antisera. The purified protein was dissolved in a 0.3 mg/ml saline and thoroughly mixed with an equal volume of complete Freund's adjuvant. Two adult rabbits were injected with this mixture in approximately 40 intracutaneous sites over the dorsal region repeatedly once during each of five consecutive weeks. Both rabbits produced the antibody successfully and they were bled 7 wk after the first injection. The gammaglobulin fractions were separated by fractional precipitation using ammonium sulfate, and antisera were absorbed specifically with Sephorose 4B (Pharmacia, Inc.) column chromatography (3.0 × 13 cm) containing albumin, α_1-acid glycoprotein, and α_1-antitrypsin. The specificity of antisera was confirmed using Ouchterlony immunodiffusion (9) and agarose gel immunoelectrophoresis (10). Antisera raised against purified protein produced a single precipitation arc when tested on immunoelectrophoresis, and migrated at the fast α_1-region. The antibody titer of the rabbit purified protein antiserum was determined by single radial immunodiffusion according to the method of Becker (11), and the antisera precipitated between 0.26 and 0.54 mg of purified protein per milliliter. The lower limit of detection for purified protein on immunoelectrophoresis, observed in stepwise dilution of purified protein, was 15 μg/ml.

Immunodiffusion and immunoelectrophoresis. Immunodiffusion using Ouchterlony's method was conducted with 1% agarose gel (Difco Laboratories, Detroit, Mich.) in veronal buffer (pH 8.6; ionic strength, 0.05) at room temperature (22°C) for 20 h, stained with 0.5% Amido Black 10B (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for 10 min, and destained with 5% acetic acid solution. Immunoelectrophoresis was carried out with the method of Grabar and Williams (10). The purified protein and its antisera were applied in various combinations to sera from the domestic chicken, mouse, rat, rabbit, dog, calf, cow, goat, sheep, and horse, as well as β_2-microglobulin (Fujizoki Co., Tokyo), and anti-β_2-microglobulin (Dakopatts, Copenhagen, Denmark).

Lymphocyte separation procedure. The procedure for obtaining T and B cells from human tonsil and peripheral blood has been described in detail previously (12, 13) and reviewed briefly here. Tonsillar and peripheral blood lymphocytes were first depleted of phagocytic cells by carbonyl-iron (3–6 μm, Wako Pure Chemicals, Osaka, Japan) ingestion. Mononuclear cells were separated by Ficoll-Hypaque (d = 1.077, Pharmacia, Inc.) sedimentation, centrifuged at 450 g for 20 min, and were designated as 'unfractionated.' Unfractionated cells contained virtually no monocytes or macrophages (<0.1%). These cells were then applied to a nylon fiber column (Semidull Nylon Staple, Dupont Instruments, Wilmington, Del.), incubated at 37°C for 45 min, and eluted with Eagle's Minimum Essential Medium (Nissui Seiyaku Co., Tokyo) supplemented with 2% fetal calf serum (FCS, Flow Laboratories, Rockville, Md.) Each nylon fiber column-passed and nylon fiber column-retained cell fraction was collected separately, and cultured for erythrocyte (E)-rosette[1] formation with sheep erythrocytes (Japan Biological Material Center, Tokyo), incubated at 37°C for 15 min, spun down, and reincubated at 4°C for at least 2 h. The cells were then gently resuspended and layered over a Ficoll-Hypaque and centrifuged at 450 g for 20 min. E-rosette forming cells were obtained from the bottom of Ficoll-Hypaque, non-E-rosette forming cells from the interface. The cells thus collected were washed and further subjected to another cycle of E-rosette formation.

Lymphocyte culture. Lymphocyte concentrations were adjusted to 1 × 10^6 cells/ml in a culture medium (RPMI 1640, Nissui Seiyaku, Tokyo) containing 6% FCS, and incubated in a Tissue Culture Flask (Lux 5375, 250 ml, Lux Scientific Corp., Calif.) for 72 h in 5% CO_2, 95% atmosphere. Phytohemagglutinin (Difco Laboratories) was used as a T-cell mitogen at a concentration of 10 μg/ml, whereas *Staphylococcus aureus* Cowan I organisms (SpA, provided by Dr. T. Matsuhashi, Institute of Medical Science, University of Tokyo) were used at 0.01% vol/vol as a B-cell mitogen. All doses of mitogens were used at optimal concentration for mitogenicity.

Preparation of pepsin-digested fragment of anit-α_1-microglobulin antibody. Anti-α_1-microglobulin antibody (0.7 g) was mixed with 14 mg of crystallized pepsin in 0.01 M sodium acetate buffer at pH 4.5 and allowed to react for 20 h at 37°C (14, 15). The mixture was adjusted to pH 8.0 to inactivate the pepsin, and dialysed in cold, 0.2 M borate buffer at pH 8.0. The pepsin-digested fragment was separated by Sephadex G-150 (40–120 μm) column chromatography (2.5 × 100 cm), equilibrated with 0.2 M borate buffer at pH 8.0 and containing 0.01 M sodium chloride. The fraction at the second peak was collected. After concentration, the antigenicity of the Fab'$_2$ of the antibody immunoglobulin was confirmed by immunoelectrophoresis using anti-rabbit immunoglobulin (Ig)G antiserum and the purified antigen (10). Its molecular weight was estimated by SDS-polyacrylamide gel electrophoresis. Protein concentration was measured by the method of Lowry et al. (16).

Indirect immunofluorescence of lymphocytes and erythrocytes. The isolation lymphocytes (2 × 10^6/ml) were washed thee times with phosphate-buffered saline (PBS), followed by incubation in 100 μl of the specific antiserum (1:5 in PBS) at 37°C for 30 min. After washing three times with cold PBS, 100 μl of fluorescein isothiocyanate (FITC, Isomer 1, Sigma Chemical Co.)-conjugated anti-rabbit gammaglobulin goat serum (1:5 in PBS, Hoechst Co., West Germany) was added, and incubated at 37°C for 30 min. The cells were again washed at least three more times with PBS and then mounted directly. Nonimmunized rabbit serum was used as a control in place of the first antibody solution. For the observation of the cytoplasm, the cells were suspended in 7% bovine serum albumin (Hyland Diagnostics Div., Travenol Laboratories, Inc., Costa Mesa, Calif.) and spread on glass slides. These slides were then fixed with 95% ethanol and incubated at 4°C for 30 min. The procedure of the following experiment was similar to that described above.

Lymphoblastoid B-cell lines (B-ALL) (17) and P10/Shibata

[1] *Abbreviations used in this paper*: E-rosette, erythrocyte rosette; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline; protein HC, human complex-forming glycoprotein; SDS, sodium dodecyl sulfate.

(18), maintained in our laboratory, were also used. Both cell lines were grown in RPMI 1640 containing 20% (vol/vol) heat-inactivated FCS, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. The cells were maintained in a logarithmic growth phase at 1–2 × 10^6 cells/ml. Both cell lines were stained similarly as described above. The reading was made under fluorescence microscope (Olympus Co., Tokyo).

The anti-α_1-microglobulin serum that had been absorbed by purified α_1-microglobulin lost its reactivity against human viable lymphocytes as judged by indirect immunofluorescent staining. The purified α_1-microglobulin was added to aliquots of the antiserum in amounts corresponding to two, four and eight times its antibody titer. Purified IgG (available at our laboratory), β_2-microglobulin, human albumin (Miles Laboratories, Inc., Ill.), and fibrinogen (AB Kabi, Stockholm, Sweden) in molar amounts equivalent to the various purified protein additions were also added to separate aliquots of the anti-α_1-microglobulin serum. After incubation for 1 h at 37°C followed by incubation overnight at 4°C, the absorbed antiserum aliquots were cleared by centrifugation.

Furthermore, indirect immunofluorescent staining of α_1-microglobulin on the surface of human erythrocytes was studied as follows. Heparinized venous blood was obtained from healthy adults. After centrifugation, the plasma and buffy coat were removed and the remaining erythrocytes were washed six times with PBS. Then 5 × 10^6 cells in 50 μl PBS were treated with the specific antisera in the manner previously described. Preparations with antisera that had absorbed α_1-microglobulin were produced in the manner described above.

Immunofluorescent method for studying tissue distribution. Highly purified T and B lymphocytes were used for immunofluorescent staining. Other human tissues were obtained from an adult patient who had died of gastric carcinoma. Autopsy specimens taken from the thymus, palatine tonsils, lymph nodes, spleen, kidney, liver, pancreas, ileum, lungs, and adrenal glands within 3 h postmortem, were fixed with 95% cold ethanol and embedded in paraffin (19, 20). The following procedure was then carried out as described by Hamashima et al. (21). Paraffin sections (4 μm thick) of these tissues were deparaffinized and washed with cold PBS. They were then incubated with anti-α_1-microglobulin serum (diluted 1:2 with PBS) for 60 min at 37°C, washed with PBS for 15 min, and incubated with FITC-labeled anti-rabbit gammaglobulin goat serum with an FITC: protein molar ratio of 1.45 and a protein concentration of 0.8 mg/ml for 60 min at 37°C. They were then washed again with PBS for 15 min, mounted in buffered glycerol, and examined under a fluorescence microscope (Zeiss Illuminator I equipped with exciter filter UG 1 and barrier filter 44, Carl Zeiss, Inc., New York). The anti-α_1-microglobulin serum had previously been absorbed with human liver powder to remove nonspecific staining (22). This absorbed antiserum still reacted well with purified α_1-microglobulin on immunoelectrophoresis and Ouchterlony immunodiffusion. Nonimmunized rabbit serum was used for control staining.

RESULTS

Purification of α_1-microglobulin. After a series of column chromatographies with Con-A-Sepharose and Sephadex G-75, the final separation was accomplished by ion-exchange chromatography. Upon elution, the α_1-microglobulin formed a rather broad peak. SDS-polyacrylamide gel electrophoresis was used to determine its purity. SDS-polyacrylamide gel electrophoretic patterns of the starting material, of normal human serum, and of the sample obtained after three runs are shown in Fig. 1. Many proteins were present in the starting material, but a single protein band was observed after the final run, suggesting size homogeneity. The brown color of the purified sample remained even after the final purification.

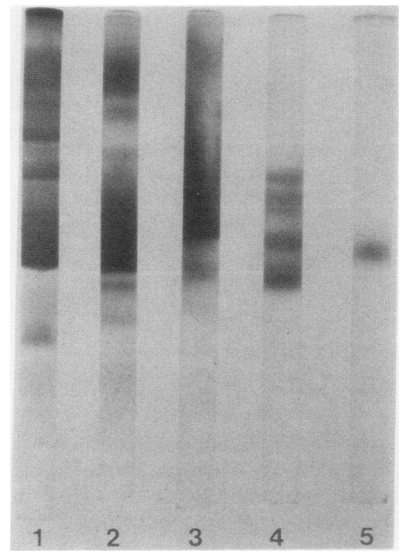

FIGURE 1 SDS-polyacrylamide gel electrophoresis of (1) normal human serum; (2) concentrated urine as the starting material; (3) pooled α_1-microglobulin-rich fraction obtained by Con-A-Sepharose chromatography; (4) pooled α_1-microglobulin-rich fraction obtained by Sephadex G-75 chromatography; (5) purified α_1-microglobulin obtained by pooling the peak fractions after ion-exchange chromatography. The process of purification was made clear in these patterns from Nos. 3 to 5. One narrow zone, suggesting size homogeneity, was found in No. 5.

Examination of physicochemical properties. Molecular weight, as determined by SDS-polyacrylamide gel electrophoresis, was 33,000. The results of amino acid and carbohydrate analyses are shown in Table I. Carbohydrate content was 21.76%, occupying a relatively large part of the molecule.

Immunodiffusion and immunoelectrophoresis. The purity of the isolated α_1-microglobulin was assessed by Ouchterlony immounodiffusion analysis and by immunoelectrophoresis. Only a single precipitation line and a single continuous arc were observed when the purified α_1-microglobulin was tested against the antiserum obtained from immunized rabbits. A single continuous arc migrated at the α_1-region on immunoelectrophoresis. Anti-α_1-microglobulin serum reacted against normal human urine concentrated 100 times, normal human serum, and the serum and 50 times concentrated urine from a patient with tubular proteinuria. It did not, however, react against any of the animal sera listed in Methods. Neither did the purified α_1-microglobulin react with antisera against albumin, α_1-

TABLE I
*Properties of Human α_1-Microglobulin**

Composition		
Amino acid	*residues/molecules*	%
Aspartic acid	15	
Threonine	17	
Serine	11	
Glutamic acid	23	
Proline	12	
Glycine	15	
Alanine	10	
Valine	10	
Half-cystine		
Methionine	4	
Isoleucine	15	
Leucine	11	
Tyrosine	8	
Phenylalanine	10	
Histidine	5	
Lysine	7	
Arginine	10	
Carbohydrate		
Hexose (galactose:mannose)		6.80
Hexosamine		9.20
Sialic acid		5.76
Molecular weight	33,000	

* Values obtained from 24- and 72h hydrolyses.

antitrypsin, α_1-acid glycoprotein, or α_1-antichymotrypsin.

The supernates of each T- and B-lymphocyte culture medium, concentrated 120 times by Minicon Ultrafiltration Apparatus (Amicon Corp., Lexington, Mass.), clearly demonstrated a single precipitin line against the monospecific anti-α_1-microglobulin serum, and showed complete identity as illustrated in Fig. 2.

The culture media with and without 6% FCS used for lymphocyte culture were tested also against anti-α_1-microglobulin serum after being concentrated 100 times, though no precipitin line appeared. Neither was any cross reactivity demonstrated between β_2-microglobulin and purified α_1-microglobulin.

Immunofluorescence examination of tissue distribution. A positive stain for α_1-microglobulin was demonstrated on the surface of most of the lymphocytes, as shown in Fig. 3. Both T and B lymphocytes were also stained positively, and surface staining of B lymphocytes was more intense than that of T lymphocytes. The fluorescence of most of the lymphocytes was dispersed evenly over the cell surface (Fig. 3A), sometimes appearing as small aggregates or spots or caplike formations (Fig. 3B, C). No fluorescence, however, was found in the cytoplasm. No fluorescent cells observed

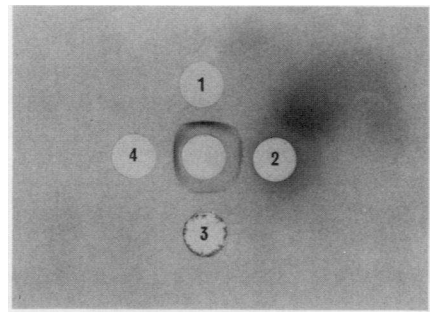

FIGURE 2 Ouchterlony immunodiffusion analysis of purified α_1-microglobulin (1), concentrated supernate of T-lymphocyte culture medium (2) and B-lymphocyte culture medium (3), and concentrated urine of a patient with tubular proteinuria (4). The center well contained anti-α_1-microglobulin serum. The α_1-microglobulin from different sources produced reactions of immunological identity.

when the lymphocyte preparations were incubated with PBS instead of the rabbit antiserum. Most of the cells did not fluoresce positively when the rabbit antiserum was replaced by nonimmunized rabbit serum. When the anti-α_1-microglobulin serum was absorbed with increasing amounts of the pure α_1-microglobulin, the number of fluorescent lymphocytes diminished significantly. Established human lymphocyte lines tested for the surface α_1-microglobulin clearly showed a positive result with the cells of B-cell lines. Almost all the cells in the B-ALL and P10/Shibata lines were fluorescent.

When isolated lymphocytes were incubated with the Fab'$_2$ fragment of anti-α_1-microglobulin rabbit IgG and then with FITC-conjugated anti-rabbit IgG goat serum, practically all lymphocytes were fluorescent. However, when replaced by an equal concentration of Fab'$_2$ fragments of nonimmunized rabbit IgG very few cells fluoresced. When the Fab'$_2$ fragments against α_1-microglobulin were diluted serially, the number of fluorescent cells decreased. Absorption of the rabbit antiserum with the purified α_1-microglobulin in amounts equivalent to two, four, and eight times the antibody titer drastically decreased the numbers of the cells showing a positive fluorescence. However, absorption of the rabbit antiserum with equivalent molar amounts of other plasma proteins, such as albumin, β_2-microglobulin, fibrinogen, and IgG, had no diminishing effect on the number of fluorescent cells.

As to α_1-microglobulin on the surface of erythrocytes, no cells showed membrane fluorescence. When the rabbit antiserum against α_1-microglobulin was replaced by normal rabbit serum or by rabbit antiserum against human albumin, no cells showed membrane fluorescence. A similar result was obtained when the antiserum against α_1-microglobulin was absorbed with increasing amounts of the pure α_1-microglobulin. Various tissues obtained from the autopsy materials were simi-

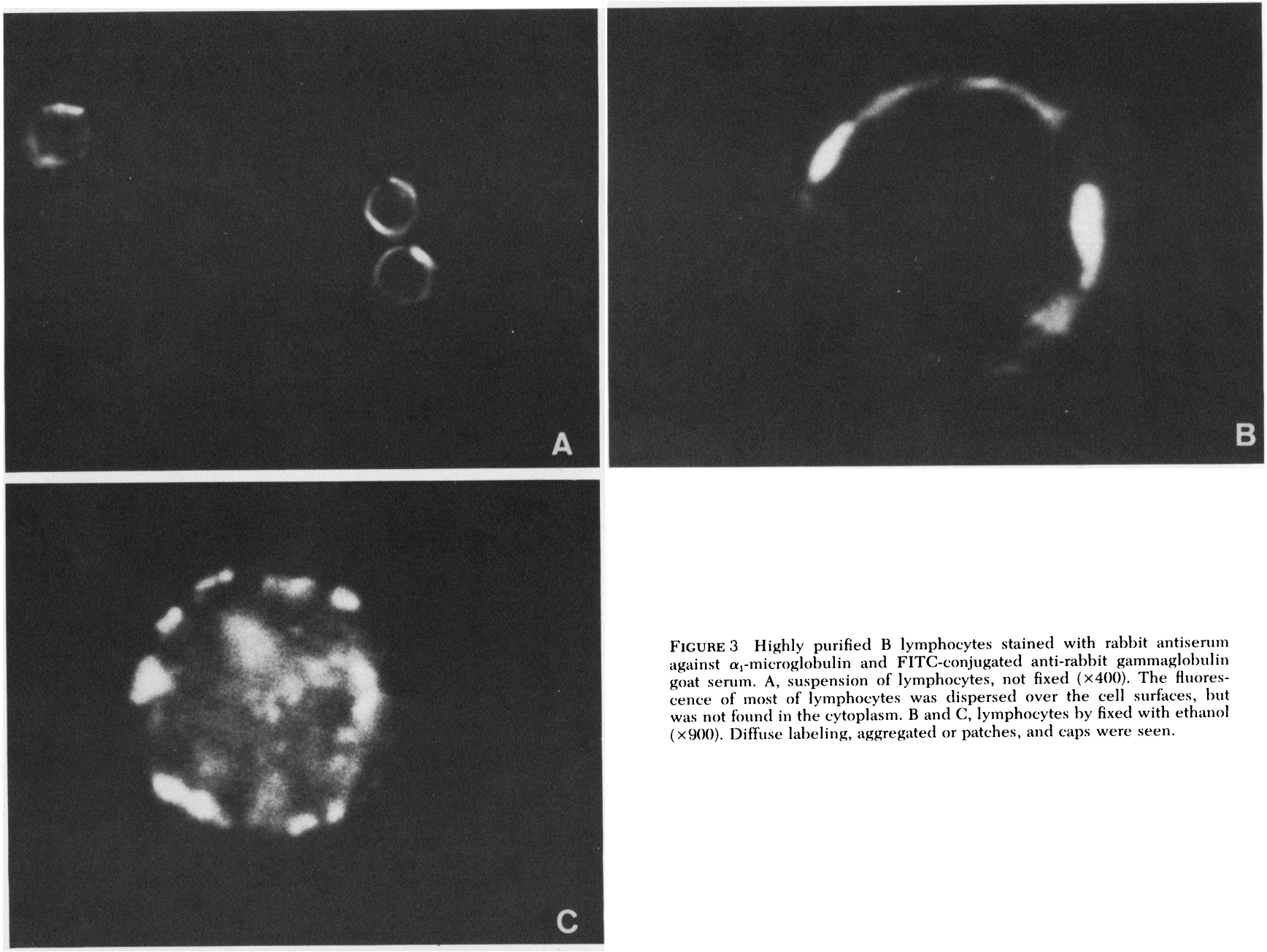

FIGURE 3 Highly purified B lymphocytes stained with rabbit antiserum against α_1-microglobulin and FITC-conjugated anti-rabbit gammaglobulin goat serum. A, suspension of lymphocytes, not fixed (×400). The fluorescence of most of lymphocytes was dispersed over the cell surfaces, but was not found in the cytoplasm. B and C, lymphocytes by fixed with ethanol (×900). Diffuse labeling, aggregated or patches, and caps were seen.

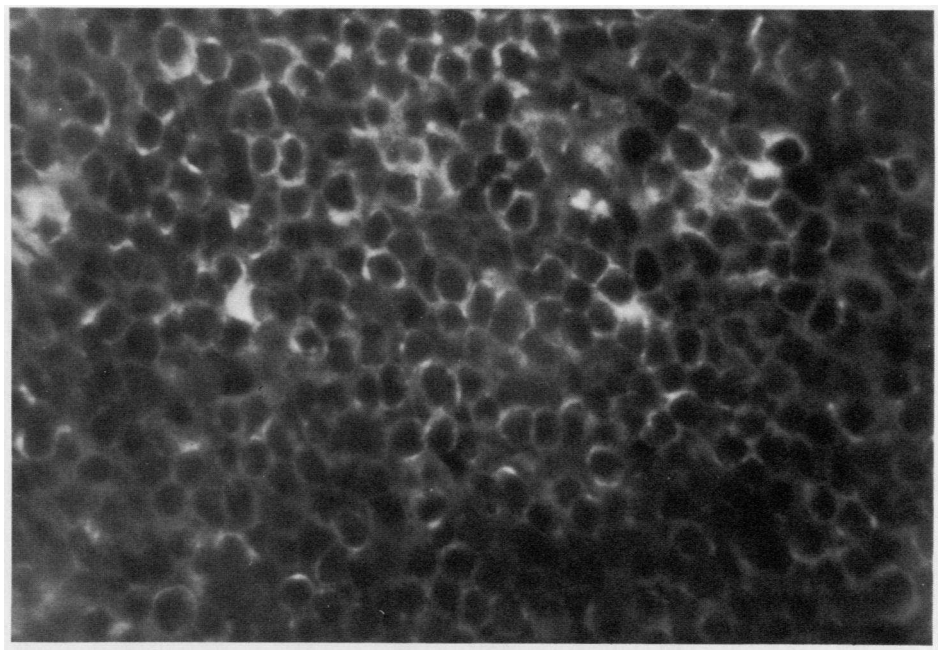

FIGURE 4 Fluorescence patterns of the lymph node (×400). Most of the lymphocytes in the lymph node were stained positively, with the evenly distributed surface fluorescence. These lymphocytes were diffusely and evenly distributed in the organ, and displayed a honey-comb appearance under fluorescence microscopy.

larly tested as described above. Positively stained lymphocytes were diffusely scattered throughout in various lymphoid organs such as the thymus, palatine tonsils, lymph nodes, and spleen, and produced a honeycomb appearance under the fluorescence microscope (Fig. 4). Cells other than lymphocytes, especially hepatocytes, glomerular endothelial, mesangial, and various epithelial cells, as well as uriniferous tubular epithelium were all negative. These lymphoid tissues became completely negative when replaced by nonimmunized rabbit serum or the rabbit antiserum blocked by the purified α_1-microglobulin.

DISCUSSION

Plasma proteins with a molecular weight of <50,000 are present only in quite low concentrations in the blood of healthy individuals. Such proteins seem to pass the glomerular basement membrane in the kidney with relative ease and are probably reabsorbed and catabolized in the renal tubules (23). Only a small amount of plasma proteins is normally excreted in final urine. Patients with tubular proteinuria as a result of renal tubular dysfunction, however, excrete considerable amounts of low molecular weight plasma proteins (24–27).

More recently, Ekström et al. (1, 3) reported a urinary α_1-glycoprotein of low molecular weight and designated it α_1-microglobulin. Subsequently, other investigators have reported on some properties of α_1-microglobulin (2).

A glycoprotein from the urine of patients with tubular proteinuria was purified by the method of Svensson and Ravnskov (2), and its physicochemical properties were examined in the present study. The final material, purified with ion-exchange chromatography, showed a single band on SDS-polyacrylamide gel electrophoresis, as illustrated in Fig. 1. This protein has migrated at the α_1-region, showing a broad, symmetric precipitin arc against the antiserum of this protein on immunoelectrophoresis. The brown color, again, did not disappear, even after final purification. The reason for the brown color of this protein, it has been suggested, is the possible presence of a tightly linked and unidentified chromophore material (3).

The results of amino acid and carbohydrate analyses of the purified protein were extremely similar to those reported by other investigators (1–3) (Table I), though there was one very minor difference. The molecular weight value of this protein, determined by SDS-polyacrylamide gel electrophoresis, was close to the value of 31,500 daltons obtained by Svensson and Ravnskov (2). Ekström et al. (3) reported its molecular weight to be 26,700 by sedimentation equilibrium ultracentrifugation, or 24,800 by gel chromatography in 6 M guanidine hydrochloride. The reason for this discrepancy may be the presence of a carbohydrate moiety that increased the water of hydration and thus effective size, as well as the seeming resistance of α_1-microglobulin to transform in the presence of SDS into compact rodlike molecules. Both these properties could result in

overestimation of molecular weight upon SDS-gel electrophoresis (28–30). These results suggest that the purified protein closely corresponds to α_1-microglobulin.

Antisera from the immunized rabbits were prepared. All antisera were appropriately absorbed equivalently, yielding a single precipitation line in immunoelectrophoresis and Ouchterlony gel diffusion. Anti-α_1-microglobulin serum did react against normal human serum, with the development of only one precipitin line. The presence of α_1-microglobulin in both normal human serum and concentrated normal human urine was established through Ouchterlony immunodiffusion analyses using anti-α_1-microglobulin serum. The reactions indicated identical serum, namely α_1-microglobulin. Various animal sera mentioned above, however, did not react against anti-α_1-microglobulin serum.

Knowledge of the origin of α_1-microglobulin is still scarce. In the attempt to shed more light on this problem, the present study delved into the tissue distribution of α_1-microglobulin. The concentrated supernates of both T- and B-lymphocyte culture media showed a specific precipitation reaction against anti-α_1-microglobulin rabbit serum and displayed complete identity (Fig. 2). On immunofluorescence study, the surface of the lymphocytes were stained positively (Fig. 3), with both T and B cells taking stains of different intensities; B cells were more intensely stained than T cells.

Observing the tissue distribution of α_1-microglobulin under immunofluorescence, lymphocyte-rich organs such as the thymus, palatine tonsils, lymph nodes, and spleen were stained strongly (Fig. 4), revealing a typical honey-comb appearance. However, no other cellular nor supporting element in any of the organs displayed a positive stain.

The study by Svensson and Ravnskov (2) found no hint concerning the function or site of α_1-microglobulin synthesis, although it seemed unlikely that α_1-microglobulin was synthesized in the liver, like most other plasma proteins. Several possibilities follow from the present study. First, the presence of α_1-microglobulin in the supernate of lymphocyte culture medium indicates that lymphocytes produce α_1-microglobulin. This would mean that mitogen-stimulated T and B lymphocytes in culture produce and secrete α_1-microglobulin into the medium. Furthermore, most of the lymphocytes were stained positively, whereas no other cells displayed a similar staining pattern. It is thus suggested that these cells are the primary producers of α_1-microglobulin. We know that β_2-microglobulin, one of the low molecular weight plasma proteins, is present on the surface of cultured human lymphoid cells and it is actively produced into their culture medium (12, 31–35). Tejler et al. (36, 37) have also reported a similar protein which they termed protein HC: human complex-forming glycoprotein, heterogeneous in charge (36–38). Further work is needed to elucidate the exact relation between α_1-microglobulin and protein HC because they differ considerably in their amino acid composition and carbohydrate content (1–3, 36), though they were shown to be closely immunochemically related by double radial immunodiffusion (36). Also, Tejler et al. (37) and Pearstein et al. (39) reported the presence of protein HC on the cell surface of lymphocytes, erythrocytes, and human fibroblast lines, and they suggested that the protein HC was a surface component of many normal human cell types. However, in the present study, α_1-microglobulin was not found on the surface of human erythrocytes with immunofluorescence, though fibroblast lines were not studied. Second, there is the question of what role, if any, is played by α_1-microglobulin in cellular and humoral immunity in vivo. The present findings suggest that, although its function is still unknown, α_1-microglobulin plays some role in immunological reactions and(or) shares some structural roles with lymphocytes. It is thus suggested that α_1-microglobulin and protein HC are related to other cell surface markers like β_2-microglobulin, human histocompatibility antigens, immunoglobulin, and so on. Indeed, Bernier at al. (40) have reported a strong reaction between protein HC and anti-histocompatibility antigen-A9 antisera. Protein HC has been known to have a complex-forming capacity. It would thus be conceivable that both glycoproteins (perhaps largely identical with only minor discrepancy) could be "carrier" proteins with a great affinity for some membrane proteins.

Accordingly, α_1-microglobulin remains an interesting protein with regard to its immunological role. There is a clear need for further elucidation of its biological functions. Clinically, this protein is found at elevated levels in the serum and urine of patients with renal failure (1–3), so it can be taken as an indicator in studies of pathophysiological aspects of renal disorders.

This research is being expanded to include its quantitation in supernates of various cell lines by radioimmunoassay and its correlation to DNA synthesis in cells.

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