Tissue Distribution of Human α₁-Microglobulin

KIMITERU TAKAGI, KOHJIN KIN, YOSHIHISA ITOH, TADASHI KAWAI, TADASHI KASAHARA, TOSHIHIKO SHIMODA, and TOSHIKO SHIKATA, Department of Clinical Pathology, Medical Biology, and Parasitology, Jichi Medical School, Tochigi-Ken, Japan, 329-04 and Department of Pathology, Nihon University School of Medicine, Tokyo, Japan, 110

A B S T R A C T Human α₁-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-α₁-microglobulin serum was prepared and observed to react monospecifically in gel diffusion to purified α₁-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-α₁-microglobulin serum in immunodiffusion. The lymphocyte culture supernate was found to contain α₁-microglobulin. Both thymus-derived(T)- and bone marrow-derived(B)-lymphocyte culture media clearly displayed a specific precipitin line against anti-α₁-microglobulin serum when tested with the Ouchterlony immunodiffusion method. The tissue distribution of α₁-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 α₁-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 α₁-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 α₁-microglobulin (1) because it migrated at the α₁-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 α₁-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 α₁-microglobulin. Following the method of Svensson and Ravnklov (2), concentrated urine was analyzed with affinity chromatography using a column (2.5 × 40 cm) packed with Con-A-Sepharose (Pharmacia, Inc., Uppsala, Sweden) equilibrated with a 0.1 M acetate buffer at pH 6.0 containing 1 M NaCl and 10 mM MnCl₂, MgCl₂, and CaCl₂. 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 the estimation of molecular weight. Reference proteins included trypsin inhibitor (Sigma Chemical Co.), bovine serum albumin (Seikagaku Kogyo Co., Tokyo), RNA-polymerase (Boehringer Mannheim GmbH, West Germany), myoglobin (Sigma Chemical Co.), and cytochrome c (Seikagaku Kogyo 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 hydrolyzation with 0.1 M H2SO4 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 hydrolyzation 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 Sephorese 4B (Pharmacia, Inc.) column chromatography (3.0 × 13 cm) containing albumin, α1-acid glycoprotein, and α1-antitrypsin. The specificity of antisera was confirmed using Ouchterlony immunodiffusion (9) and agarose gel immunoelectrophoresis (10). Antiserum raised against purified protein produced a single precipitation arc when tested on immunoelectrophoresis, and migrated at the fast α1-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 β2-microglobulin (Fujizoki Co., Tokyo), and anti-β2-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 (Semi-dull 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)-rosette1 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 × 106 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% CO2, 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. Matsushashi, 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 anti-α1-microglobulin antibody. Anti-α1-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 inactive 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 0.1% sodium chloride. The fraction at the second peak was collected. After concentration, the antigenicity of the Fab'2 fragment of the antibody immunoglobulin was confirmed by immunoelectrophoresis using anti-rabbit immunoglobulin (IgG) 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 × 10⁶/ml) were washed three times with phosphate-buffered saline (PBS), followed by incubation with 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

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

**RESULTS**

Purification of α-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-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

**figure 1** SDS-polyacrylamide gel electrophoresis of (1) normal human serum; (2) concentrated urine as the starting material; (3) pooled α-microglobulin-rich fraction obtained by Con-A-Sepharose chromatography; (4) pooled α-microglobulin-rich fraction obtained by Sephadex G-75 chromatography; (5) purified α-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.

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.

**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-microglobulin was assessed by Ouchterlony immundiffusion analysis and by immunoelectrophoresis. Only a single precipitation line and a single continuous arc were observed when the purified α-microglobulin was tested against the antiserum obtained from immunized rabbits. A single continuous arc migrated at the α-region on immunoelectrophoresis. Anti-α-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-microglobulin react with antisera against albumin, α-
TABLE I
Properties of Human $\alpha_1$-Microglobulin

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

* Values obtained from 24- and 72h hydrolyses.

antitrypsin, $\alpha_1$-acid glycoprotein, or $\alpha_1$-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-$\alpha_1$-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-$\alpha_1$-microglobulin serum after being concentrated 100 times, though no precipitin line appeared. Neither was any cross reactivity demonstrated between $\beta_2$-microglobulin and purified $\alpha_1$-microglobulin.

**Immunofluorescence examination of tissue distribution.** A positive stain for $\alpha_1$-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 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-$\alpha_1$-microglobulin serum was absorbed with increasing amounts of the pure $\alpha_1$-microglobulin, the number of fluorescent lymphocytes diminished significantly. Established human lymphocyte lines tested for the surface $\alpha_1$-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-$\alpha_1$-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 $\alpha_1$-microglobulin were diluted serially, the number of fluorescent cells decreased. Absorption of the rabbit antiserum with the purified $\alpha_1$-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, $\beta_2$-microglobulin, fibrinogen, and IgG, had no diminishing effect on the number of fluorescent cells.

As to $\alpha_1$-microglobulin on the surface of erythrocytes, no cells showed membrane fluorescence. When the rabbit antiserum against $\alpha_1$-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 $\alpha_1$-microglobulin was absorbed with increasing amounts of the pure $\alpha_1$-microglobulin. Various tissues obtained from the autopsy materials were simi-
FIGURE 3 Highly purified B lymphocytes stained with rabbit antiserum against \( \alpha_{1} \)-microglobulin and FITC-conjugated anti-rabbit gammaglobulin goat serum. A, suspension of lymphocytes, not fixed (\( \times 400 \)). 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 (\( \times 900 \)). Diffuse labeling, aggregated or patches, and caps were seen.
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 $\alpha_1$-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 1), 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 $\alpha_1$-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 \( \alpha_1 \)-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-\( \alpha_1 \)-microglobulin serum did react against normal human serum, with the development of only one precipitin line. The presence of \( \alpha_1 \)-microglobulin in both normal human serum and concentrated normal human urine was established through Ouchterlony immunodiffusion analyses using anti-\( \alpha_1 \)-microglobulin serum. The reactions indicated identical serum, namely \( \alpha_1 \)-microglobulin. Various animal sera mentioned above, however, did not react against anti-\( \alpha_1 \)-microglobulin serum.

Knowledge of the origin of \( \alpha_1 \)-microglobulin is still scarce. In the attempt to shed more light on this problem, the present study delved into the tissue distribution of \( \alpha_1 \)-microglobulin. The concentrated supernates of both T- and B-lymphocyte culture media showed a specific precipitation reaction against anti-\( \alpha_1 \)-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 \( \alpha_1 \)-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 \( \alpha_1 \)-microglobulin synthesis, although it seemed unlikely that \( \alpha_1 \)-microglobulin was synthesized in the liver, like most other plasma proteins. Several possibilities follow from the present study. First, the presence of \( \alpha_1 \)-microglobulin in the supernate of lymphocyte culture medium indicates that lymphocytes produce \( \alpha_1 \)-microglobulin. This would mean that mitogen-stimulated T and B lymphocytes in culture produce and secrete \( \alpha_1 \)-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 \( \alpha_1 \)-microglobulin. We know that \( \beta_2 \)-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 \( \alpha_1 \)-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 immunologically 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, \( \alpha_1 \)-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 \( \alpha_1 \)-microglobulin in cellular and humoral immunity in vivo. The present findings suggest that, although its function is still unknown, \( \alpha_1 \)-microglobulin plays some role in immunological reactions and(or) shares some structural roles with lymphocytes. It is thus suggested that \( \alpha_1 \)-microglobulin and protein HC are related to other cell surface markers like \( \beta_2 \)-microglobulin, human histocompatibility antigens, immunoglobulin, and so on. Indeed, Bernier et 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, \( \alpha_1 \)-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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