LEUKOCYTE SURFACE ORIGIN OF HUMAN α_1-ACID GLYCOPROTEIN (OROSOMUCOID)*

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Human α_1-acid glycoprotein (orosomucoid) (α_1-AG)[1] constitutes the main component of the seromucoid fraction of human plasma. It belongs to the acute phase proteins, which increase under conditions such as inflammation, pregnancy, and cancer (1, 2). α_1-AG has previously been found to be synthesized in liver (3), and after removal of terminal sialic acids, it is cleared from the circulation by binding to a receptor protein on liver cell plasma membranes (4).

The structure of α_1-AG is well known. It is composed of a single polypeptide chain and contains ≅45% carbohydrate including a large amount of sialic acid. The carbohydrate is located in the first half of the peptide chain linked to asparagine residues (5, 6).

The function of α_1-AG is unclear. However, Schmid et al. (5) and Ikenaka et al. (7) and reported that the amino acid sequence of the protein shows a significant homology with human IgG. This finding and the striking increase in inflammatory and lymphoproliferative disorders made us consider the possibility that leukocytes could be directly involved in the synthesis and release of α_1-AG.

We report here the presence of a membrane form of α_1-AG, with an apparent mol wt of 52,000, on normal human lymphocytes, granulocytes, and monocytes. By the use of internal labeling with [^{3}H]leucine in vitro, we demonstrate that the membrane protein is synthesized by lymphocytes. It is apparently subsequently cleaved and released as the soluble serum form with the normal mol wt of 41,000.

Materials and Methods

Isolation of α_1-AG. α_1-AG was isolated from the urine of patients with acute infectious mononucleosis by modifications of previously published methods (8). 6 liters of urine were collected, dialyzed against tap water overnight, and lyophilized. The powder was dissolved in 0.02 M sodium phosphate, pH 7.2, and applied to a 50-ml column of DEAE-cellulose made in the same buffer. After washing, a linear 200-ml gradient of 0.02 M sodium phosphate (200 ml of 1 M NaCl, 0.02 M sodium phosphate) was applied. The α_1-AG-containing fractions, which were identified by polyacrylamide slab gel electrophoresis, eluted with an NaCl concentration of 0.05–0.15 M. These were combined, dialyzed against water, and lyophilized. The powder was dissolved in 95 ml of

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[1] *Abbreviations used in this paper:* α_1-AG, α_1-acid glycoprotein; AET, 2-amino ethylisothiouronium bromide; buffer A, 0.15 M NaCl, 0.01 M sodium phosphate pH 7.4, 1% Triton X-100, 2 mM phenyl methyl sulfonyl fluoride, 1% ethanol; CNBr, cyanogen bromide; FITC, fluorescein isothiocyanate, LBL, lymphoblastoid B-cell line; MGG, May-Gruenwald-Giemsa stain; MLC, mixed lymphocyte culture; PBS, 0.15 M NaCl, 0.01 M sodium phosphate, pH 7.4; SDS, sodium dodecyl sulfate; SRBC, sheep erythrocytes.

H_2O, and 5 ml of 1 M sodium acetate was added. After addition of ammonium sulfate to a final concentration of 2.73 M, the mixture was left at 4°C for 16 h. After centrifugation at 12,500 rpm in a Sorvall SS-34 rotor for 15 min, the supernate was recovered. The pH of the supernate was adjusted to 4.9 with 2 N HCl, and this was left again at 4°C for 16 h. After centrifugation, the pH of the supernate was adjusted to 3.7 with 2 N HCl, and the sample was left at 4°C for 16 h. After centrifugation, the supernate was removed and the sediment was dissolved in H_2O, dialyzed against H_2O, and lyophilized. The sample was then passed through a 15-ml column of concanavalin A-Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) in 0.15 M NaCl-0.01 M sodium phosphate, pH 7.4 (PBS), and after washing it was eluted with 0.1 M α-methyl mannopyranoside (Calbiochem, San Diego, Calif.). The peak fractions eluted with the sugar were pooled, dialyzed against H_2O, and lyophilized. This sample was then passed over an Ultrogel AcA54 column (LKB Produkter, Stockholm, Sweden) in PBS, and the peak fractions were combined, dialyzed against H_2O, and lyophilized. The final yield of purified protein was 33 mg.

Isolation and Cultivation of Human Leukocytes. The following main populations of blood cells were isolated: granulocytes, monocytes, platelets, T lymphocytes, and B lymphocytes. They were isolated from buffy coats supplied by the Finnish Red Cross Blood Transfusion Service, Helsinki, Finland. The platelets and mononuclear cells were separated from the granulocytes and erythrocytes by a one-step Ficoll-Isopaque (density 1.077; Pharmacia Fine Chemicals) gradient centrifugation (9), at 400 *g* for 40 min.

Purification of Granulocytes. The pellets obtained after Ficoll-Isopaque centrifugation that contained erythrocytes and granulocytes were suspended in 20 ml of PBS. 10 ml of 0.15 M NaCl containing 6% dextran (Macrodex 6%; Leiras, Turku, Finland) was added, and the cell suspension was kept for 40 min at 37°C. The granulocyte-rich buffy coat was then collected, and contaminating erythrocytes were lysed by incubation in 0.017 M Tris-0.84% NH_4Cl, pH 7.45. The leukocytes were then washed three times with PBS. This procedure yielded a cell population which contained >97% granulocytes as judged from May-Gruenwald-Giemsa (MGG)-stained cytocentrifuged cell smears. The cell viability was close to 100%, as seen in the trypan blue exclusion test.

Purification of Platelets. The platelets were purified from the cell population obtained from the interphase after Ficoll-Isopaque centrifugation. This cell population was suspended in PBS, centrifuged for 10 min at 200 *g*, and the platelet-rich supernate was recovered. This procedure was repeated once, and the platelets were then pelleted at 400 *g* for 20 min. The purity of the platelet preparation approached 100%, and the contamination by other blood cells was always <0.002%.

Purification of Monocytes. The blood monocytes were separated from the cell population recovered at the interphase after Ficoll-Isopaque centrifugation. The platelets were depleted by three washes with PBS at 200 *g* for 10 min. The mononuclear cell population was mixed at a ratio of 1:50 with 2-amino ethylisothiouronium bromide (AET) (Sigma Chemical Co., St. Louis, Mo.) treated sheep erythrocytes (SRBC) (10). After incubation for 15 min at 37°C, the mixture was centrifuged for 10 min at 200 *g* and the T cells were allowed to rosette with the AET-SRBC for 1 h on ice. The pellet was then gently suspended in cold PBS containing 30% newborn calf serum, and the rosette-forming cells were separated from the non-rosette-forming cells by Ficoll-Isopaque centrifugation. The cell population recovered from the interphase mainly contained monocytes and non-T lymphocytes. The monocytes were then purified from the contaminating lymphocytes by a 1-*g* velocity sedimentation (11). After sedimentation for 4 h at 4°C, the gradient was drained into 20-ml fractions, and the cell content of each fraction was analyzed from MGG-stained smears. The early fractions which contained mainly monocytes were pooled. The cell preparation thus obtained contained 90–95% monocytes, as judged by conventional morphological criteria. The viability always exceeded 98%. Slight contamination was caused by occasional lymphoblasts, myeloid precursor cells, and some granulocytes.

Purification of T lymphocytes. The platelet-depleted mononuclear cell population recovered from the Ficoll-Isopaque centrifugation was passed over a human Ig–rabbit anti-human-Ig column as described by Wigzell et al. (12). The column-passed lymphocyte population contained <1% surface immunoglobulin-bearing cells, as judged by staining with fluorescein isothiocyanate (FITC)-conjugated polyvalent sheep anti-human immunoglobulin obtained from Professor Astrid Fagraeus, State Bacteriology Laboratory, Stockholm, Sweden. The viability of the T-cell population was always >98%, and >95% of the cells formed rosettes with AET-SRBC.

Purification of B Lymphocytes. The mononuclear cells obtained after Ficoll-Isopaque centrifugation were suspended into RPMI culture medium supplemented with 10% normal human AB

plasma. Carbonyl iron was added and the suspension was incubated for 1 h at 37°C. The majority of the phagocytic cells was then removed with a magnet. The phagocyte-depleted cell population was allowed to rosette with AET-SRBC as described above. The T cells were depleted by centrifugation of the rosette-forming cell-containing cell suspensions on a Ficoll-Isopaque gradient. The non-T lymphocytes recovered from the interphase were further purified by velocity sedimentation as described above. The later fractions obtained after sedimentation for 4 h contained mainly small lymphocytes, as judged from MGG-smears. These fractions were pooled and the cell population thus obtained was contaminated by <3% nonlymphocytic cells. This lymphocyte suspension was further fractionated over an Ig-anti-Ig column at 4°C to minimize Fc binding. The lymphocytes retained by the column were mechanically eluted by shaking the glass beads in PBS. 86% of the eluted lymphocytes stained positively for surface Ig, and <2% bound AET-SRBC. This preparation was designated B lymphocytes.

Establishment of Continuous B Lymphoblastoid Cell Lines (LBL) from Peripheral Blood. LBL were established by cultivation of T-lymphocyte-depleted blood leukocytes from different patients with acute infectious mononucleosis (13). The cell lines studied in this work were grown continuously for more than 4 mo before use in RPMI-1640 medium supplemented with 10% calf serum.

Mixed Lymphocyte Culture (MLC). Human blood T lymphocytes were purified and cultivated in RPMI-1640 medium supplemented with fetal calf serum or rabbit serum with mitomycin C (30 μg/ml) treated allogeneic leukocytes at an initial density of 10^6 responder cells and 2×10^6 stimulator cells/ml.

Labeling of Allogen-Activated T Lymphocytes with [^{3}H]Leucine. MLC cells obtained after 5 days in culture were washed in Dulbecco's PBS and suspended in 15 ml of leucine and serum-free Eagle's minimal essential medium. To this was added 1.5 mCi [^{3}H]L-leucine (58 Ci/mmole; The Radiochemical Centre, Amersham, England) and the cells were incubated at 37°C for 16 h. The cells were then pelleted by centrifugation and the medium was recovered. The medium was extensively dialyzed against H_2O and lyophilized. It was then dissolved in 5 ml of PBS and centrifuged at 100,000 *g* for 60 min in a Beckman L2-50 centrifuge, and the supernates were recovered. The cells were washed three times in PBS and lysed in PBS containing 1% Triton X-100, 2 mM phenyl methyl sulfonyl fluoride (Sigma Chemical Co.), 1% ethanol (buffer A). After centrifugation at 100,000 *g* for 60 min, the supernate was recovered. For subsequent immune precipitations, both the growth medium and the cell extract were passed through 2-ml columns of Lens culinaris-Sepharose, and the absorbed glycoproteins were eluted with 0.1 M α-methyl mannoside in buffer A. Lens culinaris beans were obtained from Dr. M. J. Crumpton, Medical Research Council, Mill Hill, England, and the lectins were purified by affinity chromatography on Sephadex G-50 (14) and coupled to Sepharose 4B (Pharmacia Fine Chemicals) by CNBr activation (15). Aliquots of the fractions were counted for radioactivity in a Wallac-LKB 81000 liquid scintillation counter (16). The radioactive fractions obtained after elution with the sugar hapten were pooled and used for immunoprecipitations.

Preparation of α_1-AG Antiserum. Rabbits were injected three times at 2-wk intervals with 0.5 mg of α_1-AG boiled in 1% sodium dodecyl sulfate (SDS), emulsified in Freund's adjuvant (Difco Laboratories, Detroit, Mich.), and bled 10 days after the last injection.

Surface Labeling of Cells. Cells were labeled with ^{3}H by treatment with neuraminidase and galactose oxidase followed by NaB^3H_4 (16). The labeling conditions have been described in detail previously (17). The *Vibrio cholerae* neuraminidase (500 U/ml; Behring-Werke AG, Marburg-Lahn, W. Germany) and galactose oxidase (Kabi AB, Stockholm, Sweden) preparations did not contain proteolytic activity when assayed as described (16). NaB^3H_4 (26 Ci/mmol) was obtained from The Radiochemical Centre. After labeling, the cells were dissolved in buffer A and centrifuged at 10,000 *g* for 15 min, and the supernates were recovered.

Chemical Determinations. Protein was determined according to Lowry et al. (18), with bovine serum albumin used as a standard. Amino acid analysis was performed with a Beckman 120 C amino acid analyzer (Beckman Instruments, Inc., Fullerton, Calif.) after hydrolysis in 6 N HCl under vacuum at 110°C for 22 h. No correction was made for the destruction of amino acids. Methionine and 1/2 cystine were determined after performic acid oxidation (19). Sialic acids were determined as described (20) with *N*-acetyl neuraminic acid (Sigma Chemical Co.) as a standard. Amino sugars were quantitated by the Elson-Morgan reaction (21), and the proportions of *N*-acetyl glucosamine and *N*-acetyl galactosamine were estimated by the use of the amino acid

analyzer (22). Neutral sugars were determined after hydrolysis in 4 N H_2SO_4 at 100°C for 4 h as alditol acetates (23).

Labeling of Cell Culture Medium and Purified α_1-AG with ^{125}I. MLCs carried on for 5 days in RPMI-1640 culture medium supplemented with 1% rabbit preimmunization serum were done as described above. The cells were pelleted by centrifugation, and the supernate was centrifuged at 100,000 *g* for 60 min. After dialysis against H_2O at 4°C for 24 h, 10 μl of the medium and 10 μg of purified urinary α_1-AG were iodinated by the chloramine-T method (24) using 0.5 mCi carrier-free ^{125}I (Amersham Corp.). Fractions were collected after passing through a 0.5 × 10-cm Sephadex G-25 column made in PBS and counted in a Wallac-LKB 80000 gamma sample counter.

Immunoprecipitations Using Protein A Containing Staphylococcus aureus (Strain Cowan I). Immunoprecipitations were made from Triton X-100 extracts of [^{3}H]leucine-labeled cells or [^{3}H]leucine-labeled culture medium which were eluted with sugar from the Lens culinaris columns. Immunoprecipitations were also done directly from Triton X-100 extracts of surface-labeled cells and ^{125}I-labeled culture medium. The samples were pretreated with 5 μg mouse IgG and 5 μl rabbit anti-mouse IgG (prepared by standard techniques) for 1 h at 0°C. Then, 100 μl of a 10% suspension of *S. aureus* Cowan I strain, obtained from Dr. P. Landwall of the Karolinska Institute, Stockholm, Sweden (25) was added, and the samples were further incubated at 0°C for 60 min. 100 μl of the staphylococcal suspension bound IgG from 6 μl of rabbit serum. The tubes were then centrifuged at 3,000 *g* for 10 min in a table centrifuge at 4°C, and the supernates were recovered. To identical aliquots of this supernate were added either 5 μl rabbit anti-α_1-AG antiserum, or 5 μl rabbit preimmunization serum, and the tubes were incubated at 0°C for 2 h. Then, 200 μl of the staphylococcal suspension was added, and the incubation was continued at 0°C for 1 h. The staphylococci were then washed three times by centrifugation in 0.15 M NaCl, 0.05% Triton X-100, 5 mM EDTA, 0.02% sodium azide, pH 7.4, and the absorbed antigens were eluted by boiling for 2 min in 1% SDS. The staphylococci were pelleted by centrifugation, and the supernates were recovered and used for polyacrylamide gel electrophoresis.

Radioimmunoassay of α_1-AG from Supernates of Cell Cultures. One of the lymphoblastoid B-cell lines was cultured at an initial cell density of 5 × 10^5 cells/ml in RPMI-1640 culture medium containing 10% fetal calf serum (Flow Laboratories, Glasgow, Scotland). At indicated times, 1.5-ml samples were taken, the cell number was counted, and 0.5-ml aliquots of the supernates were used for radioimmunoassay.

A radioimmunoassay standard curve was made by using 0.00001–10 μg of purified α_1-AG in 0.5 ml of the same fresh cell culture medium. 10 μl of rabbit preimmunization serum was added as carrier, and the samples were incubated with ≅ 30,000 cpm of ^{125}I-labeled α_1-AG and 0.1 μl of rabbit anti-α_1-AG antiserum for 16 h at 4°C. The antiserum was calibrated to bind ≅ 30% of the total radioactivity. Then 100 μl of sheep anti-rabbit IgG antiserum was added, and after 1 h at 4°C, the samples were centrifuged and the pellets were counted for radioactivity. The test samples were made in the same way, except that the purified α_1-AG was omitted. The standard curve was linear for 0.0005–0.5 μg of α_1-AG.

Cleavage of Proteins with Cyanogen Bromide (CNBr). ^{125}I-labeled purified α_1-AG and labeled proteins eluted from cylindrical polyacrylamide gels were cleaved by treatment with CNBr in 70% formic acid for 24 h at room temperature (26). After dilution with 20 vol of H_2O, the samples were lyophilized and run on cylindrical polyacrylamide gels.

Polyacrylamide Gel Electrophoresis. Slab and cylindrical polyacrylamide gels were run according to Laemmli (27) in the presence of SDS with an acrylamide concentration of 8%. Slab gels were stained with Coomassie brilliant blue according to Weber and Osborn (28). Cylindrical gels were sliced and counted (16). Slab gels were treated for fluorography (29) and the gels were vacuum-dried and covered with Kodak RP X-Omat film (Eastman Kodak Co., Rochester, N.Y.) and exposed for 1–14 days at −70°C. The films were photographed and scanned with a Joyce-Loebl Chromoscan (Joyce, Loebl and Co., Ltd., Gateshead-on-Tyne, England). ^{14}C-labeled standard proteins were prepared as described (30).

Indirect Immunofluorescence. To exclude nonspecific IgG binding to Fc receptors, F(AB)2 fragments were prepared from anti-α_1-AG anti-serum. 20 mg of IgG was digested with 0.5 mg of porcine pepsin (Schwarz/Mann Div. Becton, Dickinson & Co., Orangeburg, N.Y.) for 16 h at 37°C in 0.1 M sodium acetate buffer, pH 4.5, and the F(AB)2 fragments were isolated by gel filtration on Ultrogel AcA34 (LKB Produkter). The F(AB)2 preparation appeared pure on polyacrylamide gel electrophoresis.

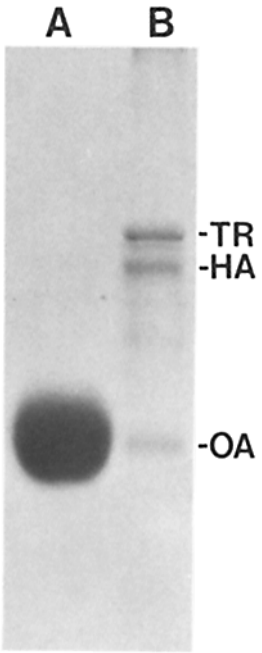

FIG. 1. Polyacrylamide gel electrophoresis of purified α_1-AG. (A), 50 μg of purified α_1-AG was run on a polyacrylamide slab gel in the presence of SDS and 2-mercaptoethanol and stained with Coomassie brilliant blue. (B), standard proteins: TR, transferrin; HA, human albumin; OA, ovalbumin.

Cells were washed and suspended in 100 μl of ice-cold PBS containing 20% fetal calf serum. 100 μg anti-α_1-AG F(AB)2, 10 μl anti-α_1-AG antiserum or 10 μl preimmunization serum were added. After 30 min on ice, the cells were washed twice with 10 ml of cold fetal calf serum in PBS and suspended in 20 μl of 1:20 diluted FITC-conjugated IgG (1 mg/ml) isolated from sheep anti-rabbit IgG antiserum. After 20–30 min on ice, the cells were washed and examined in a Zeiss Universal fluorescence microscope (Carl Zeiss, Inc., New York) equipped with epi-illuminator III RS and a high pressure mercury lamp (200 W). For capping experiments, the FITC-stained cells were incubated at 37°C.

Results

Purity, Amino Acid and Carbohydrate Compositions of Urinary α_1-AG. When α_1-AG was isolated from urine and analyzed by slab polyacrylamide gel electrophoresis, only one band was obtained with an apparent mol wt of 41,000 (Fig. 1). Our antiserum gave a single precipitation line of normal human serum on immunoelectrophoresis, and the same result was obtained with a commercially available anti-α_1-AG antiserum (Behringwerke).

Table I shows the amino acid content and the carbohydrate composition of the purified urinary protein. The amino acid composition is identical to that reported by other groups for α_1-AG.

Indirect Immunofluorescence with Anti-α_1-AG Antiserum. Isolated T and B lymphocytes, monocytes, and granulocytes showed a slightly granular membrane fluorescence pattern after staining with rabbit anti-α_1-AG F(AB)2 and FITC-conjugated sheep anti-rabbit IgG. To exclude the possibility of α_1-AG adsorption from human serum, four lymphoblastoid cell lines cultivated for

TABLE I
*Amino Acid and Carbohydrate Compositions of α_1-Acid Glycoprotein**

Amino acids	Present study‡	Calculated from amino acid sequence (5)
Lys	13.6 ± 0.2	12.8
His	3.1 ± 0.0	3.0
Arg	8.0 ± 0.4	9.5
Asx	20.3 ± 0.4	20.6
Thr	14.5 ± 0.4	14.5
Ser	6.7 ± 0.5	7.3
Glx	33.0 ± 0.8	29.5
Pro	9.4 ± 0.6	7.0
Gly	7.7 ± 0.6	7.3
Ala	9.0 ± 0.4	9.8
Val	9.2 ± 0.5	8.6
Ile	9.2 ± 0.2	9.0
Leu	14.9 ± 0.1	14.5
Tyr	9.7 ± 0.2	10.8
Phe	9.4 ± 0.1	8.8
½ cys§	4.1 ± 0.2	4.0
Met§	0.9 ± 0.0	1.3
Trp	n.d.	3.0
	Carbohydrates‖	
N-acetyl neuraminic acid		14.3
N-acetyl glucosamine		17.5
N-acetyl galactosamine		0
Fucose		7.2
Mannose		19.1
Galactose		27.2

* mol/mol, assuming a mol wt of 41,000.
‡ Mean of four determinations ± standard deviations.
§ Determined after performic acid oxidation.
‖ Mean of two determinations.

several months in the absence of human serum were also studied. All of the four cell lines had a clear membrane fluorescence, which showed redistribution or capping after incubation at 37°C (Fig. 2). No fluorescence was detected in cells treated with preimmunization rabbit serum.

Surface Labeling of Leukocytes. T and B lymphocytes, granulocytes, monocytes and MLC lymphoblasts were labeled with ^3H after treatment with neuraminidase and galactose oxidase followed by NaB^3H_4, and then run on slab gels and exposed for fluorography. The cells contain a large number of labeled characteristic surface proteins. The GP52 region is weakly labeled in all resting cells, and this protein is evidently a minor component. Immunoprecipitation of surface-labeled T lymphoblasts from MLC with anti-α_1-AG anti-serum showed one band with an apparent mol wt of 52,000 (Fig. 3, column G).

Immunoprecipitations of [^3H]leucine-labeled cells and culture medium with anti-α_1-AG antiserum.

To show that the cell surface antigen and the cell medium antigen which

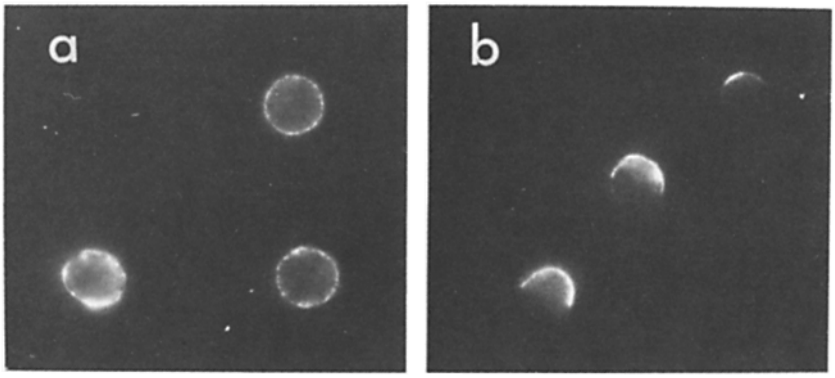

FIG. 2. Indirect immunofluorescence of cells of a lymphoblastoid line treated with rabbit anti-α_1-AG F(AB)2 and FITC-conjugated sheep anti-rabbit IgG. (a), Cells were incubated at 0°C; (b), redistribution of the fluorescence after incubation for 30 min at 37°C.

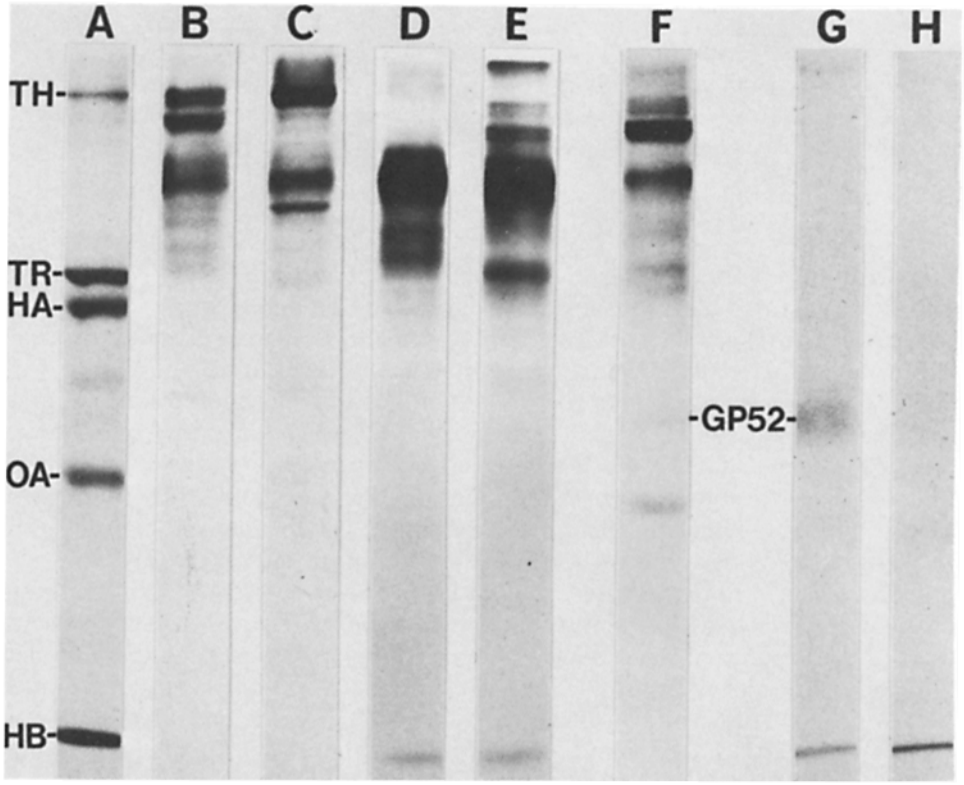

FIG. 3. Polyacrylamide gel electrophoresis patterns of surface-labeled leukocytes and leukocyte antigen precipitated with anti-α_1-AG antiserum. (A), ^{14}C-labeled standard proteins: TH, thyroglobulin; TR, transferrin; HA, human albumin; OA, ovalbumin; HB, hemoglobin. (B), T lymphocytes; (C), B lymphocytes; (D), granulocytes; (E), monocytes; (F), MLC lymphoblasts; (G), pattern of immunoprecipitation from surface-labeled MLC blasts with anti-α_1-AG antiserum; (H), control with preimmunization serum.

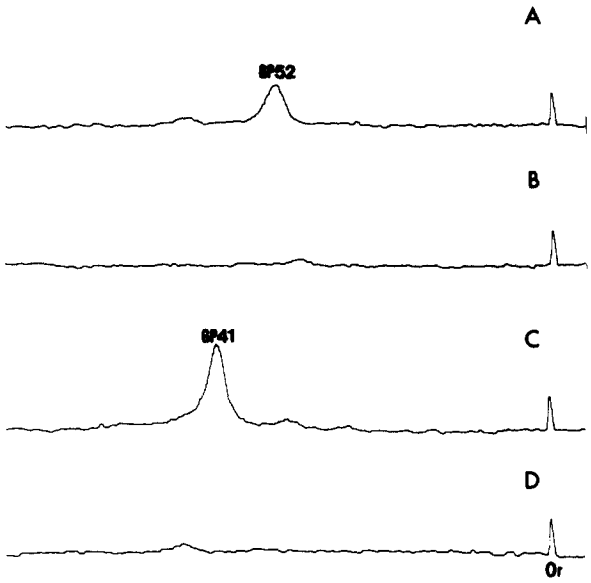

FIG. 4. Scanning patterns of immunoprecipitates obtained with anti-α_1-AG antiserum from [^{3}H]leucine-labeled MLC lymphoblasts and culture medium separated by slab gel electrophoresis and visualized by fluorography. (A), from MLC lymphoblasts with anti-α_1-AG antiserum; (B), from MLC lymphoblasts with preimmunization serum; (C), from MLC culture medium with anti-α_1-AG antiserum; (D), from MLC culture medium with preimmunization serum. Or, origin.

react with anti-α_1-AG antiserum are synthesized by lymphocytes, Triton X-100 extracts from MLC blasts labeled in culture with [^{3}H]leucine and medium from the same cultures were treated with anti-α_1-AG antiserum or preimmunization serum and staphylococci, eluted, and run on polyacrylamide gels. Fig. 4 A shows the scanning pattern of the immunoprecipitated antigen from cells. The apparent mol wt was 52,000. No radioactive band was obtained with preimmunization serum (Fig. 4 B). When the cell culture medium was treated with antiserum and staphylococci, eluted, run on gels and scanned, the pattern of Fig. 4 C was obtained. The apparent mol wt of the major peak was 41,000. Again no labeled protein was precipitated with preimmunization serum (Fig. 4 D).

Immunoprecipitations of Surface-Labeled Leukocytes. Triton X-100 extracts from surface-labeled MLC blasts were precipitated with anti-α_1-AG antiserum and staphylococci and analyzed on cylindrical gels. Two peaks were obtained with apparent mol wt of 52,000 and 41,000 (Fig. 5 A). The size of the smaller peak varied in different preparations. No peaks were obtained with preimmunization serum (Fig. 5 B). Similar patterns were obtained with labeled granulocytes (Figs. 5 C and D). Monocytes showed one major peak with a mol wt of 52,000 (Figs. 5 E and F). No detectable amounts of labeled protein could be precipitated from surface-labeled resting T and B lymphocytes, platelets, or erythrocytes.

Immunoprecipitation of ^{125}I-labeled Cell Culture Medium with Anti-α_1-AG Antiserum. To characterize the shedded antigen found in culture medium, an MLC was set up in RPMI-1640 containing rabbit preimmunization serum, the

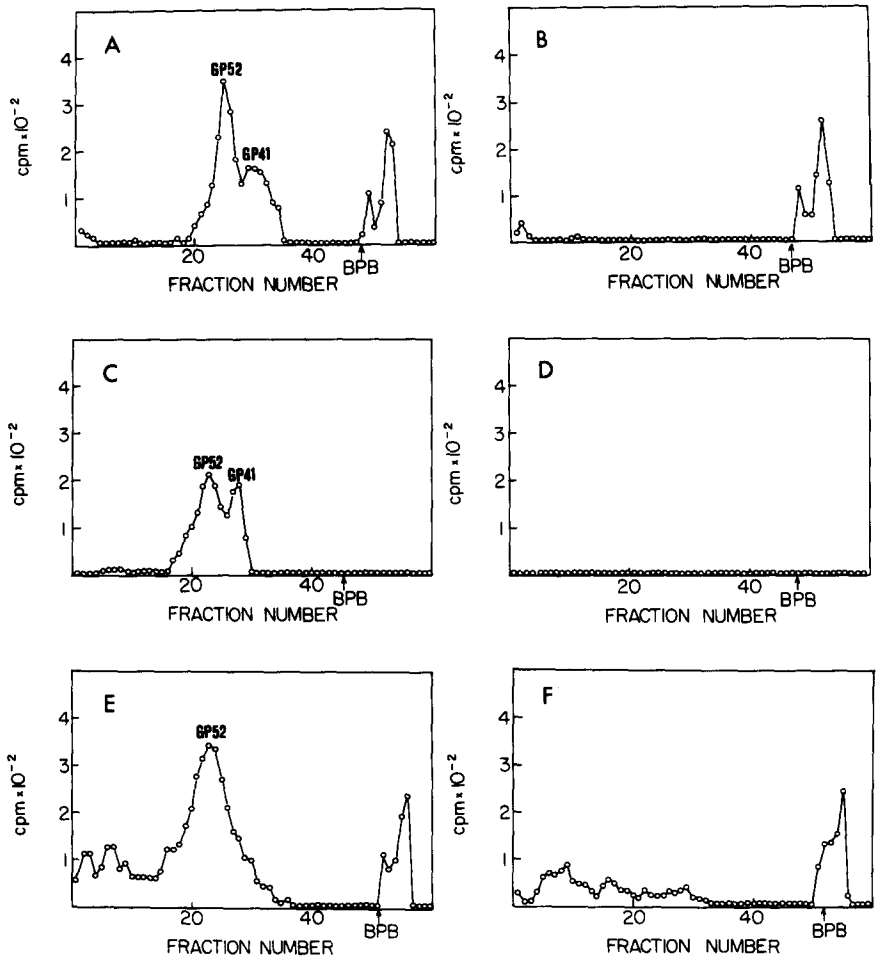

FIG. 5. Cylindrical polyacrylamide gel patterns of radioactive antigens precipitated with anti-α_1-AG antiserum from surface-labeled leukocytes. (A), MLC blasts with anti-α_1-AG antiserum; (B), MLC blasts with preimmunization serum; (C), granulocytes with anti-α_1-AG antiserum; (D), granulocytes with preimmunization serum; (E), monocytes with anti-α_1-AG antiserum; (F), monocytes with preimmunization serum. C and D were passed over a Lens culinaris column and eluted with α-methyl mannoside before immune precipitation. This procedure removed the nonspecific material of low molecular weight running in the front. BPB, position of bromphenol blue marker dye.

medium was labeled with ^{125}I, and immunoprecipitated with anti-α_1-AG antiserum. The pattern of Fig. 6 was obtained with the major peak corresponding to a mol wt of 41,000.

Accumulation of α_1-AG in the Medium of Lymphoid Cell Cultures. The rate of accumulation of α_1-AG in the culture medium during cell proliferation was studied with radioimmunoassay. For these experiments, a lymphoblastoid cell line of high viability was used to avoid contamination by α_1-AG originating from human serum and to minimize accumulation of products from disaggregating cells. Fig. 7 shows the increase of α_1-AG when determined by radioimmu-

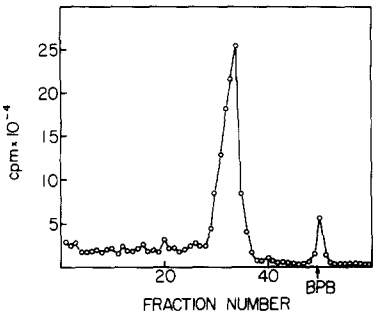

FIG. 6. Cylindrical polyacrylamide gel pattern of immune precipitated radioactive antigen obtained with anti-α_1-AG antiserum from ^{125}I-labeled MLC culture medium supplemented with rabbit preimmunization serum.

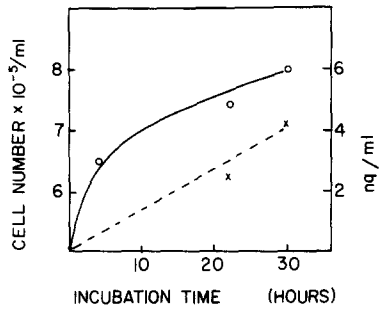

FIG. 7. Accumulation of α_1-AG in the culture medium of a B-lymphoblastoid cell line as determined by radioimmunoassay. (×--×), cell number × 10^{-5}/ml; (O——O), ng/ml.

noassay from the culture medium. After an initial, more rapid increase, there was a slower accumulation.

Cleavage with CNBr of α_1-AG and of Molecules Recovered by Anti-α_1-AG Antiserum from Cells and Culture Medium. To prove that our anti-α_1-AG antiserum was monospecific for α_1-AG, we performed CNBr cleavage of the immunoprecipitated molecules. Purified, ^{125}I-labeled α_1-AG from urine ran on cylindrical gels as a single peak (Fig. 8 A). Cleavage of this protein with CNBr resulted in three peaks (Fig. 8 B). Cleavage of the iodinated, immunoprecipitated protein isolated from the culture medium of MLC in rabbit serum gave a pattern similar to that of CNBr-treated purified α_1-AG (Fig. 8 C). When the membrane-bound form of α_1-AG was isolated by immunoprecipitation from [^{3}H]leucine-labeled cells and cleaved by CNBr treatment, the pattern of Fig. 8 D was obtained. Again, the peaks of highest molecular weights correspond to those of CNBr-cleaved pure α_1-AG.

Discussion

We have purified α_1-AG or orosomucoid from the urine of patients with acute infectious mononucleosis. Anti-α_1-AG antiserum and its F(AB)2 fragments reacted with normal T and B lymphocytes, T lymphoblasts, granulocytes, and monocytes and lymphoblastoid B-cell lines as shown by immunofluorescence. We were not able to find this antigen on platelets, erythrocytes, or cultured

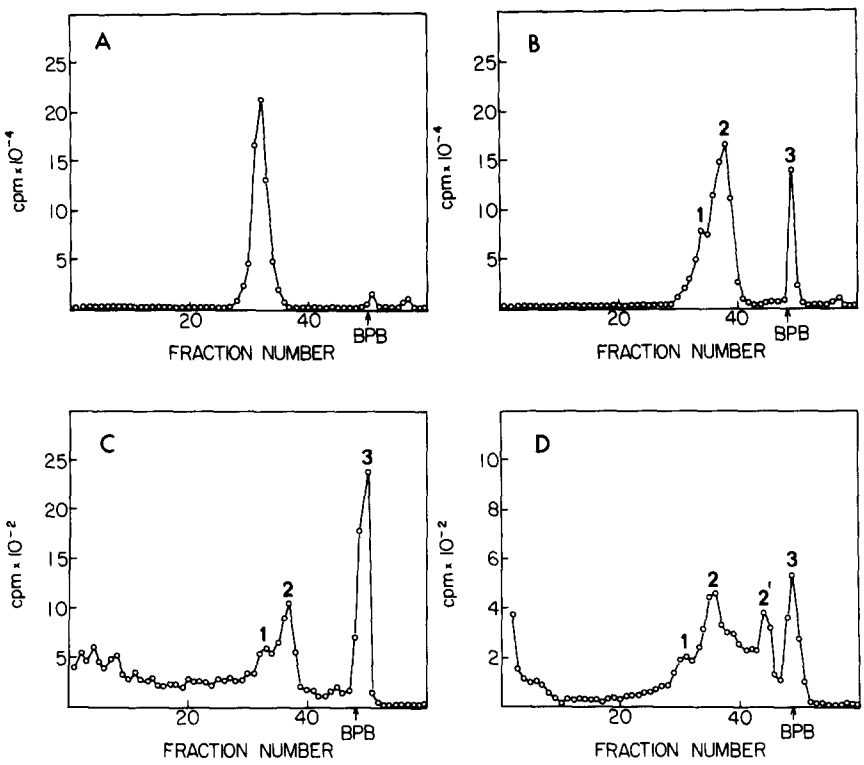

FIG. 8. Cylindrical polyacrylamide gel patterns of pure ^{125}I-labeled α_1-AG (A), its CNBr fragments (B), the CNBr fragments of the antigens precipitated with the anti-α_1-AG antiserum from ^{125}I-labeled MLC culture medium (C), and [^{3}H]leucine-labeled MLC blasts (D).

normal human fibroblasts.[2] The immunofluorescence and capping experiments indicate that the antigen is closely associated with the lipid bilayer of the membrane (31). Surface radiolabeling of cells followed by immunoprecipitation and polyacrylamide gel electrophoresis revealed that the membrane form of α_1-AG has a mol wt of 52,000. The same molecule was obtained after metabolic labeling of lymphoblasts in MLC with [^{3}H]leucine. On the other hand, the molecule isolated from the culture medium either after [^{3}H]leucine labeling or after ^{125}I-iodination of the medium, had an apparent mol wt of 41,000 which corresponds to that of α_1-AG in serum and urine. Although the α_1-AG was detectable on resting T and B lymphocytes by indirect immunofluorescence, sufficient radioactivity could not be introduced by surface labeling to yield a distinguishable band in the fluorography patterns of cells, or to allow isolation of significant amounts by immunoprecipitation. In the fluorography patterns of surface-labeled MLC T blasts and LBL (data not shown, 32), the GP 52,000 band was clearly seen and could be specifically recovered by the antiserum. This indicates that the α_1-AG constitutes only a minor component on the surface of resting lymphocytes, whereas it is more abundantly expressed on activated and

[2] C. G. Gahmberg and L. C. Andersson. Manuscript in preparation.

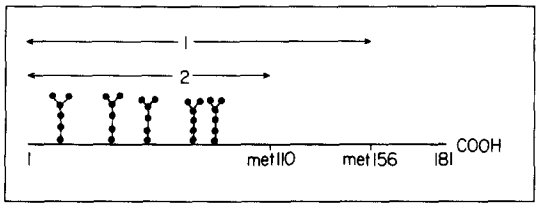

FIG. 9. Scheme of the cleavage points of α_1-AG with CNBr. The CNBr-sensitive methionine residues are at positions 110 and 156, resulting in peptides 2 and 1 (Fig. 8). The five oligosaccharide side chains are indicated.

proliferating T and B cells. The 41,000 mol wt protein found in variable amounts on freshly isolated blood granulocytes might partially represent adsorbed serum α_1-AG.

The difference seen in molecular weights between the membrane-bound and secreted molecules may be due to a hydrophobic fragment, which anchors the protein to the membrane. This would thus resemble the HLA-molecules where the membrane-bound form apparently has a hydrophobic intramembrane portion (33).

The structure of soluble α_1-AG is well known from the studies by Schmid et al. (2, 5) and Ikenaka et al. (7). It is rich in carbohydrate, containing five asparagine-linked oligosaccharide side chains. The carbohydrate is all in the first part of the molecule, and in this respect it resembles another well-studied membrane glycoprotein, the major sialoglycoprotein of human erythrocytes (34).

Elucidation of the primary structure of α_1-AG showed one constant methionine residue at position 110, and variably one more at residue 156 (5). Treatment with CNBr therefore results in cleavage at position 110 and in addition, in part of the molecules at position 156 giving large and easily distinguishable fragments. A schematic drawing of the cleavage points is shown in Fig. 9. The molecular weights of fragments 1 and 2 seen on the gels (Fig. 8) correspond to those expected from the primary structure. Fragments 1 and 2 both contain carbohydrate, which was seen after cleavage of α_1-AG labeled with ^3H by the galactose oxidase method.[2]

The antigen on granulocytes and monocytes with an apparent mol wt of 52,000 reacting with anti-α_1-AG antiserum had the same apparent molecular weight as that of lymphocytes. We have not studied the molecule from the nonlymphoid cells as extensively as that of lymphoid cells, but it is reasonable to assume that they are identical or nearly similar.

The elevated serum concentrations of α_1-AG under various conditions have been somewhat difficult to explain by stimulation of liver synthesis alone (2). Inflammation, major surgery, and cancer are associated with proliferation of leukocytes, and at least part of the increased serum α_1-AG may originate from such cells.

Serum α_1-AG has been shown to bind different steroids (35). The cell surface-located form of α_1-AG would also be expected to do it. Steroids have profound effects on lymphocyte functions in vivo and in vitro (36), and both T and B lymphocytes contain steroid receptors (37). Although the cytoplasmic and

nuclear binding of steroids are well documented (38), there may also be surface-located receptors.

Isoelectric focusing resolves desialylated α_1-AG from one individual into several bands, indicating heterogeneity (5). Whether these variants of serum α_1-AG are specific products of different types of cells or tissues remains to be established. α_1-AG might be important for intracellular communication and recognition by leukocytes. Such a role for α_1-AG is supported by the recent findings of Chiu et al. (39) that addition of α_1-AG to lymphocyte cultures altered the MLC response.

Summary

Specific antibodies against human α_1-acid glycoprotein reacted with human lymphocytes, granulocytes, and monocytes. The antigen on the leukocytes is an externally located integral membrane glycoprotein which is made by the cells and has an apparent mol wt of 52,000. It is released from cells in vitro to the culture medium. The mol wt of the soluble fragment is 41,000, which corresponds to that of α_1-acid glycoprotein in serum and urine. Peptide mapping confirmed that the main part of the cellular membrane antigen consists of α_1-acid glycoprotein with an additional, probably hydrophobic fragment. This finding may partially explain the increase in the serum levels of α_1-acid glycoprotein observed in many disorders involving leukocyte proliferation. In addition, the known sequence homology of α_1-acid glycoprotein with immunoglobulins can now be more easily understood by their origin in similar cell types.

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