CULTURED HUMAN MONOCYTES SYNTHESIZE AND SECRETE α_2-MACROGLOBULIN*

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Alpha$_2$-macroglobulin is a wide-spectrum protease inhibitor (1) present in normal human plasma in high concentrations, especially in children and young adults (2, 3). The site(s) of its synthesis in vivo is not known; liver has been considered to be the most plausible candidate organ for this activity (4). In vitro, diploid fibroblast-like cell strains are known to synthesize and secrete α_2-macroglobulin (5), and there is evidence that tissue slices from several organs may produce the protein (6).

Association of α_2-macroglobulin with the surface of a subpopulation of mononuclear leukocytes, probably B lymphocytes, has been reported in rabbits (7), mice, and men (8, 9). Cultures of human embryonic blood (10) and of adult leukocytes (11) were reported to incorporate amino acid precursors into protein precipitable by anti-α_2-macroglobulin. Here we report experiments which suggest that most, if not all, of the α_2-macroglobulin produced in human leukocyte cultures is synthesized by cells of the monocyte-macrophage lineage.

Materials and Methods

Cultures of Mononuclear Leukocytes and Their Subpopulation. Mononuclear leukocytes were isolated from the buffy coat fraction of blood from healthy donors, kindly provided by the Finnish Red Cross Blood Transfusion Center, Helsinki. The standard dextran sedimentation (12) and subsequent Ficoll-Isopaque centrifugation (13) were used as described previously (14). Finally the cells were suspended in prewarmed growth medium: RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 2 mM glutamine, 100 IU/ml penicillin, 50 μg/ml streptomycin, and 10% fetal calf serum. Mononuclear leukocytes comprised more than 90% of all living (trypan blue-excluding) cells in these suspenions.

Populations enriched in T lymphocytes or B + T lymphocytes were prepared by passing mononuclear leukocyte suspensions through nylon wool (NW)[1] (15) or cotton wool (CW) (16, 17), respectively. The resulting nonadherent cell populations will later be referred to as NW$^-$ and CW$^-$ cells, respectively. Mononuclear leukocytes, NW$^-$ cells, and CW$^-$ cells were cultured at 37°C in 30 ml Falcon plastic flasks (Falcon Plastics, Division of BioQuest, Oxnard, Calif.) at 2–3 × 10^6 cells/ml of growth medium.

Monocyte-enriched cell cultures were prepared by incubating mononuclear leukocyte suspensions in medium 199, supplemented with 2 mM glutamine and antibiotics, on glass coverslips for

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[1] *Abbreviations used in this paper:* CW, cotton wool; CW$^-$ cells, cells not retained by cotton wool; NW, nylon wool; NW$^-$ cells, cells not retained by nylon wool; SDS, sodium dodecyl sulfate; SmIg, surface membrane immunoglobulin.

TABLE I
Percentages of Lymphocytes and Monocytes in Different Subpopulations of Peripheral Blood Leukocytes

Subpopulation	Cell type marker*		
	SRBC-rosetting‡ T lymphocytes	$SmIg^+$§ B lymphocytes	Phagocytic cells‖ Monocytes
	%	%	%
Mononuclear leukocytes (unfractionated)	50–60	10–15	20–25
CW^- cells	70–80	10–15	1–5
NW^- cells	85–90	1–2	1–5
Adherent cells	0–1	1–3¶	85–95

* Tests for the markers were performed before starting the cultures.

‡ Sheep erythrocytes (SRBC) rosetting was carried out as recommended by Aiuti et al. (17).

§ Cells were washed three times with phosphate-buffered saline and stained for surface membrane immunoglobulin (SmIg) at +4°C, by using fluorescein-isothiocyanate-conjugated swine anti-human immunoglobulin (17). Small lymphocyte-like cells with bright granular fluorescence in epi-illumination were considered as B cells.

‖ Phagocytic cells were identified by incubating for 60 min at 37°C in RPMI + 50% fetal calf serum containing polystyrene particles (diameter 1.01 μm; Polysciences, Inc., Warrington, Pa.), and examining in a phase contrast microscope after washing with phosphate-buffered saline.

¶ By incubating the culture for 3 days in medium 199 practically all $SmIg^+$ cells disappeared.

1–2 h at 37°C. After washing carefully four times with phosphate-buffered saline the adherent cell cultures were refed with the serum-free medium 199, and incubated at 37°C. The medium was changed daily or every 2nd day. Alternatively the cells were refed with medium 199 supplemented with 10% fetal calf serum, and incubated without medium changes. In some later experiments a 1:1 mixture of medium 199 and RPMI-1640 medium (Grand Island Biological Co.) was used, as it was found that this mixture was optimal for the survival of monocyte-macrophage cultures. Percentages of different types of mononuclear leukocytes in these preparations are shown in Table I.

Radioimmunoassay of α_2-Macroglobulin. Alpha$_2$-macroglobulin was purified from human plasma by a modification of the procedure described by Roberts et al. (18) and iodinated by the method of Krohn et al. (19) by using minimal concentrations of chloramine-T. Antiserum against highly purified α_2-macroglobulin was purchased from Behringwerke, AG, Marburg-Lahn, W. Germany, absorbed with lyophilized fetal calf serum (40 mg/ml), and tested for monospecificity towards human α_2-macroglobulin by double immunodiffusion and immunoelectrophoresis. The absorption removed all detectable antibodies against bovine α_2-macroglobulin. Of the iodine label in α_2-macroglobulin, 89% was precipitated by the absorbed antiserum in a double antibody immunoprecipitation. The dose-response curve for α_2-macroglobulin in a double antibody type radioimmunoassay was linear between 2.4 and 200 ng/ml. Details of purification and labeling of α_2-macroglobulin, as well as of the radioimmunoassay are reported elsewhere.[2]

Immunofluorescence. Cells on coverslips or in suspension were fixed with 3.5% formaldehyde (20 min, +20°C) and cold acetone (20 min, −20°C). Alternatively, the cells were fixed with the formaldehyde alone (20). Indirect immunofluorescence studies for α_2-macroglobulin were carried out by using calf serum-absorbed rabbit anti-α_2-macroglobulin serum and fluorescein-isothiocya-

[2] Mosher, D., O. Saksela, and A. Vaheri. Manuscript submitted for publication.

nate-conjugated anti-rabbit-γ-globulin. Rabbit antiserum against human plasma fibronectin (5), a glycoprotein present in plasma and on cultured fibroblasts (21), was used in a similar immunofluorescence technique to identify possibly contaminating fibroblasts in the culture. The tests with fixed cells were carried out at room temperature and those with living cells at +4°C.

Metabolic Labeling. Adherent cell cultures were transfered to a modified basal medium of eagle for diploid cells containing $^1/_{10}$th of the normal methionine concentration and supplemented with antibiotics as above plus 0.05% bovine serum albumin (or plus 10% fetal calf serum thoroughly dialyzed against phosphate-buffered saline). The cells were then labeled with 10 μCi/ml ^{35}S-methionine (Radiochemical Centre, Amersham, England, sp act 200–400 Ci/mmol) for 24 h at 37°C.

Analysis of Radiolabeled Proteins. Supernates of radiolabeled cell cultures were clarified by centrifugation for 10 min at 800 *g* at 4°C and supplemented with phenylmethyl sulfonylfluoride, 1 mM, and Trasylol (aprotinin, Bayer AG, Leverkusen, W. Germany), 100 U/ml. Cells were dissolved in a mixture of 6 M urea, 0.1% Triton X-100, 0.01% NaN_3, and 2 mM phenylmethyl sulfonylfluoride, and subsequently mixed with 1 vol of normal human serum.

For Ouchterlony double diffusion-autoradiography portions of the supernates were concentrated 10-fold by precipitation with 50% saturated $(NH_4)_2SO_4$ together with carrier human serum. Concentrated supernates and cell extracts were analyzed by double diffusion in agarose gel by standard techniques (22). Radioactivity in the precipitates was localized by autoradiography. Preliminary experiments revealed that monocytes produce several antigens reacting with anti-human serum, and an antiserum against the human complement component C_3'(anti β_1C/β_1A) was used to identify one of these (23). For electrophoretic polypeptide analysis the precipitates were cut out from the gels, suspended in 1 vol of 4% sodium dodecyl sulfate (SDS) plus 10% 2-mercaptoethanol, buffered to pH 6.8 with Tris-HCl, and boiled for 3 min immediately before applying on electrophoresis.

For double antibody precipitation,[2] 1-ml portions of the supernates were shaken for 2 h at room temperature with 15 μl of specific antisera against α_2-macroglobulin, or fibronectin, or with 15 μl of normal rabbit serum. The antigen-antibody complexes were precipitated at 4°C by a 16-h incubation with 375 μl of sheep anti-rabbit globulin mixed with an equal volume of 20 mM Tris-HCl buffer, pH 8.0, 2 mM phenylmethyl sulfonylfluoride, 200 U/ml Trasylol, and 1% sodium deoxycholate. The precipitate was collected by centrifugation (20 min, 15,000 *g*), washed three times with 10 mM Tris-HCl buffer, pH 8.0, and once with water. The above protease inhibitors and deoxycholate were present through the buffer washes. The final precipitate was dissolved in 250 μl of 4% SDS, 10% 2-mercaptoethanol, and buffered to pH 6.8 with Tris-HCl.

Electrophoresis in discontinuous polyacrylamide slab gels containing SDS (24) was used to identify the labeled polypeptides in immunoprecipitates. Radioactivity in the migrating bands was assayed by autoradiography. The following molecular weight standards were used: plasma fibronectin ($M_r = 2.0 \times 10^5$), α_2-macroglobulin ($M_r = 1.6 \times 10^5$), phosphorylase a globulin ($M_r = 9.3 \times 10^4$), bovine serum albumin ($M_r = 6.8 \times 10^4$), and ovalbumin ($M_r = 4.3 \times 10^4$), all labeled with [^{14}C]formaldehyde according to Rice and Means (25), and dissolved in 4% SDS and 10% 2-mercaptoethanol in Tris-HCl buffer, pH 6.8.

Commercial Reagents and Chemicals. Dextran T500 and Ficoll 400 were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden; Isopaque was supplied by Medica Ltd, Helsinki; nylon wool (Leukopak) was from Fenwall Laboratories, Inc., Morton Grove, Ill. Media and fetal calf serum were from Gibco-BioCult, Paisley, Scotland, tissue culture vials were from Falcon Plastics, Ltd., Oxnard, Calif. Fluorescein-isothiocyanate-conjugated globulin fraction of swine antiserum towards human immunoglobulin was from Meloy Laboratories, Inc., Springfield, Va. Rabbit antiserum against human β_1C/β_1A was from Behringwerke.

Results

Release of α_2-Macroglobulin by Cultured Mononuclear Leukocytes. Cultures of mononuclear leukocytes, NW^- leukocytes, and CW^- leukocytes were incubated at 37°C in medium RPMI + 10% fetal calf serum, and portions of cell-free supernates were collected daily and kept at −20°C until assay. Initial cell concentrations in different cultures were identical within each experiment. Radioim-

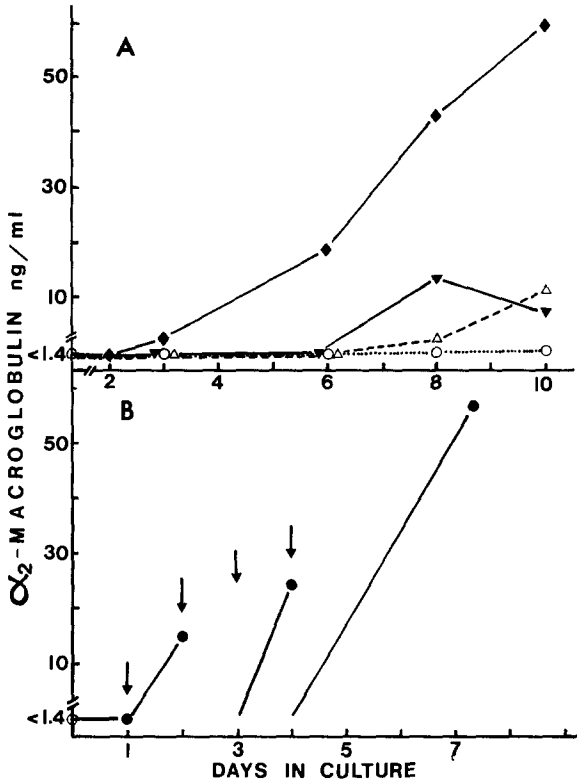

FIG. 1. Production of α_2-macroglobulin by human leukocyte cultures. A. 15 × 10^6 leukocytes were cultured in 5 ml of RPMI-medium (Grand Island Biological Co.) supplemented with 10% fetal calf serum. Samples of culture medium were drawn as indicated and assayed for α_2-macroglobulin by radioimmunoassay. Symbols: ◆—◆, unfractionated mononuclear leukocytes; ▼—▼, CW^-; △—△, NW^- cells, and ○—○, blank medium. B. 1.5–2.0 × 10^6 adherent cells were cultured in 5 ml medium 199 without serum. Arrows indicate complete medium change. ●—●, α_2-macroglobulin in the cell supernate.

munoassays of the samples showed increasing concentrations of α_2-macroglobulin in supernates of unfractionated mononuclear leukocytes (Fig. 1A). Low concentrations of α_2-macroglobulin were also found in both NW^- and CW^- cultures (but only at the later stages of the incubation [Fig. 1A]).

This result suggested that monocytes, which were removed by passage through nylon wool or cotton wool, might be responsible for the appearance of α_2-macroglobulin in the culture medium of unfractionated mononuclear leukocytes. Therefore, adherent cell cultures enriched in monocytes (Table I) were incubated at 37°C in serum-free medium 199, and portions were assayed for α_2-macroglobulin. Relatively large amounts of α_2-macroglobulin appeared in the medium (Fig. 1B). It should be noted that the cell number per milliliter medium in the adherent cell cultures was only about 10% of that in the unfractionated mononuclear leukocyte cultures.

Metabolic Labeling of Protein(s) Precipitable by Anti-α_2-Macroglobulin. Adherent cell cultures of mononuclear leukocytes were labeled overnight with ^{35}S-methionine. Supernatant protein was coprecipitated with human se-

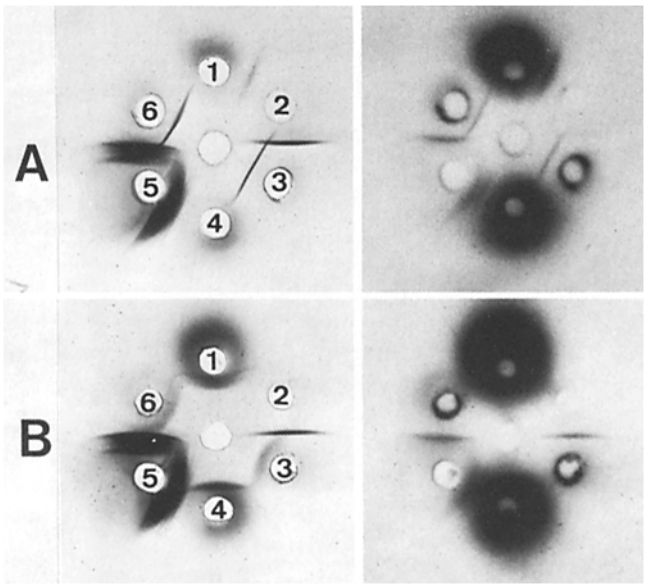

FIG. 2. Ouchterlony double diffusion assay for immunoreactive α_2-macroglobulin and fibronectin synthesized in adherent cell cultures. Left panels: protein staining with ponceau red. Right panels: autoradiography. The respective wells contained: A. Center well, anti-α_2-macroglobulin; 1 and 4, cell extract; 2, anti-C_3'; 3 and 6, cell supernate; 5, anti-human serum. B. Center well, anti-fibronectin; 1 and 4, cell extract; 2, anti-α_2-macroglobulin; 3 and 6, cell supernate; 5, anti-human serum.

rum proteins in 50% ammonium sulfate, dissolved in phosphate-buffered saline, and assayed for immunoreactive α_2-macroglobulin by Ouchterlony double diffusion.

The carrier human serum globulins formed several precipitation lines with anti-human serum but only a single line when tested against anti-α_2-macroglobulin or anti-fibronectin. Autoradiography revealed radioactivity in the single precipitation line between the cell supernate and anti-α_2-macroglobulin (Fig. 2). The respective line between the cell supernate and anti-human serum also was radioactive. A faint line of radioactivity (not visible in the photograph) was seen at the precipitate formed by the cell supernate and anti-human-C_3' serum. Radioactivity from the cell extract was precipitated by anti-human-C_3' and in several lines by anti-human serum but not by anti-α_2-macroglobulin. Radioactivity was not precipitated when the cell extracts or the cell supernates were tested against anti-fibronectin (Fig. 2). These results indicate that α_2-macroglobulin and a number of other serum proteins, including C_3' but probably not fibronectin, are synthesized in adherent cell cultures prepared from human blood mononuclear leukocytes.

Labeled Polypeptides Precipitated by Anti-α_2-Macroglobulin. Adherent cell cultures of human peripheral blood mononuclear leukocytes were incubated at 37°C in serum-free medium with daily medium change, or in medium supplemented with 10% fetal calf serum without medium change. In both types of the cultures morphological transformation of the initially round cells to enlarged extension-sending macrophages was seen. The proportion of altered cells in-

creased with increasing incubation time (days). Parallel cultures were labeled with ^{35}S-methionine at 1–2 or 4–5 days of incubation. Supernates were collected, and portions were treated with anti-α_2-macroglobulin, anti-fibronectin, or normal rabbit serum and subsequently with anti-rabbit immunoglobulin. The precipitates were dissolved and electrophorezed in discontinuous polyacrylamide gel slabs containing SDS.

In addition, samples of concentrated supernates were reacted against anti-α_2-macroglobulin or anti-fibronectin in Ouchterlony double diffusion, the precipitates were cut out, dissolved, and electrophorezed as above. Autoradiography of the gels showed a major radioactive band comigrating with authentic subunit of α_2-macroglobulin, in channels containing material precipitated by anti-α_2-macroglobulin (Fig. 3, channels 2, 5, and 7). Greater amounts of label migrating at this band were seen in supernates from cultures incubated for 4 days before labeling than in those from cells labeled after 1 day in culture (channels 2 and 5). Cells incubated in the serum-containing medium produced greater amounts of this material than cultures in the serum-free medium (not shown).

Material precipitated by anti-fibronectin contained a weak band migrating at α_2-macroglobulin but never a band of 2.2×10^5 daltons, the size of fibronectin subunit (21). Several weak bands with lower molecular weight were produced by precipitates of both antisera (barely visible in Fig. 3). Precipitates formed with normal rabbit serum did not show any reproducible radioactive bands in electrophoresis.

Analysis of supernates and cell extracts of labeled monocytes by electrophoresis showed that α_2-macroglobulin is not a major labeled cellular protein under these conditions (Fig. 3, channels 9 and 10). Staining of the gels for proteins (not shown) resulted in a similar conclusion.

Immunofluorescence Studies. Suspensions of mononuclear leukocytes or adherent cell cultures on coverslips, either unfixed or fixed with formaldehyde or with formaldehyde-acetone, were stained for α_2-macroglobulin and fibronectin by the indirect technique. The results were repeatedly negative while tests for fibronectin on human skin fibroblasts were regularly positive (21).

Discussion

These results show that α_2-macroglobulin is synthesized in human leukocyte cultures. When the leukocyte cultures were depleted of monocytes it was found that less α_2-macroglobulin appeared into the medium. Conversely, adherent cells enriched in monocytes produced much greater quantities of α_2-macroglobulin per cell than any of the populations more rich in lymphocytes. These results suggest that monocytes or a subpopulation of the monocyte-macrophage lineage is responsible for the production of α_2-macroglobulin in this system. Metabolic labeling of supernatant α_2-macroglobulin in adherent cell cultures proved that the protein was a product of synthesis by the cells rather than a result of release of previously endocytosed material. SDS-polyacrylamide gel electrophoresis of the immunoprecipitates showed that anti-α_2-macroglobulin specifically precipitates a polypeptide from the supernate which migrates at the position of authentic α_2-macroglobulin subunit. This indicates that the material precipitated by the antiserum is true α_2-macroglobulin and not, e.g., labeled protease-unlabeled α_2-macroglobulin complex.

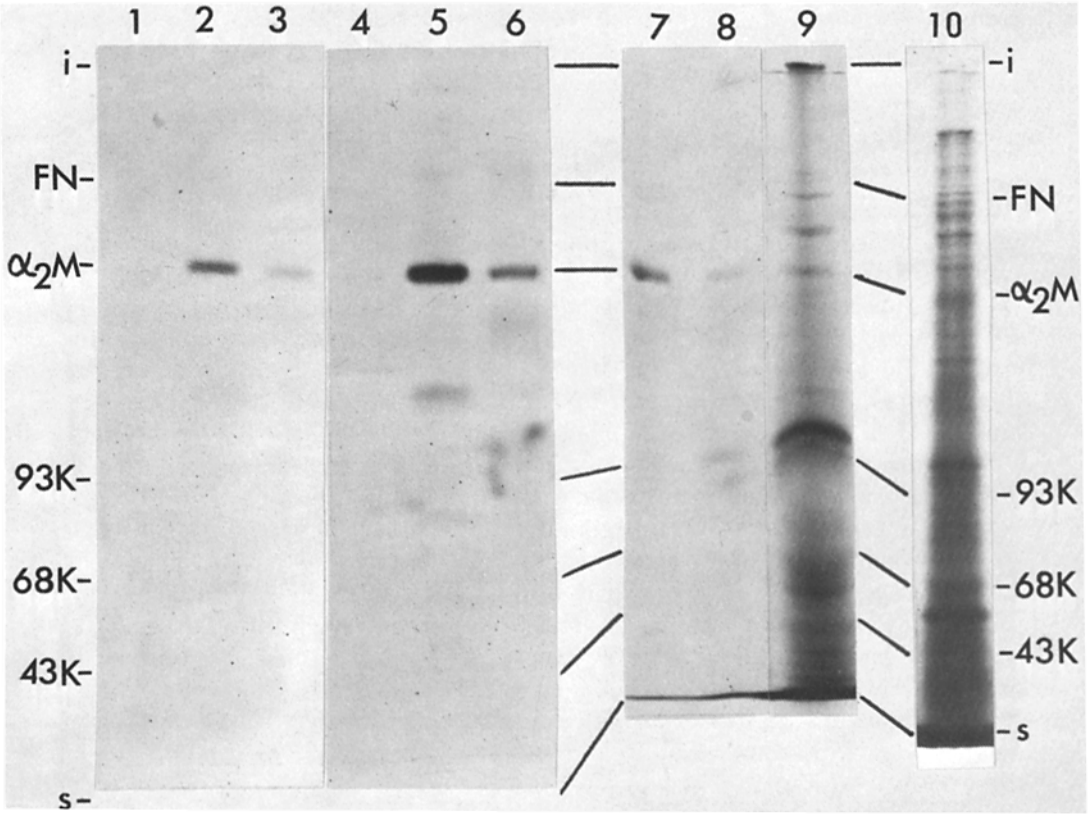

FIG. 3. SDS-polyacrylamide gel electrophoresis of ^{35}S-labeled polypeptides from monocyte supernates and cell extracts. Adherent cell cultures of human mononuclear leukocytes were incubated on glass coverslips in a 1:1 mixture of medium 199 and RPMI-1640 (Grand Island Biological Co.) supplemented with 10% of dialyzed fetal calf serum. After 1 or 4 days in culture the medium was replaced with the ^{35}S-methionine-labeling medium and incubation was continued for another 24 h. Supernates were harvested and subjected for immunoprecipitation either by the double antibody technique or Ouchterlony double diffusion. The immunoprecipitates were redissolved in a reducing SDS-containing buffer and portions, together with cell extracts, were analyzed in three parallel discontinuous (3 and 5% acrylamide) slab gels. After a 20-h run at 10 mA the gels were stained with Coomassie Blue, and migration of radiolabeled polypeptides was analyzed by autoradiography. Part of the autoradiogrammes are shown in this figure. i, interphase between the two acrylamide concentrations, FN, fibronectin; α_2M, α_2-macroglobulin; 93K, phosphorylase a; 68K, human serum albumin: 43K, ovalbumin; s, bromphenol blue tracker dye; 1–6, double antibody precipitates of supernates from cultures incubated for 1 day (1–3) or for 4 days (4–6) before labeling. Double antibody precipitates produced by normal rabbit serum (1, 4), anti-α_2-macroglobulin (2, 5), or anti-fibronectin (3, 6); 7 and 8, Ouchterlony immunoprecipitates produced by supernate from cultures incubated for 4 days, tested against anti-α_2-macroglobulin (7) or anti-fibronectin (8); 9, $(NH_4)_2SO_4$-precipitate (50% saturation) of supernate from cultures incubated for 1 day before labeling; 10, cell extract from a culture incubated for 1 day before labeling.

Skin fibroblasts have been shown to synthesize small amounts of α_2-macroglobulin in culture,[2] and it is theoretically possible that fibroblasts contaminate leukocyte cultures as a result of skin wounding by venipuncture. However, skin fibroblasts also synthesize large amounts of fibronectin (21). Lack of metabolic

labeling of fibronectin indicates that contamination by fibroblasts cannot be responsible for the synthesis of α_2-macroglobulin in adherent blood cell cultures. In addition, our cultured adherent cells did not contain fibronectin detectable by indirect immunofluorescence while skin fibroblasts are readily positive in the test.

Surface α_2-macroglobulin has been suggested for a marker for B lymphocytes (7–9). We could show very little synthesis and secretion of α_2-macroglobulin by the lymphocyte preparations. The NW^- cells produced as much α_2-macroglobulin as CW^- cells, making it unlikely that the small amount of α_2-macroglobulin in the lymphocyte preparations originated from B cells. In addition, we could not detect α_2-macroglobulin on cultured lymphocytes in the immunofluorescence assays. Our results do not, however, exclude the possibility that B cells bind α_2-macroglobulin from the plasma to their surface or synthesize but do not secrete α_2-macroglobulin.

There are several reasons to believe that α_2-macroglobulin synthesized and secreted by adherent cells may have an important function. Studies of in vitro cultures of fibroblast-like cells suggest a positive correlation between the secretion of proteases and α_2-macroglobulin.[2] Activated macrophages are known to shed plasminogen activator (26), lysosomal hydrolases (27), elastase (28), and collagenase (29), all of which are inhibited by α_2-macroglobulin (1). Rabbit peritoneal macrophages have been shown to specifically phagocytose α_2-macroglobulin protease complexes (30). It is interesting to note that the monocytes were found to release significant amounts of α_2-macroglobulin only after some days in culture. During this incubation period, there was a partial transformation to macrophages as judged by morphology. Similarly, Gordon and co-workers (31) reported that only activated monocytes produce plasminogen activator. Simultaneous secretion of proteases and protease inhibitors and phagocytosis of protease-inhibitor complexes may help in controlling the active protease levels in the microenvironment of cell surface and allow both spatial and temporal differences in the protease levels to occur. Protease activity is considered to be important in migration of macrophages in tissues (32). In addition, there is evidence (33–34) that α_2-macroglobulin may participate in the regulation of in vitro phenomena thought to be representative of portions of the in vivo immune response.

Summary

Alpha$_2$-macroglobulin levels in the supernates of cultures of different subpopulations of human peripheral blood mononuclear leukocytes were assayed by a radioimmunoassay. Unfractionated mononuclear leukocytes produced greater amounts of the macroglobulin (4.0 vs. 0.8 ng/10^6 cells) than did subpopulations enriched in T or B + T lymphocytes, by passage through nylon wool or cotton wool columns, respectively. Still higher concentrations of α_2-macroglobulin (40 ng/10^6 cells) were measured in the supernates of glass-adherent mononuclear leukocyte cultures. These results suggest that cells of monocyte-macrophage lineage are mainly, if not exclusively, responsible for the appearance of α_2-macroglobulin in the supernate of human peripheral blood leukocyte cultures.

The *de novo* synthesis and release of α_2-macroglobulin by cultured monocytes was demonstrated by immunoprecipitation of radioactivity from supernates of

^{35}S-methionine-labeled glass-adherent cells. Antiserum against purified α_2-macroglobulin was used in both Ouchterlony double diffusion and double antibody precipitation tests. SDS-polyacrylamide gel electrophoresis of immunoprecipitates showed that most of the radioactivity comigrated with authentic α_2-macroglobulin subunit at about 160,000 daltons.

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References

1. Barrett, A. J., and P. M. Starkey. 1973. The interaction of α_2-macroglobulin with proteinases. Characterization and specificity of the reaction, and a hypothesis concerning its molecular mechanism. *Biochem. J.* **133**:709.
2. Adham, N. F., P. Wilding, J. Mehl, and B. J. Haverback. 1968. Alpha$_2$-macroglobulin levels in human serum. I. The effect of sex and estrogen. II. The role of alpha$_2$-macroglobulin as a carrier protein for insulin. *J. Lab. Clin. Med.* **71**:271.
3. Tunstall, A. M., J. M. L. Merriman, and K. James. 1975. Normal and pathological levels of α_2-macroglobulins in men and mice. *J. Clin. Pathol. (Lond.).* **28**:133.
4. Laurell, C.-B., and J.-O. Jeppson. 1975. Protease inhibitors in plasma. *In* Plasma Proteins. F. V. Putnam, editor. Academic Press, Inc., New York. 229–264.
5. Mosher, D. F., and D. A. Wing. 1976. Synthesis and secretion of α_2-macroglobulin by cultured human fibroblasts. *J. Exp. Med.* **143**:462.
6. Williams, D. F., Jr., R. Asofsky, and G. J. Thorbecke. 1963. Plasma protein formation in vitro by tissues from mice infected with staphylococci. *J. Exp. Med.* **118**:315.
7. Sell, S. 1970. Stimulation of rabbit peripheral lymphocytes with antisera to α-macroglobulin. *Int. Arch. Allergy. Appl. Immunol.* **38**:150.
8. McGormick, J. N., D. Nelson, A. M. Tunstall, and K. James. 1973. Association of α_2-macroglobulin with lymphoid cells. *Nat. New Biol.* **246**:78.
9. Gelder, F., P. Hurtubise, J. Scillian, and S. Murphy. 1975. The association of α_2-macroglobulin with the Fc receptor on B lymphocytes. *Clin. Res.* **23**:291A.
10. Gitlin, D., and A. Biasucci. 1969. Development of γG, γA, γM, β1C/β1A, C'1 esterase inhibitor, ceruloplasmin, transferrin, hemopexin, haptoglobin, fibrinogen, plasminogen, α_1-antitrypsin, orosomucoid, β-lipoprotein, α_2-macroglobulin, and prealbumin in the human conceptus. *J. Clin. Invest.* **48**:1433.
11. Tunstall, A. M., and K. James. 1974. Preliminary studies on the synthesis of alpha$_2$-macroglobulin by human lymphocytes in vitro. *Clin. Exp. Immunol.* **17**:697.
12. Skoog, W. A., and W. S. Bech. 1956. Studies on the fibrinogen, dextran and phytohemagglutinin methods of isolating leukocytes. *Blood.* **11**:436.
13. Böyum, A. 1968. Separation of leukocytes from blood and bone marrow. *Scand. J. Clin. Lab. Invest.* **21**:(Suppl. 97).
14. Hovi, T., A. C. Allison, and S. C. Williams. 1976. Proliferation of human peripheral blood lymphocytes induced by A23187, a Streptomyces antibiotic. *Exp. Cell. Res.* **97**:92.
15. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3**:645.
16. Lamvik, J. O. 1966. Separation of lymphocytes from human blood. *Acta Haematol. (Basel).* **35**:294.
17. Aiuti, F., J.-C. Cerottini, R. R. A. Coombs, M. Cooper, H. B. Dickler, S. Frøland, H. H. Fudenberg, M. F. Greaves, H. M. Grey, H. G. Kunkel, J. Natvig, J.-L.

Preud'homme, E. Rabellino, R. E. Ritts, D. S. Rowe, M. Seligman, F. P. Siegel, J. Stjernswärd, W. D. Terry, and J. Wybran. 1975. International Union of Immunological Societies (IUIS), Report-July 1974. Identification, Enumeration and Isolation of B and T lymphocytes from Human Peripheral Blood. *Clin. Immunol. Immunopathol.* 3:584.

18. Roberts, R. C., W. A. Reisen, and P. K. Hall. 1974. Studies on the quaternary structure of human serum α_2-macroglobulin. *In* Proteinase Inhibitors. H. Fritz, H. Tschesche, L. J. Greene, and E. Truscheit, editors. Springer-Verlag, New York Inc., New York. 63–71.
19. Krohn, K., L. Sherman, and M. Welch, 1972. Studies of radioiodinated fibrinogen. I. Physicochemical properties of the ICl, chloramine-T and electrolytic reaction products. *Biochim. Biophys. Acta.* 285:404.
20. Biberfeld, P., Biberfeld, G., Molnar, Z., and Fagraeus, A. 1974. Fixation of cell-bound antibody in the membrane immunofluorescence test. *J. Immunol. Methods* 4:135.
21. Vaheri, A., and E. Ruoslahti. 1975. Fibroblast surface antigen produced but not retained by virus-transformed human cells. *J. Exp. Med.* 142:530.
22. Ouchterlony, Ö., and L. Å. Nilsson. 1973. Immunodiffusion and immunoelectrophoresis. *In* Handbook of Experimental Immunology. D. M. Weir, editor. Blackwell Scientific Publications Ltd., Oxford, England. 2nd edition 1:1–20.
23. Stecher, V. J., and G. J. Thorbecke. 1967. Sites of synthesis of serum proteins. I. Serum proteins produced by macrophages in vitro. *J. Immunol.* 99:643.
24. Laemli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680.
25. Rice, R. H., and G. E. Means. 1971. Radioactive labeling of proteins in vitro. *J. Biol. Chem.* 246:831.
26. Unkeless, J. C., S. Gordon, and E. Reich. 1974. Secretion of plasminogen activator by stimulated macrophages. *J. Exp. Med.* 139:834.
27. Cardella, C. J., P. Davies, and A. C. Allison. 1974. Immune complexes induce selective release of lysosomal hydrolases from macrophages. *Nat. New Biol.* 247:46.
28. Werb, Z., and S. Gordon. 1975. Elastase secretion by stimulated macrophages. Characterization and regulation. *J. Exp. Med.* 142:361.
29. Werb, Z., and S. Gordon. 1975. Secretion of a specific collagenase by stimulated macrophages. *J. Exp. Med.* 142:346.
30. Debanne, M. T., R. Bell, and J. Dolovich. 1975. Uptake of proteinase-α-macroglobulin complexes by macrophages. *Biochim. Biophys. Acta.* 411:295.
31. Gordon, S., J. C. Unkeless, and Z. A. Cohn. 1974. Induction of macrophage plasminogen activator by endotoxin stimulation and phagocytosis. Evidence for a two-stage process. *J. Exp. Med.* 140:995.
32. Vassalli, J.-D., J. Hamilton, and E. Reich. 1976. Macrophage plasminogen activator: Modulation of enzyme production by anti-inflammatory steroids, mitotic inhibitors, and cyclic nucleotides. *Cell.* 8:271.
33. Calder, E. A., S. J. Urbaniak, W. J. Irwine, and K. James. 1975. The effect of anti-alpha$_2$-macroglobulin on K-cell cytolysis and T- and B-cell rosette formation. *Clin. Exp. Immunol.* 22:112.
34. Renold, H. G., and R. D. Rosenberg. 1975. Enhancement of migration inhibitory factor activity by plasma esterase inhibitors. *J. Biol. Chem.* 250:6608.