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Determination of Subpopulations of Leukocytes Involved in the Synthesis of α_1-Antitrypsin *in Vitro* (40832)[1]

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α_1-Antitrypsin (A_1AT) is a highly polymorphic glycoprotein which consists of a single polypeptide chain containing 12% carbohydrate and has a molecular weight (MW) of 54,000 (1). A_1AT is the major inhibitor of serine proteases such as trypsin, elastase, and leukocyte proteases (1). Since the first identification of different phenotypes of A_1AT, 26 different alleles and more than 45 different phenotypes have been discovered (2, 3). Of these 26 alleles (Pi types) some, such as Pi^Z, are associated with reduced serum levels of A_1AT (1). Reduced levels of A_1AT can result in a predisposition to life-threatening disorders such as chronic obstructive pulmonary disease and cirrhosis of the liver (review in Ref. (4)); this indicates strongly that A_1AT is required for the maintenance of normal homeostatic mechanisms in these organs.

Recently Gupta *et al.* (5) were able to identify A_1AT in pulmonary macrophages obtained from sputum specimens from individuals who smoked or who had chronic obstructive pulmonary disease. Pulmonary macrophages (MØ) from nonsmokers did not contain detectable A_1AT. No attempt was made to determine why the pulmonary MØ from individuals with pulmonary disease had demonstrable A_1AT, i. e., whether it was due simply to increased uptake of A_1AT-protease complexes by MØ as a result of excessive protease release by MØ or neutrophils promoting tissue damage, or whether the pulmonary MØ actually synthesized A_1AT as a result of increased activation in response to the disease process.

In another study, Lipsky *et al.* (6) reported that A_1AT could be demonstrated on the surface of lymphocytes by immunofluorescence. Although they found A_1AT on lymphocytes and demonstrated that it was present only after activation by concanavalin A (ConA), they did not investigate its origin. Presumably, either lymphocytes or monocytes might synthesize A_1AT *de novo*. Lymphocytes may synthesize it after direct activation by ConA, or monocytes may make it after activation by mediators of cellular immunity (MCI) released by ConA-activated T or B lymphocytes. It is also possible that neither cell type actually synthesizes A_1AT *in vitro*. Instead, it may be present already as a consequence of endocytosis *in vivo* and expressed only when the cells are fully stimulated either by phytolectins (6) or by MCI. To learn more about the origin of the A_1AT found on lymphocytes and in macrophages (monocytes), we undertook in the present study to answer two basic questions: (i) Is A_1AT synthesized *de novo* or just released by activated white blood cells? (ii) If it is synthesized *in vitro* by white blood cells, which cell type is responsible for making it?

Materials and methods. Mononuclear leukocyte cultures. Heparinized venous blood (50 units/ml Panheparin) was obtained from adult human volunteers who had given their informed consent. All the participants in this study were healthy and had white blood cell counts within the normal range (5,000–10,000/mm^3). All were PiM as shown by isoelectric focusing (3).

Mononuclear leukocytes (MNL) were isolated by density gradient centrifugation following the method of Böyum (7) with two modifications: (i) a commercially available solution of Ficoll and sodium dia-

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trizoate was employed (LSM solution; Bionetics, Kensington, Md.), and (ii) the blood was diluted 1:2 with Dulbecco's phosphate-buffered saline [PBS; Ca^{2+}, Mg^{2+} free; Grand Island Biological Company (Gibco, Grand Island, New York] prior to layering over the LSM solution. The isolated MNL were washed vigorously three times in PBS, counted, and then either resuspended in RPMI 1640 special medium (RPMI-S) if they were to be cultured as a mixed population of cells, or in complete medium 199 if they were to be separated into adherent and nonadherent cells. Complete RPMI-S culture medium consisted of a specially prepared RPMI 1640 medium depleted of L-leucine, L-glutamic acid, L-aspartic acid, L-phenylalanine, and L-valine (Gibco) and supplemented with 1 m*M* L-glutamine, 1% bovine serum albumin (BSA; Cohn fraction V; Sigma Chemicals, St. Louis, Mo.), 2 μCi/ml ^{3}H-labeled protein hydrolysate (yeast mixture; Schwarz/Mann, Orangeburg, N.Y.), and penicillin and streptomycin (100 U/ml and 100 μg/ml). Complete medium 199 (M199-C) consisted of medium 199 with Earle's salts (Gibco) supplemented with antibiotics, L-glutamine, and 10% fetal calf serum (FCS) exactly as described previously (8).

MNL were cultured as 1.0-ml aliquots containing 10^6 cells/ml in complete RPMI-S in 16 × 125 mm Falcon tissue culture tubes (No. 3033; Falcon Plastics, Oxnard, Calif.) for up to 6 days with or without the addition of 2.0 μg purified phytohemagglutinin (PHA; Wellcome Research Laboratories, Beckenham, England) per milliliter. At the end of the culture, the cell-free supernatants were harvested for analysis of total protein and A_1AT content after removal of cells and filtration of the medium through a 0.45-μm needle filter (Acrodisc, Gelman; Ann Arbor, Mich.). Experiments by Wilson and Bahm (9) and Polet and Spieker-Polet (10) have previously documented that when BSA is substituted for FCS or autologous serum, peak responses of MNL to mitogen and antigen stimulation are still observed.

Separation of nonadherent lymphocytes and adherent mononuclear cells. Monocytes were removed from MNL by adherence to plastic or glass upon incubation at 37°C (11), using the method described previously by Wilson *et al.* (12). Briefly, 2.0 ml MNL (6.0×10^6 cells/ml) were incubated in M199-C in a 35 × 10-mm Falcon tissue culture petri dish precoated by incubating 1.0 ml fresh M199-C in the dish for 15 min at 37°C prior to adding the MNL. After 30 min at 37°C, the nonadherent lymphocytes (NA-MNL) were removed by vigorously washing the dishes with PBS. The procedure was repeated a minimum of three times. The NA-MNL obtained were washed in PBS three times and cultured in complete RPMI-S as described for MNL. The adherent monocytes were cultured for 24–48 hr in fresh M199-C to ensure complete detachment of lymphocytes, washed several times with PBS, and then cultured in fresh RPMI-S alone or supplemented with either 2.0 μg/ml PHA or 1.0–100 μg *Escherichia coli* endotoxin (Difco Laboratories, Detroit, Mich.) for up to 6 days.

Monocytes and NA-MNL were >90 and >99% pure, respectively, when separated using the above methods, as determined by latex particle engulfment and by histochemical staining for acid phosphatase (9, 12). Each plate contained approximately 8 to 10×10^5 monocytes.

Determination of total protein content. Quantitation of total protein synthesized by either MNL, NA-MNL, or monocytes was performed following the procedure described by Einstein *et al.* (13). Briefly, ^{3}H-labeled protein and unlabeled BSA in the cell-free supernatant were precipitated by the addition of 4 vol 20% trichloroacetic acid (TCA) to 1 vol culture medium, followed by incubation of the mixture for 15 min at 4°C and then centrifugation at 1000*g* for 15 min at 4°C. The supernatant was removed and the TCA precipitate was dissolved in NaOH by heating at 37°C for 15–30 min. The procedure was repeated a minimum of three times. A control consisting of fresh RPMI-S was analyzed with each set of cultures to determine the background counts per minute (cpm). The final dissolved material was added to 5.0 ml

scintillation fluid (PCS; Amersham Corp., Arlington Heights, Ill.) and counted in a scintillation counter.

Determination of total A_1AT synthesized in vitro. The amount of A_1AT synthesized by 6-day cultures of MNL, NA-MNL, or monocytes was determined as follows. One volume of normal human serum was added to 10 vol of culture medium containing goat antiserum to human A_1AT (Meloy Laboratories, Springfield, Va.). The mixture was incubated for 1 hr at 37°C and then for 16 hr at 4°C. The precipitate was harvested by centrifugation at 1000*g* for 15 min and washed three times in cold PBS (4°C). The final precipitate was dissolved in a small volume of 1 *N* NaOH, and the amount of ^{3}H-labeled material was determined as noted previously for the TCA-precipitated material. Controls consisted of (i) determining how much ^{3}H-labeled material could be nonspecifically adsorbed to either of two different antigen–antibody complexes formed between antibodies and antigens known not to be synthesized by human MNL [BSA and human serum albumin (HSA)], and (ii) determining how much ^{3}H-labeled material was precipitated by antibody to A_1AT when the culture medium was pretreated with an excess of soybean trypsin inhibitor (SBTI, Sigma Chemicals) (4.0 mg/ml) prior to the addition of antibody or human serum. SBTI is known to inhibit a variety of proteases made by cells or found in serum which might bind to A_1AT (14); when SBTI is added first, the ^{3}H-labeled proteases secreted by the cells will not bind to the A_1AT found in the antiserum or human serum and thus will not provide erroneous values for labeled A_1AT which in reality is labeled protease bound to unlabeled A_1AT. It is known that proteases must be active and not complexed to another protease inhibitor in order to be bound by A_1AT (1).

Gel filtration chromatography and SDS acrylamide gel electrophoresis. Aliquots of culture medium from 6-day PHA-stimulated cultures of MNL (2.0 ml) were fractionated by gel permeation chromatography on a 1.2 × 100-cm column of Sephadex G-200 (Pharmacia Fine Chemicals, Piscataway, N. J.) using descending flow at a rate of 6 ml/hr (15). Potassium phosphate buffer (0.01 *M*, pH 7.2) containing 0.15 *M* NaCl was employed as eluent, and tube volumes of 2.0 ml were collected. The column was calibrated using proteins of known molecular weight (Fig. 2). The column effluent was monitored by absorbance at 280 nm, and each tube was analyzed for ^{3}H-labeled protein by counting 200-μl aliquots as described previously. Five pools were made for subsequent testing (Fig. 2) either by sodium dodecyl sulfate (SDS)–acrylamide gel electrophoresis or by immune precipitation using antibody specific for human A_1AT. The five pools were lyophilized and reconstituted to 1.0 ml to concentrate the samples.

SDS–acrylamide gel electrophoresis was performed essentially as described by Fehrnström and Moberg (16) using the LKB 2117 muliphor. After completion of an electrophoresis run, the fixed and stained gel was cut into slices (5.0 × 10 mm) for analysis for ^{3}H-labeled protein. Prior to counting each slice was hydrated in 50 μl distilled water, and then protein was solubilized by incubation of the slice in 200 μl of Soluene 100 (Packard Instrument Co., Downers Grove, Ill.) (17). After an overnight incubation at 25°C, 10 ml of PCS scintillation fluid (Amersham Corp.) was added and the mixture was allowed to stabilize for at least another 24 hr prior to counting. Each sample was incubated with SDS and 2-mercaptoethanol prior to electrophoretic analysis (16), and a series of proteins of known molecular weight were employed to determine the size of the various ^{3}H-labeled products present in crude culture supernatants or material from Sephadex G-200 gel filtration (Fig. 2).

Results. Total protein synthesis. The results obtained for total ^{3}H-labeled protein secreted over 6 days by PHA-stimulated and unstimulated MNL and NA-MNL are shown in Table I. The optimal amount of PHA for use had been determined in previous studies (9). In each experiment, a significant increase in the amount of ^{3}H-labeled protein was obtained for both MNL and NA-MNL cultures (stimulation index

TABLE I. PROTEIN SYNTHESIS BY MONONUCLEAR LEUKOCYTES STIMULATED WITH PHA, OR ENDOTOXIN DURING 6-DAYS OF CULTURE

Cell type[a]	Expt	Donor	Stimulant[b]	^{3}H-labeled protein per 10^6 cells (cpm × 10^{-3})[c] Unstimulated	Stimulated	SI[d]
MNL	1	R.C.	PHA	4.61	9.73	2.07
	2	D.W.	PHA	5.27	8.71	1.65
	3	H.H.	PHA	3.65	12.28	3.36
	4	P.T.	PHA	2.98	6.32	2.12
	5	J.H.	PHA	4.02	11.65	2.90
	Mean			4.11	9.74	2.42
	±SD			±0.88	±2.39	
NA-MNL	1	P.T.	PHA	1.65	5.01	3.04
	2	R.C.	PHA	5.38	12.68	2.36
	3	L.S.	PHA	3.00	8.77	2.92
	Mean			3.34	8.82	2.77
	±SD			±1.89	±3.84	
Monocytes	1	G.W.	Endo	9.20	20.10	2.18
	2	H.H.	Endo	12.13	23.95	1.97
	3	J.H.	Endo	10.90	22.50	2.06
	4	H.H.	PHA	9.80	11.05	1.13

[a] MNL, mononuclear leukocytes; NA-MNL, nonadherent MNL (lymphocytes).
[b] PHA, phytohemagglutinin (20 μg/ml); Endo, *E. coli* endotoxin (25 μg/ml).
[c] Total counts after 6 days in culture.
[d] SI, stimulation index = (cpm in stimulated culture)/(cpm in unstimulated culture).

(SI) >1.4), indicating that the culture conditions employed were sufficient to promote adequate uptake and incorporation of ^{3}H-labeled amino acids into TCA-precipitable material. In order to promote protein synthesis in monocytes, we used a substance known to stimulate increased metabolic activity in monocytes, namely, *E. coli* endotoxin (18). When endotoxin was tested over a range of concentrations (Fig. 1), maximal stimulation was obtained with 25 μg/ml; this amount was used in all subsequent studies. Although endotoxin promoted more than a twofold increase in protein synthesis by monocytes, comparing favorably to the SI for PHA-stimulated MNL or NA-MNL, PHA failed to promote any significant increase in protein synthesis by monocytes. The results obtained in a representative experiment are shown in Table I.

Synthesis of A_1AT. Table II shows representative results for our analysis of SBTI-treated or untreated medium obtained from 6-day cultures of MNL, NA-MNL, or monocytes, either stimulated or unstimulated. Several points are illustrated by these results: (i) The amount of A_1AT found in stimulated MNL cultures increased 2.3-fold over that in unstimulated MNL cultures. Similar SI values were obtained for MNL cultures with or without prior treatment of the medium with SBTI, indicating that the

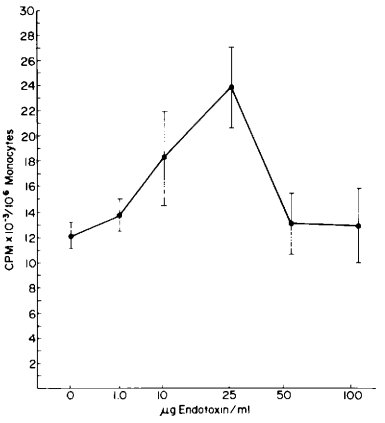

FIG. 1. Incorporation of ^{3}H-labeled amino acids into protein by monocytes after stimulation with various amounts of *E. coli* endotoxin. Each point represents the mean (± SEM) cpm incorporated into TCA-precipitable protein by 10^6 monocytes over a 6-day period.

TABLE II. A_1AT SYNTHESIS BY MONONUCLEAR LEUKOCYTES STIMULATED WITH PHA, OR ENDOTOXIN DURING 6 DAYS OF CULTURE

Cell type[a]	Donor	Stimulant[b]	A_1AT per 10^6 cells (cpm × 10^{-3})[c]		SI[d]
			Unstimulated	Stimulated	
Medium not treated with SBTI[e]					
MNL	R.C.	PHA	0.15	0.55	3.61
	J.H.	PHA	0.26	0.38	1.48
	H.H.	PHA	0.16	0.29	1.80
	Mean		0.19	0.41	2.30
	±SD		±0.06	±0.13	
NA-MNL	P.T.	PHA	0.17	0.16	0.95
	J.H.	PHA	0.21	0.24	1.15
	H.H.	PHA	0.15	0.18	1.19
	Mean		0.18	0.19	1.10
	±SD		±0.03	±0.04	
Monocytes	H.H.	Endo	0.39	1.20	3.08
	J.H.	Endo	0.42	0.91	2.17
Medium treated with SBTI[e]					
MNL	R.C.	PHA	0.11	0.40	3.59
	J.H.	PHA	0.09	0.19	2.11
NA-MNL	P.T.	PHA	0.05	0.06	1.38
	J.H.	PHA	0.09	0.09	1.00
Monocytes	J.H.	Endo	0.20	0.80	4.00
	H.H.	Endo	0.21	0.51	2.45

[a] As in Table I.
[b] As in Table I.
[c] Total ^{3}H-labeled protein associated with immune precipitates of human A_1AT and a goat antiserum specific for human A_1AT. 100 μl human serum was added per ml of culture as a source of unlabeled A_1AT.
[d] As in Table I.
[e] SBTI, soybean trypsin inhibitor (Sigma, Type IIS), 4 mg/ml. Where indicated, SBTI was incubated with the culture medium for 15 min at 37°C before serum was added.

counts per minute associated with the A_1AT immune precipitate were not due only to ^{3}H-labeled protease binding to unlabeled A_1AT already in the serum. (ii) NA-MNL cultures did not show an increase in A_1AT levels when stimulated by PHA, whereas monocyte cultures contain increased amounts of ^{3}H-labeled A_1AT when stimulated by endotoxin. (iii) Treatment of culture supernatants with SBTI indicated that some but not all of the radioactive material found in the A_1AT–anti-A_1AT immune precipitate was probably due to the binding of ^{3}H-labeled protease to the unlabeled A_1AT in the serum (e. g., compare values for H. H. monocytes with and without SBTI treatment in Table II).

The finding that NA-MNL culture medium still contained significant levels of labeled material even after SBTI treatment required an explanation. One possibility was that the counts remaining simply represented material passively adsorbed to the immune complex. To determine the extent of nonspecific adsorbtion of ^{3}H-labeled material, we employed two other immune complexes, BSA–anti-BSA and HSA–anti-HSA. Representative results are shown in Table III. Although some material coprecipitated in each instance, the nonspecifically bound material amounted to only 30–40% of the counts per minute in unstimulated SBTI-treated MNL cultures (compare results for MNL of R. C. and J. H. in Tables II and III) and to only 10–15% of the counts per minute in stimulated cultures.

Documentation of the presence of A_1AT by Sephadex G-200 gel filtration chromatography and SDS–acrylamide gel electrophoresis. The data presented in Tables II and III appeared to indicate that A_1AT is

TABLE III. NONSPECIFIC ADSORPTION OF ^{3}H-LABELED PROTEIN TO IMMUNE PRECIPITATES IN CULTURES OF MONONUCLEAR LEUKOCYTES (MNL) STIMULATED WITH PHA (2.0 μg/ml) DURING 6-DAYS OF CULTURE

MNL donor	Antiserum[a]	^{3}H-protein per 10^6 cells (cpm)[b]		SI[c]
		Unstimulated	Stimulated	
J.H.	Goat anti-A_1AT	260	384	1.48
R.C.	Goat anti-A_1AT	152	549	3.61
J.H.	Rabbit anti-BSA	75	62	0.83
R.C.	Rabbit anti-BSA	45	52	1.16
J.H.	Goat anti-HSA	30	26	0.87
R.C.	Goat anti-HSA	27	37	1.37

[a] Goat anti-A_1AT, goat antiserum specific for human A_1AT; rabbit anti-BSA, rabbit antiserum specific for bovine serum albumin; goat anti-HSA, goat antiserum specific for human serum albumin.

[b] Total counts precipitated from culture supernatants over 6 days. Human serum (100 μl) was added before each antiserum. The human serum was not treated with SBTI (see Table II).

[c] SI, as in Table I.

indeed synthesized *de novo* by monocytes. The possibility remained to be excluded, however, that the A_1AT detected was due to the release of A_1AT accumulated by endocytosis *in vivo* (6). To confirm that A_1AT was indeed being synthesized and labeled *in vitro*, we attempted to demonstrate the presence of labeled components of MW equal to that of A_1AT (54,000). A typical elution profile for culture medium from 6-day PHA-stimulated MNL is shown in Fig. 2. As can be seen, significant amounts

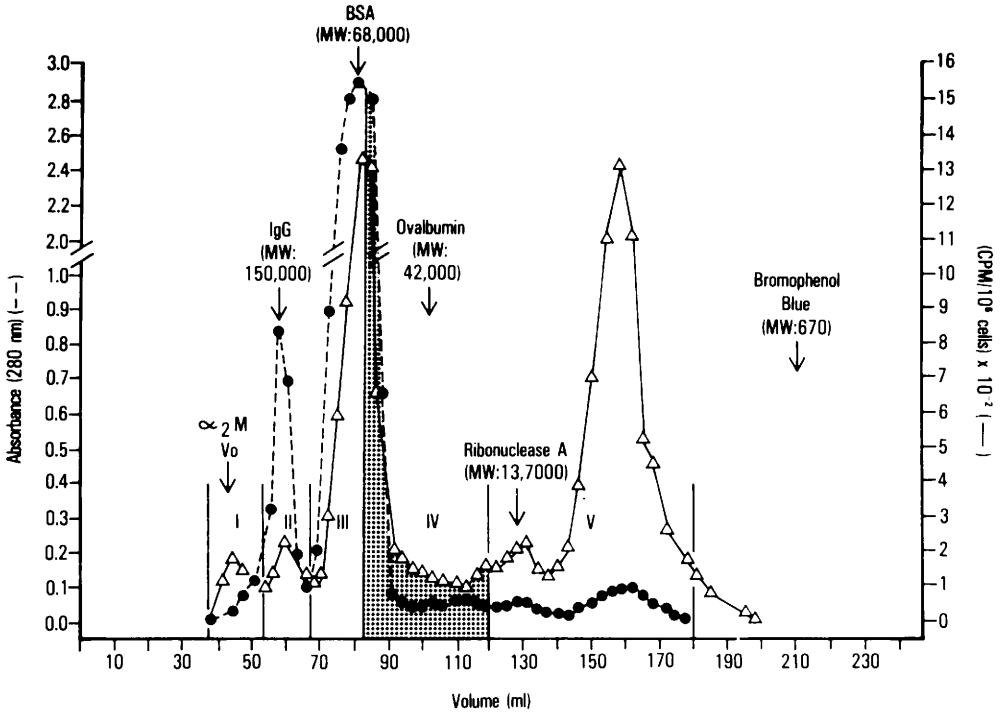

FIG. 2. Elution profile obtained for 2.0 ml of culture medium from 6-day cultures of PHA-stimulated mononuclear leukocytes on a 1.2×100-cm column of Sephadex G-200. Pools made for subsequent analyses are numbered I to V. The column was calibrated with substances of known molecular weight (MW) as indicated. A_2M, α_2-macroglobulin (MW near 725,000); BSA, bovine serum albumin; Vo, void volume (effluent volume containing substances totally excluded from the gel).

of ^{3}H-labeled material eluted in the MW range of A_1AT (between ovalbumin and BSA). To confirm the presence of A_1AT, the following experiment was performed. The Sephadex G-200 eluate was divided into five pools (I–V), and an aliquot of pool IV (Fig. 2) was treated with antibody specific for human A_1AT or BSA. The immune precipitate obtained in each case was then dissolved by treatment with SDS and mercaptoethanol in preparation for SDS–acrylamide gel electrophoresis. As controls, we also analyzed aliquots of the crude culture supernatant; the Sephadex G-200 fraction IV (not treated with antibody to A_1AT); the other Sephadex G-200 column fractions (I–III, V; Fig. 2); and the BSA–anti-BSA immune precipitate. The results obtained for the crude supernatant, the untreated Sephadex G-200 pool IV and the A_1AT–anti-A_1AT immune precipitate are shown in Fig. 3. ^{3}H-Labeled material with a MW equal to that of A_1AT was readily demonstrated in the crude supernatant (peak 4) and in Sephadex G-200 pool IV and could be recovered from the specific immune precipitate (Fig. 3), indicating that A_1AT was indeed synthesized *de novo*. The BSA–anti-BSA immune precipitate did not contain ^{3}H-labeled material in the MW range of A_1AT.

Discussion. A_1AT, like α_2-macroglobulin

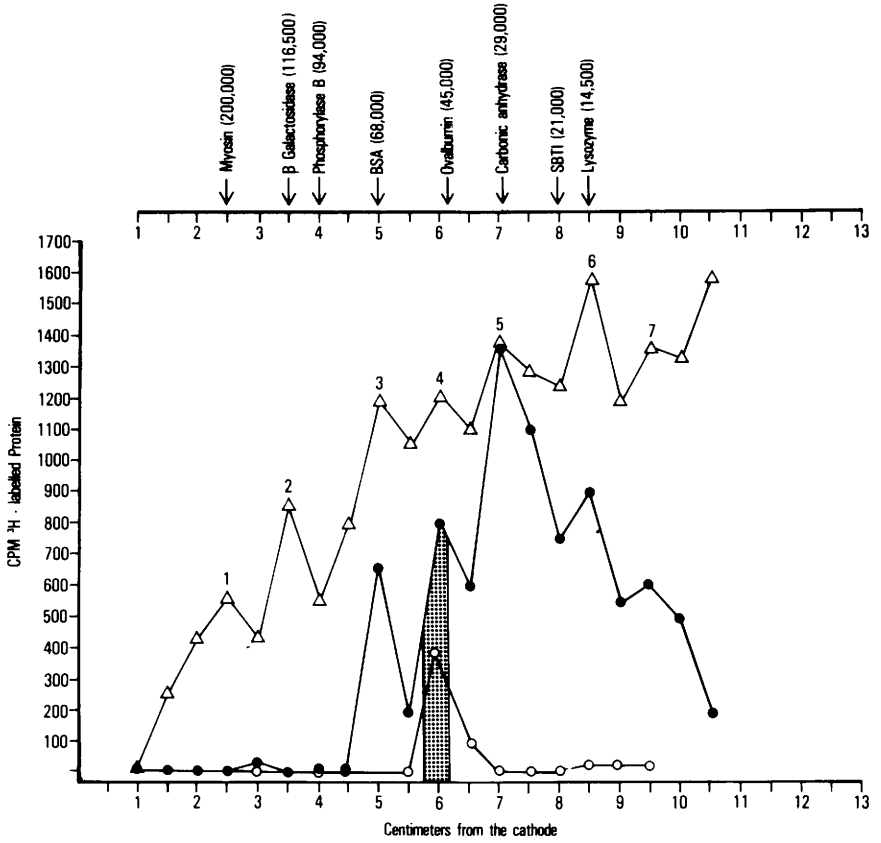

FIG. 3. Analysis of ^{3}H-labeled protein by SDS-acrylamide gel electrophoresis. The position (cm from the cathode) of the protein markers of known MW are indicated. (●), Crude supernatant from 6-day PHA-stimulated MNL. (△), Sephadex G-200 pool IV (Fig. 2). (○), Material obtained from treatment of Sephadex G-200 pool IV with antibody specific for A_1AT. The position at which A_1AT should be present (MW 54,000) is indicated as a crosshatched region under peak 4 just before the 45,000-MW marker.

(A_2M) and some other serum proteins (most notably the complement components C1q, C2, C3 and C4), is thought to be synthesized primarily by the liver *in vivo* (1). Early studies by Gitlin and Biasucci (19) established that A_1AT is synthesized *in vitro* by cultures of hepatocytes, as is A_2M. Later, it was shown that A_2M was associated with the surface of a subpopulation of MNL (probably B lymphocytes) by immunofluorescence (20), and that it could be synthesized by human MNL *in vitro* (21). Hovi *et al.* (22) and Mosher *et al.* (23) established that the monocyte macrophage was the cell type primarily involved in the synthesis of A_2M. Interestingly, complement component C3, originally believed to be synthesized only by hepatocytes (24), was also shown recently to be synthesized by human monocytes *in vitro* (13). Following an evolving path of investigations similar to those of A_2M but somewhat delayed in time, A_1AT has recently been demonstrated on the surface of MNL by immunofluorescence (6). In the present study evidence has been presented that A_1AT, like A_2M, is synthesized by MNL and is secreted primarily by monocytes.

Evidence for the *de novo* synthesis of A_1AT by MNL was provided by demonstration that (i) accumulation of ^3H-labeled A_1AT was increased in 6-day MNL cultures stimulated with PHA as compared to unstimulated cultures (similar results (data not shown) were obtained in preliminary studies with pokeweed mitogen); (ii) in the presence of SBTI, although labeled proteases were inhibited, A_1AT levels were still increased, indicating that the presence of labeled A_1AT was not due merely to binding of labeled protease by A_1AT in the carrier serum; (iii) supernatants from PHA-stimulated NA-MNL did not contain elevated levels of A_1AT (Table II), although total protein content was increased to the same extent as in MNL cultures (Table I); and (iv) immune precipitates formed between proteins such as HSA or BSA, known not to be synthesized by MNL, did not passively adsorb significant amounts of ^3H-labeled material (Table III). In addition, the use of BSA instead of FCS or autologous serum as a culture supplement helped rule out the possibility that the amounts of A_1AT obtained might represent labeled protease bound to A_1AT from serum. The fact that NA-MNL did contain small amounts of labeled A_1AT may be explained by the release of membrane-bound labeled protease complexed-A_1AT derived from donor plasma *in situ;* however, this clearly could account for only a fraction of the total A_1AT present (Tables II and III), and the amount did not increase significantly with stimulation as seen for MNL and monocytes (Table II). Further evidence that A_1AT was synthesized by monocytes *de novo* was obtained by demonstrating the presence of material of MW equal to that of A_1AT, using SDS–acrylamide gel electrophoresis and Sephadex G-200 gel filtration chromatography (Figs. 2 and 3).

It is likely that A_1AT synthesis by monocytes is regulated by some sort of feedback mechanism. Figure 4 presents one possible explanation for how secretion of A_1AT by monocytes might be affected by other cells, predominantly lymphocytes: (i) Lymphocytes are stimulated by specific antigen (or in this study, lectins or mitogens such as PHA) and secrete mediators of cellular immunity (MCI). (ii) Among these are MCI which can activate monocytes in the same manner as endotoxin or latex particles (18). (iii) Activated monocytes can secrete a variety of substances (25), including A_1AT. A_1AT may then inhibit human proteases or bacterial proteases, thus preventing tissue damage, and may cause immune suppression, as described previously for A_2M–

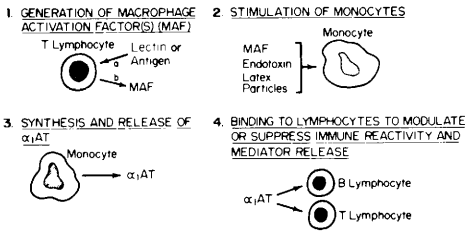

FIG. 4. A possible mechanism for the induction of increased synthesis of A_1AT by monocytes. Binding of A_1AT to the lymphocytes is proposed to inhibit continued monocyte activation by decreasing or stopping the secretion of macrophage activation factors (MAF) by lymphocytes. See Discussion for further explanation.

protease complexes (26). Unlike A_2M, however, we speculate that A_1AT probably is immunosuppressive alone (without bound protease), binding directly to the lymphocyte surface as demonstrated by Lipsky *et al.* (6). (iv) Such binding could serve to decrease the secretion of MCI by lymphocytes and thereby decrease monocyte activation. The mechanism presented in Fig. 4 may also explain the data of Gupta *et al.* (5) concerning the demonstration of A_1AT in pulmonary macrophages obtained from smokers or subjects with chronic lung disease but not from nonsmokers. Presumably, after persistent irritation the lung would contain significant numbers of fully activated macrophages engaged in markedly increased production of A_1AT, much like the endotoxin-stimulated monocytes evaluated in this study. Resting macrophages (from non-smokers) would make less A_1AT than either endotoxin-activated macrophages or even monocytes in unstimulated cultures, which are activated to some extent when they adhere to plastic (e.g., compare A_1AT levels for unstimulated MNL, unstimulated monocytes, and endotoxin-stimulated monocytes).

In the lung, where the pulmonary macrophages might locally produce A_1AT at a level higher than the concentration of A_1AT provided to the lung from the serum, the macrophage would be predominantly responsible for control of protease activity. Since of the two major inhibitors (A_2M and A_1AT) only A_1AT completely inhibits protease activity (A_2M inhibits only proteolysis of high-MW substrates and does not inhibit the esterolytic activity of the protease) (1), individuals deficient in A_1AT would have an impaired capacity for protease regulation, and consequently increased tissue destruction would occur due to proteolytic damage. Tissue destruction due to uncontrolled proteases is exemplified by reported cases of pulmonary emphysema (27). In the peripheral blood, unlike the lung, the monocyte's contribution to circulating A_1AT levels would be insignificant.

Summary. Cell-free culture medium from stimulated and unstimulated 6-day cultures of mononuclear leukocytes (MNL), nonadherent MNL (lymphocytes), and adherent MNL (monocytes) was evaluated for evidence of *in vitro* synthesis of protein and of α_1-antitrypsin (A_1AT). Phytohemagglutinin (PHA, 2.0 μg/ml) promoted significant increases in ^3H-labeled protein content in both MNL and lymphocyte cultures, whereas endoxotin (25.0 μg/ml) but not PHA caused significant increases in the amount of protein synthesized by monocytes. A_1AT was found to be made by MNL and monocytes in culture but was not newly synthesized by lymphocytes. Evidence for the presence of *de novo* synthesized A_1AT was obtained by Sephadex G-200 chromatography, immuno-precipitation with antibody specific for A_1AT, and SDS–polyacrylamide gel electrophoresis. Evidence was also found for the release of small amounts of A_1AT from the surface of lymphocytes after 6 days in culture, presumably complexed to labeled protease; however, this accounted for less than 10% of the A_1AT made by endotoxin-stimulated monocytes.

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