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Synthesis of α_1-anti-trypsin by human monocytes

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SUMMARY

The present report concerns the site of synthesis of α_1-anti-trypsin (α_1AT). In this study circulating cells and tissue fragments were incubated with radioactive amino acids to be incorporated during protein synthesis. The *de novo* synthesis of proteins was demonstrated by autoradiography of the immunoelectrophoretic slide. The results constitute proof that α_1AT is formed by circulating human monocytes and support those obtained in earlier immunofluorescent studies which showed that α_1AT is present in pulmonary macrophages, as could be expected because these cells derive from circulating monocytes. The culture fluid of liver tissues also showed labelled α_1AT, but it is not yet clear whether in this organ α_1AT is formed by Kupffer cells and/or parenchymal cells.

INTRODUCTION

Alpha$_1$-anti-trypsin (α_1AT), a glycoprotein, is capable of inhibiting a variety of proteolytic enzymes, for instance trypsin, chymotrypsin, kallikrein and the neutral proteases of polymorphonuclear leucocytes (Morse, 1978). Reduced levels of α_1AT are associated with such disorders as pulmonary emphysema (Laurell & Eriksson, 1963; Lieberman, 1972) and juvenile cirrhosis of the liver (Sharp *et al.*, 1969).

The site of α_1AT synthesis is not known with certainty. Some studies indicate that this protein is synthesized by liver cells (Asofsky & Thorbecke, 1961; Alper *et al.*, 1980) and others indicate that it is present or formed in monocytes (Wilson *et al.*, 1980; Isaacson, Jones & Judd, 1979) and alveolar macrophages (Cohen, 1973; Gupta *et al.*, 1979; White *et al.*, 1981); however, negative results have been reported by Papadimitriou, Stein & Papacharalampous (1980), who found positive staining for α_1AT in myelocytes and neutrophilic granulocytes but not in monocytes.

For investigations on the cellular origin of α_1AT, the culture of cells or tissue fragments *in vitro* in the presence of labelled amino acids which are incorporated during protein synthesis offers a satisfactory method (van Furth, 1978) if use is made of extremely pure cell suspensions. Because there are a number of indications that α_1AT is formed by macrophages, we would have preferred to use human pulmonary macrophages. However, because an adequate number of such cells is difficult to obtain, and monocytes are the direct precursors of macrophages (Blussé van Oud Alblas & van Furth, 1979), the investigation was done in peripheral blood monocytes.

The present report concerns a study on the synthesis of α_1AT by human peripheral blood monocytes, lymphocytes, and various tissues in which use was made of the method of *in vitro* culture in the presence of radioactive amino acids and analysis of the culture fluid by immunoelectrophoresis and followed by autoradiography to detect *de novo* synthesis of α_1AT.

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MATERIALS AND METHODS

Peripheral blood leucocytes. Blood was obtained from healthy volunteers with the normal α_1AT phenotype (PiMM) and from individuals with an α_1AT deficiency (PiMS or PiZZ). Peripheral blood monocytes and lymphocytes were isolated by centrifugation on Ficoll-Hypaque according to Böyum (1968). Further separation of peripheral blood monocytes and lymphocytes was obtained by culture on glass coverslips in Leighton tubes in medium 199 (M.A. Bioproducts, Walkersville, Maryland, USA) containing 20% newborn calf serum (GIBCO Europe Ltd., Paisley, Scotland). Next, the cell suspension (adjusted to contain about 2×10^6 monocytes/ml) was incubated in Leighton tubes. After 2 hr of incubation to allow the cells to adhere to the glass surface the supernatant cell suspension (morphologically 95–99% lymphocytes) was removed from the tubes, pooled, washed and cultured in radioactive medium in a roller tube. The glass adherent cells were washed twice with medium 199 and incubated for a further 48 hr in radioactive medium.

Suspensions of peripheral blood leucocytes from patients with acute or chronic monocytic leukaemia, chronic lymphatic leukaemia, and hairy cell leukaemia, were isolated by sedimentation with high molecular weight dextran (mol. wt 200,000 daltons) as described elsewhere (van Furth, Schuit & Hijmans, 1966a).

Tissues. Human tissues were obtained during surgery or after abortion and immediately processed as described elsewhere (van Furth *et al.*, 1966a). Only histologically normal tissues were included in this study. The fragments of tissue prepared for culture weighed 50–200 mg.

Human cell lines. A human macrophage cell line (U937) (Sundstrom & Nilsson, 1976) and a human promyelocytic leukaemia cell line (HL60) (Gallagher *et al.*, 1979) were cultured in 25 ml tissue culture flasks (Falcon Labware, Oxnard, California, USA). For the former MEM alpha medium (GIBCO) was used and for the latter RPMI 1640 medium (Flow Laboratories Ltd., Irvine, Ayrshire, Scotland), to both of which 10% fetal calf serum (Flow Laboratories) was added.

Incorporation of ^{14}C-labelled amino acids into protein in vitro. Newly synthesized proteins in cultures of cell suspensions or tissue were detected by the incorporation of ^{14}C-labelled amino acids according to a modification of the method of van Furth (1978) and Hochwald, Thorbecke & Asofsky (1961). Fragments of tissue minced with scalpel blades, cell suspensions, or glass adherent cells were incubated in modified Eagle's medium containing 1 μCi/ml ^{14}C-L-lysine (specific activity 350 mCi/mmole) and 1 μCi/ml ^{14}C-L-isoleucine (293 mCi/mmole; New England Nuclear, Boston, Massachusetts, USA), and 100 units/ml penicillin. One millilitre of radioactive medium was added to $1–2 \times 10^6$ cells or 100–200 mg of tissue fragments. After incubation for 48 hr, the cultures were frozen at -20°C and after thawing were centrifuged for 20 min at 20,000 *g*. The cell free supernatants were dialysed against 0·015 M phosphate buffer (pH 7·6) to remove the unincorporated amino acids.

The dialysed culture fluids were concentrated approximately 10-fold by lyophilization and dissolved in distilled water. To detect the presence of labelled α_1AT, the concentrated culture fluids were studied by immunoelectrophoresis in 1·3% agar Noble (DIFCO Laboratories, Detroit, Michigan, USA) with 0·05 M veronal buffer (pH 8·6) and rabbit anti-α_1AT serum (see below), followed by autoradiography of the dried slides with a Kodak RS pan film (650 ASA). The exposure time was 6 weeks.

Since the concentrated culture fluids did not contain enough α_1AT to provide a good precipitin line, purified α_1AT (of phenotype MM) was added as a source of carrier protein. The anti-human α_1AT antiserum was prepared in rabbits by repeated immunization with α_1AT which had been purified essentially as described by Klasen (1980), i.e. by $(NH_4)_2SO_4$ precipitation, molecular sieving, ion exchange chromatography, and affinity chromatography on Sepharose bound concanavalin A. The antiserum was made monospecific by absorption with insolubilized human serum in which hardly any α_1AT was detectable.

The cultures were also studied by immunoelectrophoresis and autoradiography to investigate synthesis of other serum proteins and lysozyme (McClelland & van Furth, 1975; van Furth, 1978). Normal human sera and purified lysozyme were used as carriers; the antisera against human serum proteins were obtained from the Central Laboratory of the Red Cross Blood Transfusion Service

(Amsterdam, The Netherlands) and antiserum against human lysozyme was prepared in rabbits from Dako Immunoglobulins Ltd (Copenhagen, Denmark).

The intensity of the autoradiography was scored on an arbitrary scale with $-$ = absent; (+) = just visible line. + = clearly visible line, and + + = dark line; the mean scores were calculated by assessing values from 0 = $-$, 1 = (+), to 3 = + +.

RESULTS

α_1AT synthesis by peripheral blood leucocytes

All cultures of human peripheral blood monocytes showed synthesis of α_1AT (Table 1). The intensity of the autoradiographic lines ranged from clearly visible to rather dark. Also, in all cultures of monocytic leukaemia cells α_1AT was synthesized and gave autoradiographic lines of the same intensity as found for normal monocytes (Table 1).

Lymphocytes of normal peripheral blood, chronic lymphatic leukaemia, and hairy cell leukaemia did not synthesize α_1AT (Table 1). However, ^{14}C-labelled IgG and IgM were formed in these cultures (Table 1), which proves that the incubated cells were viable and capable of synthesizing proteins (van Furth, Schuit & Hijmans, 1966b).

Table 1. α_1AT synthesis by human cells*

Origin	Mean number of cells or weight per culture	Number of cultures	α_1AT synthesis†	Other proteins synthesized
Normal blood monocytes	$1{\cdot}2 \times 10^7$	5	+/+ +	lysozyme; C3
Normal blood lymphocytes	$2{\cdot}0 \times 10^7$	2	$-$	IgG
Monocytes of monocytic leukaemia	$7{\cdot}0 \times 10^7$	10	+/+ +	lysozyme; C3
Lymphocytes of chronic lymphatic leukaemia	$5{\cdot}0 \times 10^8$	2	$-$	IgG, IgM
Lymphocytes of hairy cell leukaemia	$4{\cdot}5 \times 10^7$	2	$-$	IgG
Cell line U937	$5{\cdot}0 \times 10^7$	1	+	lysozyme
Cell line HL60	$1{\cdot}0 \times 10^8$	1	$-$	lysozyme
Adult liver	150 mg	2	+	albumen; α and β globulins
Fetal liver	225 mg	1	+	albumen; α and β globulins
Normal skin	125 mg	4	$-$	none
Normal spleen	185 mg	2	$-$	IgG

* Detected by immunoelectrophoresis of ^{14}C-labelled culture fluid and autoradiography.

† The intensity of the autoradiographic lines is graded from $-$ = absent; (+) = just visible; + = clearly visible; to + + = dark line.

α_1AT synthesis by tissues

Cultures of adult and fetal liver tissue also showed synthesis of α_1AT. The autoradiograph of the immunoelectrophoretic slides showed an uniform labeling pattern of the α_1AT precipitation line. The spleen and skin cultures were negative in this respect (Table 1).

α_1AT synthesis by human cell lines

The macrophage cell line (U937) synthesizes α_1AT, as can be concluded from the autoradiographic results. As in the case of liver tissue cultures, a uniform label of α_1AT precipitin line was observed. The promyelocytic cell line (HL60) was negative (Table 1).

Table 2. Synthesis of α_1AT by monocytes from patients with different α_1AT phenotypes

Phenotype	Number of individuals	Mean number of monocytes	Intensity of line(s) in the autoradiographs*
MM	2	$1{\cdot}7 \times 10^7$	++
MS	1	$2{\cdot}0 \times 10^7$	+†
ZZ	2	$3{\cdot}0 \times 10^7$	+†

* The intensity of the autoradiographic lines is graded from + = clearly visible; to ++ = dark line.

† Only the cathodic part of the line was labelled.

α_1AT synthesis by monocytes from individuals with different α_1AT phenotypes

Monocytes from individuals with α_1AT phenotypes (Pi type) MM, MS, or ZZ, as determined by isoelectric focusing of serum, were cultured in medium containing ^{14}C-amino acids. The synthesis of α_1AT was evaluated by immunoelectrophoresis followed by autoradiography. The autoradiographs showed differences in intensity of the lines between the various phenotypes (Fig. 1). A clear line is observed in cultures of material from individuals with phenotype MM, whereas weaker lines were seen in case of phenotype MS and ZZ (Table 2). In cultures from individuals with phenotype

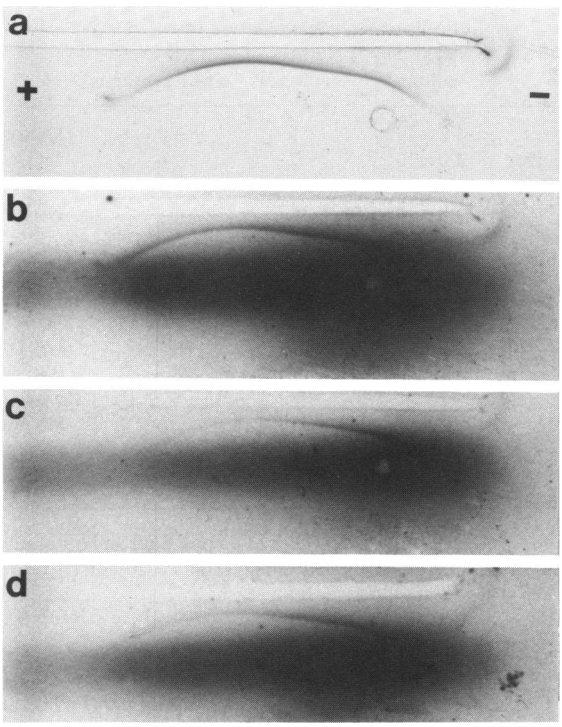

Fig. 1. Synthesis of α_1AT analysed by immunoelectrophoresis and autoradiography. (a) Immunoelectrophoresis of monocyte culture of an individual with phenotype MM and purified α_1AT as carrier developed with α_1AT antiserum and stained with amido black. (b) Autoradiograph of this immunoelectrophoresis slide showing a labelled α_1AT line. (c) Autoradiograph of immunoelectrophoresis of a monocyte culture from an individual with phenotype MS showing a labelled line with higher intensity of the cathodal part of the line. (d) Autoradiograph of an immunoelectrophoretic pattern obtained with monocyte culture fluid of an individual with phenotype ZZ, showing that the cathodal part of the line was mainly labelled.

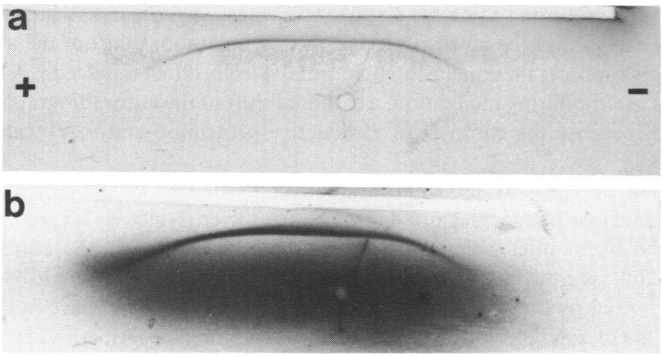

Fig. 2. Immunoelectrophoretic pattern and autoradiography after complex formation of α_1AT with porcine pancreatic elastase. (a) Immunoelectrophoresis of monocyte culture of an individual with phenotype MM, mixed with PiMM serum which served as carrier protein and next incubated with an equal molar amount of elastase before analysis at 23·5°C for 15 min. A shift in mobility of α_1AT is observed due to complex formation with elastase (compare with Fig. 1a). (b) Autoradiograph of this immunoelectrophoresis slide showing a shift of the mobility of the labelled line to cathodal side (compare with Fig. 1b).

MS and ZZ mainly the cathodal part of the line was labelled (Fig. 1). In all these experiments the same carrier protein (isolated PiMM α_1AT) was added. Analysis of serum of individuals with phenotype ZZ by immunoelectrophoresis confirmed a relatively cathodal orientation of α_1AT with this phenotype. However, the electrophoretic mobility of α_1AT of phenotype MS and MM were the same (data not shown).

Addition of porcine pancreatic elastase to a concentrated radiolabelled monocyte culture containing PiMM serum as a carrier protein, resulted in a shift in electrophoretic mobility due to formation of a complex between α_1AT and elastase (Fig. 2a). The autoradiography of this electrophoretic slide shows that the mobility of the labelled line is also shifted towards the cathodal side (Fig. 2b).

DISCUSSION

The main conclusion to be drawn from this study is that α_1AT is synthesized by circulating monocytes and not by lymphocytes. Since monocytes are the precursors of macrophages, it seems probable that α_1AT is also synthesized by pulmonary macrophages, as already indicated by studies done with immunocytochemical staining with specific anti-α_1AT antiserum (Cohen 1973; Gupta *et al.*, 1979; White *et al.*, 1981). A human macrophage cell line also synthesized α_1AT in culture, as shown in this study and previously by immunofluorescence (Isaacson *et al.*, 1979). The present results also confirm and extend those of an earlier study on α_1AT synthesis by 6 day cultures of human monocytes where the *de novo* protein synthesis (Wilson *et al.*, 1980) was demonstrated with a different technique. Very recently, the synthesis of α_1AT by human blood monocytes and the macrophage cell line U937 was reported (Isaacson *et al.*, 1981).

The possibility was considered that the protein synthesized *in vitro* was not radioactive α_1AT, but a proteinase which forms a complex with unlabelled α_1AT and is seen in the autoradiographs as a labelled line. However, two observations argue against this possibility. First, analysis of culture fluids of monocytes from individuals with phenotype ZZ or MS showed that mainly the cathodal part of the precipitin line was labelled, although the α_1AT carrier was a phenotype MM, like that used for the monocyte culture of the PiMM individual. Therefore, the shift in mobility could only be ascribed to a different electrophoretic mobility of the labelled product. Immunoelectrophoresis of serum confirmed a more cathodal orientation of α_1AT with phenotype ZZ compared with PiMM α_1AT. However, this was not found for serum α_1AT of phenotype MS, and in this case the discrepancy between the electrophoretic mobility of serum and radioactive α_1AT remains

unexplained. Second, addition of porcine pancreatic elastase, a proteinase which forms a complex with α_1AT of the carrier and the labelled product, led to a cathodal shift of the autoradiographic line, which strongly supports the conclusion that the labelled product is indeed α_1AT. If a proteinase had been labelled *in vitro* during incubation, a cathodal shift of the autoradiographic line could not have been found, because the complex of radioactive proteinase and α_1AT can not react with porcine elastase.

The production of α_1AT by fetal and adult liver tissue can be explained by synthesis by (immature) mononuclear phagocytes present in the fetal liver (Naito & Wisse, 1977; Deimann & Fahimi, 1978) and by Kupffer cells, which derive from monocytes in the adult liver (Crofton, Diesselhoff-den Dulk & van Furth, 1978) and/or synthesis by parenchymal liver cells. The last possibility is supported by findings in patients given a liver transplant, since in three out of four cases the phenotype of α_1AT became and remained the donor type (Alper *et al.*, 1980). However, this finding is curious, because C3 and factor B also became the donor type, although there is a good evidence that these two complement components are synthesized by mononuclear phagocytes (monocytes and macrophages) (Brade & Bentley, 1980) and thus should ultimately become the recipient type, because Kupffer cells are continuously replaced by monocytes originating from the recipient's bone marrow (Crofton *et al.*, 1978). The occurrence of so called α_1AT bodies in the liver of patients with α_1AT deficiency (phenotype ZZ) (Blenkinsopp & Haffender, 1977) and the recent observation that in these individuals an elevation of the α_1AT level in serum is induced by danazol (Gadek *et al.*, 1980), a drug which stimulates hepatocytes to increase protein synthesis, also suggests that α_1AT is formed by parenchymal cells of the liver. The absence of α_1AT synthesis in spleen cultures might be explained by the small number of mononuclear phagocytes in these cultures.

It is of interest to consider the difference in the intensity of the lines in the autoradiographs of samples with various α_1AT phenotypes. Clear bands were observed after electrophoresis of samples prepared from blood monocytes of PiMM individuals, and weaker bands were obtained with the samples of PiMS and PiZZ individuals. These observations suggest that the amount of α_1AT synthesized by cultured cells reflects the serum level of α_1AT.

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