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BINDING OF ALPHA 1 ANTITRYPSIN (α_1 PROTEASE INHIBITOR) TO HUMAN LYMPHOCYTES

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SUMMARY

Radioiodinated α_1 antitrypsin has been found to bind to human lymphocytes. This binding is fast and reversible, and the cells can be saturated. Each lymphocyte can bind a maximum of approximately 1.2×10^6 molecules of α_1 antitrypsin with an association constant of 0.7×10^6 M^{-1}xl. The binding is inhibited by the addition of cold α_1 antitrypsin or Soybean trypsin inhibitor, and partially by α_2 macroglobulin. The data suggest that α_1 antitrypsin is likely to bind to a cell surface-associated protease. The addition of cell-free supernatants from lymphocytes incubated at 37°C was found to decrease the binding of α_1 antitrypsin, suggesting that the receptor is released from the cell surface.

INTRODUCTION

Protease-antiprotease systems are likely to control several cellular responses to signals generated at the cell surface. For instance, lymphocyte DNA synthesis induced by mitogen stimulation can be inhibited by several natural or synthetic protease inhibitors (1,2,3,4). The presence of α_2 macroglobulin, a serum protease inhibitor, was demonstrated at the surface of B lymphocytes (5,6), although experiments in cell cultures showed that monocytes rather than lymphocytes represented the main source of α_2 macroglobulin (7). Similarly, the major serum protease inhibitor, α_1 antitrypsin (α_1AT), was recently reported to be synthetized by monocytes (8). The control of lymphocyte responses by α_1AT is demonstrated by the ability of α_1AT to prevent *in vitro* and *in vivo* primary antibody responses to sheep erythrocytes in the mouse (9), and to suppress mitogen-induced DNA synthesis by human lymphocytes (10). These data suggested that α_1AT could interact with the membrane of lymphocytes or accessory cells, but the binding of α_1AT to lymphocytes had not been investigated so far.

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In the present report we indicate evidence for binding α_1AT to lymphocyte membrane and describe the characteristics of this binding.

MATERIAL AND METHODS

Reagents. Bovine serum albumin, Soybean trypsin inhibitor and bovine pancreatic trypsin were purchased from Sigma (Saint-Louis, Missouri). α_1AT and α_2 macroglobulin were kindly provided by Dr. Baudner (Behringwerke, Marburg, Germany). α_1AT was purified from a serum of an individual with the M type. The only detectable contaminant was human albumin (1 %). The biological activity was 0.87 mg trypsin inhibited/mg α_1AT. α_1AT was labeled with [^{125}I]Na using the lactoperoxidase method (11) to a specific activity of 0.48-3.76 µCi/µg. α_1AT was also labeled using the method of Hunter and Greenwood (12).

Cell isolation techniques. Heparinized peripheral blood from healthy volunteers was mixed with dextran (Pharmacia, Uppsala, Sweden) to allow red cell sedimentation (1 hr, 20°C). The buffy coat was collected and centrifuged on Ficoll-sodium metrizoate as described by Boyum (13). Lymphocytes were collected from the interface and polymorphonuclear cells from the pellet. Erythrocytes were collected from the pellet of heparinized blood centrifuged on Ficoll-sodium metrizoate. Fragments of tonsils or thymuses were obtained from the surgical department. After gentle teasing with forceps, cells were centrifuged on Ficoll-sodium metrizoate. All cell suspensions were washed three times in Hanks' balanced salt solution and then resuspended at 2 x 10^6 cells/ml RPMI 1640 medium (Eurobio, Paris) buffered with HEPES and supplemented with bovine serum albumin at a final concentration of 30 mg/ml. No serum was added. Fractionation of tonsillar lymphocytes into T-enriched or T-depleted suspensions was performed by addition of sheep erythrocytes to allow the formation of E-rosettes by T cells, followed by centrifugation on Ficoll-sodium metrizoate as already described (14). Rosetting lymphocytes were freed of erythrocytes by hypotonic schock.

Binding assay. Five microliters of [^{125}I] α_1AT were added to 10^6 cells in 0.5 ml of RPMI medium supplemented with 3 % bovine serum albumin. Cells were incubated for 15 min at 0°C unless otherwise stated. Incubation was terminated by addition of 3 ml of ice-cold Hanks solution. Lymphocytes were then washed five times with Hanks solution (3 ml, 600 x G, 5 min, 0°C). The cell suspensions were transfered into new test tubes prior to the last washing. Tubes containing only medium and [^{125}I] α_1AT without cells were run in parallel as controls. The radioactivity was measured in a gamma spectrometer.

RESULTS

Kinetics. In a first series of experiments performed with α_1AT labeled with the chloramin T method (13) no binding could be demonstrated. All other experiments were performed with α_1AT labeled with the lactoperoxidase method (11). The binding of [^{125}I] α_1AT to peripheral blood lymphocytes reached its maximum within 1 to 3 minutes (fig.1). Addition of cold Hanks completely stopped the binding since no binding was detected after 15s incubation. It was greater at 0°C than at 37°C. The reversibility of the binding was investigated in the following ex-

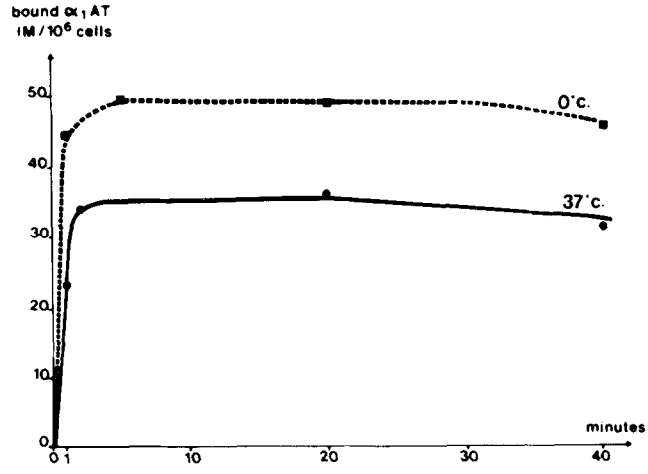

Figure 1. Kinetics of the binding of [^{125}I] α_1AT (50 pM/ml) to tonsillar lymphocytes.

periment:after binding of [^{125}I] α_1AT followed by five washings, lymphocytes were resuspended in RPMI 1640 medium and cultured at 37°C. After one hour no radioactivity was detectable in the supernatant, whereas 70 to 100 % of the radioactivity initially bound to lymphocytes was detected in the supernatant within 16 hours.

Saturation. The amount of bound α_1AT was found to depend upon the concentration of [^{125}I] α_1AT in the medium during the incubation period (fig. 2). Saturation was achieved at a concentration of 2 nM/ml or greater. A linear relationship was obtained between these variables in a double reciprocal plot. The number of receptors actually measured at saturation approximated 1.2 x 10^6/cell and the association constant was calculated at 0.7 x 10^6 M^{-1}x 1.

Binding inhibition. When radiolabeled α_1AT was incubated for 10 minutes at ambiant temperature with equimolar amounts of bovine pancreatic trypsin prior to its addition to the lymphocyte suspension, no binding could be detected. When unlabeled α_1AT was added in 100-fold excess with [^{125}I] α_1AT to the cell suspension, the amount of cell-bound radiolabeled α_1AT was reduced to 20 % of its level without unlabeled α_1AT.

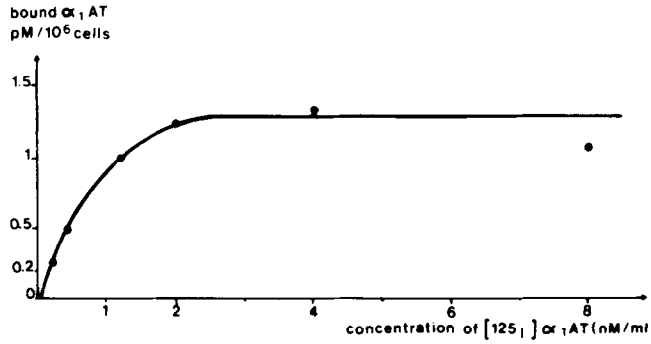

Figure 2. Binding of [^{125}I] α_1AT to tonsillar lymphocytes after 15 min-exposure to various concentrations of [^{125}I] α_1AT.

Displacement experiments. When experiments were performed in RPMI without addition of albumin, we observed an important fixation of [^{125}I] α_1AT. Addition of albumin to the culture medium decreased this binding in direct relationship with the amount of albumin bound up to a concentration of 1 % albumin. Increase of the albumin concentration in the medium up to 3 % did not further decrease the binding of α_1AT. We therefore considered that part of the binding of α_1AT to cells was non-specific and all experiments were performed in culture medium containing bovine serum albumin at a concentration of 3 % to prevent this phenomenon.

For displacement experiments, unlabeled α_1AT, Soybean Trypsin Inhibitor and α_2 macroglobulin were added 5 minutes after incubation with [^{125}I] α_1AT. The results show that the binding could be reversed only with high concentrations of protease inhibitors (table 1). Competition by soluble receptors was demonstrated in the following experiment : peripheral blood lymphocytes were incubated for 1 hr in RPMI 1640 medium (10^7 cells/ml) at 0°C or at 37°C. Two hundred microliters of supernatants were then collected and added to 10^6 lymphocytes in 0.3 ml of medium supplemented with 3 % bovine serum albumin. The binding of [^{125}I] α_1AT was significantly decreased by the addition of supernatant obtained at 0°C and completely inhibited by that of the supernatant obtained at 37°C (table 2).

Table 1. EFFECT OF PROTEASE INHIBITORS ON THE BINDING OF [^{125}I] α_1AT TO HUMAN TONSILLAR LYMPHOCYTES [a]

Protease inhibitor	Molar ratio[b] [protease inhibitor]/[^{125}Iα_1AT]	Percent binding
Unlabeled α_1AT	0	100
	15	91
	74	57
	148	0
Soybean trypsin inhibitor	46	30
	466	14
α_2 macroglobulin	9	75

[a] all inhibitors were added 5 min after [^{125}I]α_1AT (30 pM/ml).

[b] molecular weights (α_1AT 45 000, Soybean trypsin inhibitor 14 300) were obtained from the Handbook of Biochemistry, H.A. Sober ed., The Chemical Rubber Co, Cleveland 1970.

Binding of [^{125}I] α_1AT *to various cell types.* The binding of [^{125}I] α_1AT to cell suspensions from various origins, was measured with the same protocol. [^{125}I] α_1AT was always added at a final concentration of 30 pM/ml. The results presented in table 3 show important variations among the individuals tested.

Table 2. INHIBITION OF [^{125}I] α_1AT BINDING BY PRIOR ADDITION OF SUPERNATANTS FROM LYMPHOCYTES INCUBATED IN PROTEIN-FREE MEDIUM

Inhibitor	Bound [^{125}I] α_1AT	
	cpm	(fM/10^6 lymphocytes)
0	343 ± 21[a]	9.8
Supernatant 0°C[b]	264 ± 38	5.3
Supernatant 37°C[b]	161 ± 11	0.0
Cell-free control	169 ± 13	-

[a] mean ± Standard Deviation from triplicate assays.

[b] 0.2 ml of supernatant from lymphocytes (10^7/ml incubated 1 hr at 0°C or at 37°C were added to 10^6 lymphocytes in 0.3 ml. [^{125}I] α_1AT was then introduced at a final concentration of 30 pM/ml.

Table 3. BINDING OF [^{125}I] α_1AT TO VARIOUS CELLS[a)]

Cell origin	Bound [^{125}I] α_1AT (fM/10^6 cells)	Donor
Peripheral blood lymphocytes	17.3 ± 0.33	(a)
	31.8 ± 1.6	(b)
	33.3 ± 7.6	(c)
	48.8 ± 7.9	(d)
Red blood cells	7.5 ± 0.26	(a)
	6.2 ± 0.83	(c)
	28.6 ± 0.7	(b)
Polymorphonuclear cells	17.3 ± 2.6	(a)
	16.0 ± 1.4	(c)
Tonsillar lymphocytes :		
. unseparated	33.4 ± 2.2	(e)
	20.1 ± 0.5	(f)
. T cell-enriched	43.4 ± 4.5	(e)
	10.8 ± 0.6	(f)
. T cell-depleted	69.5 ± 3.3	(e)
	62.4 ± 5.3	(f)
Thymocytes	0.0 ± 0.0	(g,h,i)

a) all measurements were performed after incubation of 10^6 cells with [^{125}I] α_1AT at a concentration of 30 pM/ml.

Such variations are not accounted for by a poor repeatability of the assay on the same suspension, as shown by the standard deviations. Furthermore, the same technique was applied to all cell suspensions. A slight, but significant binding was observed with erythrocytes and polymorphonuclear cells, but the highest values were obtained with T-cell-depleted lymphocyte suspensions. No significant binding was measurable with thymocytes from three different subjects.

DISCUSSION

The present report demonstrates that α_1AT binds to human lymphocytes, erythrocytes and polymorphonuclear cells. The binding of α_1AT to lymphocytes is specific, saturable and reversible. The non specific binding of any protein to

cell surfaces in protein-free medium was avoided by addition of bovine serum albumin. The binding is prevented by addition of cold α_1AT. However, since the displacement of bound α_1AT could be achieved not only by α_1AT but also by other protease inhibitors, the receptor for α_1AT is likely to be a protease. Several other lines of evidence suggest that α_1AT can bind to a cell-surface protease. First, the preincubation of α_1AT with bovine pancreatic trypsin prevents its binding to lymphocytes. Second, no binding could be detected when α_1AT was iodinated with the method of Hunter and Greenwood (12) using chloramin T, an oxidizing agent which inactivates α_1AT (15) by oxydation of the methionine of the active site (16). Third, proteases have been demonstrated at the surface of lymphocytes (17) as well as erythrocytes (18). Finally aprotinin a low molecular weight polypeptide, extracted from bovine lung, which possesses a broad spectrum of antiprotease activity, was shown to bind to the surface of blood lymphocytes and polymorphonuclear leukocytes (19).

The binding of α_1AT was found to be impaired by addition of supernatants from lymphocytes incubated in protein-free medium. Parallel experiments have demonstrated a protease activity in these supernatants (Bata *et al.*, submitted for publication). Therefore, soluble proteases released from lymphocytes are likely to compete with surface proteases for the binding of α_1AT. Similarly, the spontaneous release of proteases from lymphocyte surface may account for the shedding of protease-[^{125}I] α_1AT complexes which was found to occur at 37°C.

The presence of receptors for α_1AT on lymphocyte surface may be relevant to the mechanisms whereby α_1AT inhibits primary antibody response (9) or lymphocyte DNA synthesis triggered by mitogens (10). The inhibitory effect of other antiproteases was more readily demonstrable on B than on T cell responses, suggesting that surface-associated proteases may contribute to B cell activation (reviewed in 20). However, since activation of B lymphocytes requires the cooperation of accessory cells, including monocytes or macrophages, the inhibitory effect of antiproteases may still be mediated by these accessory

cells. Conversely, the production of antiproteases by these cells may represent a new pathway of regulation of lymphocyte responses. Finally, the demonstration of α_1AT on concanavalin A-stimulated lymphoblasts (21) could indicate either active synthesis of α_1AT by these cells or passive binding of α_1AT produced by other cells in the culture. Experiments are in progress to clarify this important point.

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