Cell surface protease activity of human lymphocytes; its inhibition by α1-antitrypsin[1]

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Summary. Using isotopically-labelled casein as substrate, a surface-associated protease activity was demonstrated on human peripheral blood and tonsil lymphocytes. It was greater in T-depleted than in T-enriched suspensions. It could be inhibited by α1-antitrypsin purified from human serum.

Proteolytic enzymes are likely to be present on the surface of a wide variety of cells and to be involved in the regulation of cell responses and cellular interactions. The relationship of the protease-antiprotease systems to lymphocyte responses is rupported by several lines of indirect evidence, including the modulation of lymphocyte responses by addition of proteases or protease inhibitors (reviewed by Bata et al.[2]), the binding of protease inhibitors[3] and the demonstration of surface-associated proteolytic activity[4] on mouse B and T cells and human peripheral blood lymphocytes[5]. Alpha 1-antitrypsin (α1-AT), the major plasma protease inhibitor, which binds to the active site of serine proteases, was shown to prevent the in vivo and in vitro primary antibody responses to sheep erythrocytes in the mouse[6] and to be detectable by specific fluoresceinated antibodies on the surface of concanavalin-A-stimulated lymphoblasts[7]. In this study we have measured protease activity bound to the cell surface an secreted into the medium by viable T and non-T human lymphocytes, and we have investigated the inhibition of these activities by purified α1-AT.

Materials and methods. Peripheral blood lymphocytes were obtained from healthy donors. Citrated blood was mixed with a 10% dextran solution (v/v). After sedimentation of erythrocytes (1 h, room temperature), the supernatant was collected, diluted in 3 vol of Hank's balanced salt solution (HBSS) and centrifuged on Ficoll-Metrizoate (density 1.080) according to Boyum[8]. Lymphocytes collected at the interface were washed thrice in HBSS. Tonsil lymphocytes were prepared by gentle teasing of tonsils in HBSS, followed by washing thrice in HBSS. Fractionation of T lymphocytes was achieved by formation of E-rosettes with sheep erythrocytes (16 h, 4 °C, 15% human AB serum) followed by centrifugation on Ficoll-Metrizoate, hypotonic lysis of erythrocytes and washing in HBSS as already described[9]. Human erythrocytes were obtained from the pellet of citrated blood centrifuged on Ficoll-Metrizoate. The assay for proteolytic enzyme activity relied on the measurement of trichloracetic acid soluble peptides released from isotopically labeled casein as substrate[10]. Casein was labeled with ^{125}I, using the lactoperoxidase method[11]. 4 million cells suspended in 0.5 ml serum-free RPMI 1640 medium were incubated with 10 µl ^{125}I-casein (3 h, 37 °C). The protein concentration of ^{125}I-casein was measured by the method of Lowry et al.[12]. Controls with the same amounts of casein in cell-free medium were introduced measuring the spontaneous degradation, which was subtracted from the experimental values. Counts of trichloracetic acid soluble activity were measured in cell-free supernatants and surface protease activity was calculated by subtraction from total acid soluble activity.

The α1-AT preparation was found to contain traces of albumin accounting for less than 2% of the protein content without other detectable contaminants. The α1-AT had a nomal M phenotype pattern in thin layer isoelectric focusing. 1 mg of the α1-AT preparation inhibited the activity of 0.87 mg of bovine trypsin (Sigma, Saint-Louis, Missouri).

Results and discussion. A significant proteolytic activity was observed on erythrocytes from the blood of 3 different donors, but little or no protease was released from these cells (table 1). Conversely, peripheral blood and tonsil lymphocytes had protease activity in their supernatants and at the cell surface. The calculated activity represents a reasonable estimate of cell-surface activity since casein is not endocytosed by lymphocytes during the assay[5]. Calculated surface-associated activities were lower in T-enriched than in T cell-depleted tonsil lymphocytes (table 1). Since T cells were obtained by positive selection of E-rosette forming cells (E-RFC), it is still possible that the difference between T and non-T lymphocytes is to be attributed to the effect of the binding of sheep erythrocytes rather than to a genuine property of the lymphocyte surface. Alternatively, a possible contamination of the T lymphocytes by red cell membranes cannot be excluded. The greater protease activity in supernatants of E-RFC-enriched suspensions would support either interpretation.

Table 1. Surface protease activity of various cell types

Cell origin	^{125}I-Casein hydrolyzed (fmole/h)		
	Total activity	Released activity	Calculated surface activity
Red blood cells	270±20	0	270
	310± 8	0	310
	490±45	70± 1	422
Blood lymphocytes	1460±60	980±18	480
	1700±76	1300±49	390
	550±11	90± 1.5	450
	1050±29	490±30	490
	660±18	230±10	0
Tonsillar lymphocytes			
1) Unfractionated	710± 28	130± 4	580
E-RFC-enriched	1490±130	1430±110	60
E-RFC-depleted	980± 32	310± 10	760
2) Unfractionated	1110± 38	240± 1	870
E-RFC-enriched	360± 5	115± 1	245
E-RFC-depleted	330± 5	0	330

4.10^6 cells were incubated with ^{125}I-casein (230000 cpm) in RPMI 1640 medium (0.5 ml, 3 h, 37°C). Results are expressed as fmole of hydrolyzed casein per h (mean ± SD calculated from triplicate assays). Spontaneous degradation occurring in cell-free medium 23–48% of total activity) was subtracted from all samples.

Table 2. Effect of α 1-AT on surface protease activity of peripheral blood lymphocytes

α 1-AT added added (µg)	^{125}I-Casein hydrolyzed (fmole/h)		
	Total activity	Released activity	Calculated surface activity
0	1460±60	980±18	480
25	1120±15	800±30	320
100	660±56	570± 7	90

4.10^6 lymphocytes were incubated with 12 µg ^{125}I-casein in RPMI 1640 medium (0.5 ml, 3 h, 37°C), with or without α 1-AT. Spontaneous degradation in the absence of lymphocytes was subtracted from all samples.

A nearly complete inhibition of protease surface activity was achieved by addition of purified α1-AT at a concentration of 0.2 mg/ml (table 2). At the same α1-AT concentration, the total proteolytic activity was only partially suppressed. The inhibition of lymphocyte surface proteolytic activity by α1-AT is in agreement with the demonstration of a reversible binding of α1-AT to tonsil cells incubated in serum-free medium[14], and with the reported binding of other protease inhibitors, like trasylol[3] or soybean trypsin inhibitor[14], to the lymphocyte surface. Since several synthetic or natural protease inhibitors are known to prevent lymphocyte blastogenesis, the demonstration of the suppression of lymphocyte surface proteolytic activity by α1-AT provides additional indirect evidence for the role of protease-anti-protease systems in the control of lymphocyte activation.

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Lymphocyte reactivity in Imuran-treated guinea-pigs and in vitro effect of colchicine

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Summary. Imuran treatment significantly diminished macrophage migration inhibition in immunized guinea-pigs and the percentage of E-rosette forming cells in all treated animals. 10^{-6} M colchicine in vitro significantly increased the percentage of E-rosette forming cells in Imuran-treated animals. The results suggest possible alteration of the lymphocyte receptors after treatment with Imuran.

The effect of Imuran (azathioprine) on some immune reactions is well established. However, the mechanisms of the action of this drug on lymphocyte reactivity are still not well understood. Winkelstein's[2] data suggest that the suppressive effects of this immunosuppressive drug are connected with its cytostatic effects. However, Bach[3] states that such effects of Imuran are not its only mode of action. He suggests that Imuran modifies the surface receptors on lymphocytes and thus alters their function.

The role of microtubules in the receptor distribution has been suggested by many authors in recent years[4-7]. Using antimicrotubular agents, Wybran[6] has shown that they affect human lymphocyte reactions with sheep erythrocytes in forming E-rosettes.

The effect of Imuran on lymphocyte reactivity using the macrophage migration inhibition test and the rosette forming test in ovalbumin-immunized guinea-pigs was investigated in this paper. The in vitro effect of colchicine, an antimicrotubular agent, on the formation of rosettes was studied as well.

Material and methods. Guinea-pigs of both sexes, 250–300 g b.wt, were used in the experiments. The animals were divided into 6 groups: the 1st group (I) consisted of 19 animals, which served as the control group and received 0.5 ml saline i.m. Group II (23 animals) received 0.5 ml of complete Freund's adjuvant (CFA) with 0.5 ml saline into the foot pads. Group III (23 animals) was injected with 5 mg of crystallized ovalbumin in 0.5 ml saline and 0.5 ml CFA into the foot pads. Group IV (10 animals) received 20 mg/kg b.wt of Imuran (Wellcome) in 0.5 ml saline i.m. Group V (10 animals) was injected with 0.5 ml CFA and 0.5 ml saline into the food pads and 20 mg/kg b.wt of Imuran i.m. simultaneously. Group VI (19 animals) received 5 mg of crystallized ovalbumin in 0.5 ml saline and 0.5 ml CFA, and 20 mg/kg b.wt of Imuran simultaneously.

Imuran was injected into the animals of the groups IV, V and VI on the day of the immunization and during 4 consecutive days.

The macrophage migration inhibition test (MMI) was performed according to David and David[8]. Briefly, peritoneal macrophages were washed and packed into capillary tubes which were cut at the cell-fluid interface. The capillaries were put in culture chambers containing tissue culture medium RPMI 1640 (Eurobio Paris), supplemented with antibiotics and 15% inactivated horse serum. Ovalbumin was added to the test chambers at a concentration of 80 μg/ml, while control chambers were without antigen. The chambers were incubated for 48 h at 37 °C and the migration index was calculated as:

$$\text{MI} = \frac{\text{migration area in test chambers}}{\text{migration area in control chambers}} \times 100$$

The E-rosette forming test (ERF) was performed according to Wilson[9] with some modifications: 0.25 ml guinea-pig lymph node lymphocytes (3×10^6 cells/ml) were mixed with 0.25 ml of 0.8% papain-pretreated rabbit erythro-