EXPERIMENTAL BIOLOGY

Impact of Plasmin and Its Complexes with α_2-Macroglobulin or α_2-Antiplasmin on the Proliferative Activity of Human Lymphocytes

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It is shown that plasmin in doses of 0.1, 1, or 10 μg/ml did not influence significantly phytohemagglutinin-induced proliferation of mononuclear lymphocytes in a 3-day culture with these cells. Their proliferative response to pokeweed mitogen was stimulated by plasmin in the dose of 10 μg/ml only. Biogenic complexes of plasmin with α_2-macroglobulin or α_2-antiplasmin induced a moderate reduction of spontaneous proliferation after 3 days of culture, and so did plasmin after 5 days; α_2-macroglobulin induced a dose-dependent comitogenic effect with phytohemagglutinin and pokeweed mitogen, while α_2-antiplasmin induced a dose-independent comitogenic effect with pokeweed mitogen.

Key Words: *plasmin; α_2-macroglobulin; α_2-antiplasmin; lymphocytes; proliferation*

A large body of evidence now exists to indicate that proteinases are directly involved in immune responses. The major serine proteinase plasmin has been found to be an endogenous regulator of the synthesis and secretion of interleukins 1 and 6 [5]; a plasmin receptor has been identified on the surface membrane of peripheral lymphocytes [7]. Estimates of plasmin levels in biological fluids have to rely on indirect evidence because plasmin is rapidly bound by its inhibitors, primarily α_2-antiplasmin (α_2-AP) and α_2-macroglobulin (α_2-MG) [1]. The biogenic complexes between plasmin and antiplasmin (P-AP) and between plasmin and macroglobulin (P-MG) also appear to play a role in the immune system, given that receptors for P-MG on monocytes [10] and for P-AP on both monocytes and lymphocytes [7] have been identified.

In this study, we tested plasmin and its P-AP and P-MG complexes for their effects on lectin-stimulated proliferation of lymphocytes from healthy subjects.

MATERIALS AND METHODS

Macroglobulin (MG) was isolated from donor plasma using zinc-chelate chromatography on iminodiacetic agarose (*Kemoteks*, Estonia) and gel chromatography on Sephadex G-200 (Pharmacia, Sweden) [2]. Antiplasmin was obtained from donor plasma with a combination of affinity chromatography on plasminogen agarose, ion-exchange chromatography

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on DEAE-Sephadex, and chromatography on concanavalin A-Sepharose (Pharmacia) [9]. Plasminogen was extracted by affinity chromatography on a lysine-agarose column [6] and converted into plasmin by incubation with streptokinase immobilized on cyanogen bromide-activated agarose. For the production of P-MG complexes, the inhibitor was incubated with a fivefold excess of plasmin at 37°C for 60 min followed by gel chromatography on Sephadex G-200. P-AP complexes formed under conditions of a fivefold excess of the inhibitor and were then separated from other proteins by affinity chromatography on a column of cyanogen bromide-activated agarose with immobilized antibodies to plasminogen; the complexes were eluted from the gel with a 0.1 M glycine-hydrochloride buffer (pH 3.0) containing 0.5 M sodium chloride. The native proteins and their complexes were checked for purity by polyacrylamide gel electrophoresis [4].

Mononuclear cells (MNC) from Na_2-EDTA-stabilized venous blood samples of 14 healthy donors of both sexes were isolated by centrifugation in a Ficoll-Verografin gradient (1.077 g/cm^3). The isolated cells were cultured (1×10^6/ml) in RPMI-1640 medium (Vektor Company, Koltsovo, Novosibirsk Region) with 10 mM HEPES buffer (pH 7.2), 2 mM L-glutamine, 50 μg/ml gentamicin, and 10% fetal bovine serum in a CO_2 incubator (Flow Laboratories, Great Britain). The culture plates and supplements to the medium were also from Flow Laboratories. Phytohemagglutinin(PHA)-stimulated cell proliferation (1:100; Difco-M, USA) was estimated after 3 days and pokeweed mitogen(PWM)-stimulated proliferation (50 μg/ml; Sigma, USA) after 5 days. The results were read radiometrically using ^{3}H-thymidine. After sterilizing filtration, the test proteins were added to cultures in the following concentrations: P-MG at 1000, 200, 50, and 10 μg/ml (we found its normal average levels to be are 50 μg/ml for males and 100 μg/ml for females); P-AP at 2, 0.2, and 0.05 μg/ml (normal concentrations of this complex are 0.1-0.2 μg/ml [8]); plasmin at 10, 1, and 0.1 μg/ml). To take account of the possible cytotoxicity of the proteins, MNC from three donors were cultured for 18 h with the proteins in maximal doses or without them, after which dead and viable cells, stained with ethidium bromide and acridine orange, were counted [3].

The results were treated statistically on an HP-85 minicomputer (Hewlett Packard) using a software package produced by the same firm.

RESULTS

The test proteins were found not to be cytotoxic, so that the decreased proliferative responses noted

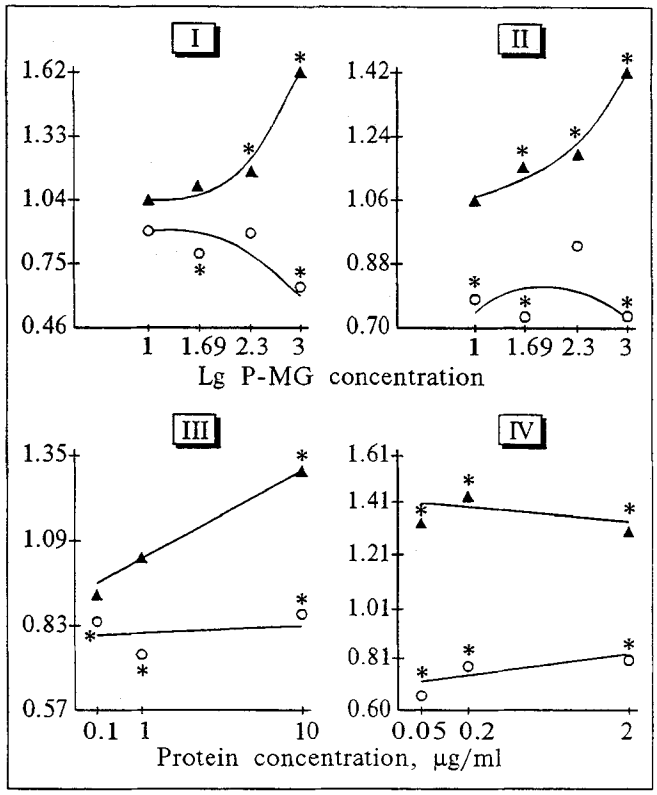

Fig. 1. Effects of plasmin (*P*) and its complexes with α_2-MG and α_2-AP on spontaneous and lectin-stimulated proliferation of lymphocytes from healthy subjects. *I*) P-MG + PHA; *II*) P-MG + PWM; *III*) P + PWM; *IV*) P-AP + PWM. Circles: spontaneous proliferation in the presence of proteins; triangles: lectin-stimulated proliferation in the presence of proteins. Asterisks denote statistically significant differences from control tests ($p<0.05$). Ordinates: quotients from dividing test values (proliferation in the presence of the test protein) by control values (proliferation in the absence of protein) for spontaneous and stimulated proliferation, respectively.

in some of the main tests were not due to cell death directly. During the 3-day culture, plasmin in the doses used did not affect significantly either spontaneous or PHA-stimulated proliferation, with the exception of cells from three donors (who were not included the main group), on which it exerted a marked dose-dependent comitogenic effect: their spontaneous proliferation was at the level of 2754±515 cpm, their proliferative responses to PHA and to plasmin at 0.1, 1, or 10 μg/ml were at the same level, whereas the responses to both PHA and plasmin in these three doses were 42,816±10,484, 44,857±10,167, and 108,418±23,170 cpm, respectively. This observation does not appear to be accidental and can presumably be attributed to some special physiological state of those three donors or to the presence of some latent abnormality in them, or else, if we assume that trace amounts of fibrin were present in the cell suspension, to the effect of its proteolytic products as inducers of

interferon synthesis [5]. During the 5-day cultivation period, spontaneous proliferation declined monotonically (by 17% on average) in response to all protein doses, and a synergistic effect with PWM occurred at the plasmin dose of 10 μg/ml (Fig. 1, *I* and *III*).

The P-AP complex did not influence either spontaneous or PHA-stimulated proliferation during the 3-day culture, except in the dose of 0.05 μg/ml, at which the mitogenic response was decreased by 21%. During the 5-day cultivation period, there occurred a small but statistically significant decrease in spontaneous proliferation and a distinct additive effect on PWM-induced stimulation. Note the monotonic nature of responses to all three doses of the P-AP complex (Fig. 1, *I* and *IV*).

The P-MG complex in doses of $\geqslant 50$ μg/ml exerted a strong synergistic effect in cell responses to both PHA and PWM. In both cases, the moderate decrease in cell proliferation was of a "mirror" character (Fig. 1, *I* and *II*).

According to current notions, lectin-induced proliferation is a model of cell cooperation based on stimulation of the synthesis and acceptance of several interleukins. The modulation of these processes by the ligands we have studied appears to reflect a more complex organization of intersystemic connections than believed hitherto.

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