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Lack of Evidence for Human Lymphokines with Thrombocyte Aggregating Activity

An *in Vitro* Study

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The ability of human lymphocytes to produce soluble mediator(s) with thrombocyte aggregating activity (TAA) was investigated in an *in vitro* technique. Isolated human mononuclear cells were stimulated with phytohaemagglutinin or purified protein derivative of tuberculin. The supernatants were investigated for lymphokine activity in a leucocyte migration inhibitory factor assay. Supernatants were then tested for their ability to aggregate human thrombocytes, and in contrast to the results of previous studies, we were not able to demonstrate any TAA. Most likely, lymphokines with TAA are not involved in the thrombotic processes seen in human cell-mediated immune inflammatory reactions.

Key words: Mononuclear cells; lymphokines; thrombocyte aggregating activity; cell-mediated immune reaction.

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Lymphokines are soluble, non-specific factors involved in cell-mediated immune reactions. They are produced by lymphocytes after non-specific stimulation with polyclonal T-cell activators or after specific stimulation with recall antigen. Lymphokines are known to possess several biological activities related to the immune inflammatory process. Lymphokines with thrombocyte aggregating activity (TAA) are generally believed to exist and TAA has been regarded as an important mechanism for the initiation of thrombotic processes in cell-mediated immune reactions.

In vitro production of rabbit lymphokines with TAA were in 1975 demonstrated by Lavelle et al. (7). In 1976 Coeugniet & Bendixen (5) were able to confirm these results in a human *in vitro* technique. In the latter study platelet aggregating activity was demonstrated in supernatants from Con A-stimulated human lymphocytes. Increasing attention has been paid in recent years to the platelets as immune inflammatory cells, but to our knowledge, no studies have as yet confirmed the existence of a human lymphokine with TAA.

In this study we reexamined the *in vitro* ability of human lymphocytes to produce a factor with TAA, following stimulation with a non-specific polyclonal T-cell activator as well as specific antigen.

MATERIAL AND METHODS

Lymphokine production

The lymphokines were produced by blood mononuclear cells (MNC) obtained from healthy adults. The cells were pulsed for 1 h at 37°C with the polyclonal T-cell activator, phytohaemagglutinin (PHA), as detailed previously (2). After removal of unbound PHA, the washed cells were cultivated at 37°C in medium

TC-199 with or without serum. After 22 h the cells were pelleted, and the supernatants were dialyzed for 3 days with 3 changes of the dialysate buffer, 50 μM NH_4HCO_3, acetic acid, pH 7.2. The supernatants were then lyophilized and kept at −20°C until use. Control materials were obtained from the supernatants of cells pulsed and cultured in parallel in medium alone. In some experiments the lymphokines were generated by MNC cultured for 22 h in the presence of 50 μg/ml of tuberculin (State Serum Institute, Copenhagen, Denmark) (PPD). These supernatants were dialyzed and lyophilized as mentioned above.

Assay for the lymphokine (LIF)

This assay has been detailed previously (1). Briefly, lyophilized lymphokine-containing and corresponding control materials were dissolved in the original volume of medium TC-199 with 10% (v/v) heat-inactivated human serum. The solutions were divided into two, each being treated with either neutralization anti-LIF rabbit immunoglobulin (anti-LIF Ig) or control immunoglobulin (control Ig) for 30 min. Then, purified blood neutrophils were added, and the ability to migrate under agarose was tested. A migration index (MI) was calculated from the results of triplicate tests using the formula:

MI = mean migration area of cells in supernatant materials treated with control Ig/ mean migration area of cells in materials treated with anti-LIF Ig.

Generally, values 0.85 denote the presence of LIF.

Assay for platelet aggregation

4.5 ml cubital vein blood from healthy donors was drawn into test tubes each containing 0.5 ml 3.13% trisodium citrate. After 5 min centrifugation at 400 *g* (20°C) the thrombocyte rich plasma was collected. The samples were centrifuged for 10 min at 1800 *g* (20°C) and the thrombocyte-depleted plasma was isolated. The thrombocyte aggregation was measured with a Dual Channel Payton Aggregometer, according to the method described elsewhere (8).

The aggregation inducers, ADP (adenosine diphosphate, Sigma Chemical Company, St. Louis, U.S.A.) and epinephrine (Gauche, Rhóne-Poulenc, Paris, France), were used at threshold concentrations as a control of the test system. The threshold concentrations were defined as the lowest concentration of ADP or epinephrine, which caused an irreversible aggregation, and at least an 80% difference in light transmission between platelet-rich and platelet-depleted plasma. ADP was tested at the concentrations 1.0, 2.0, 4.0, and 16.0 μM. Epinephrine was tested at the concentrations 0.01, 0.05, 0.1, and 1.0 μg/ml. The lyophilized supernatants were dissolved in 0.9% NaCl and used at concentrations corresponding to the initial supernatants, 5 times concentrated and 2 times diluted. The effects of the supernatants on the ADP- and epinephrine-induced thrombocyte aggregation were also investigated.

RESULTS

TAA was not seen in any of the PHA- or PPD-stimulated supernatants (Fig. 1).

A more or less pronounced inhibition of the ADP- and epinephrine-induced thrombocyte aggregation, by both PHA- and PPD-stimulated supernatants, was observed in several experiments (Fig. 2). However, in a few of the experiments the control supernatants also inhibited thrombocyte aggregation.

DISCUSSION

Thrombotic processes are a prominent feature of several immune inflammatory reactions (9). Thrombocytes may participate in these reactions by aggregation and by release of various endogenous factors, such as lyzosomal enzymes, vasoactive compounds, prostaglandins and procoagulant activity (12).

Components involved in both immediate, subacute and delayed hypersensitivity reactions have been found capable of activating thrombocytes. IgE-sensitized rabbit basophils/mast cells have been shown to release a factor with

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