

From the Pathophysiological Institute of University Medical School
of Debrecen, Hungary.

EFFECTS OF LYMPHOKINES ON FIBRINOLYSIS AND KININ GENERATION IN HUMAN PLASMA

By

SÁNDOR SIPKA & TIBOR SZILÁGYI

Lymphokines, the biological active products of lymphoid cells responding to antigen or mitogens, are considered to be potential mediators of cellular immunity (13). Their role is manifested in delayed-type hypersensitivity reactions, in protective immunity and in cell-cooperation effects (6).

During recent years a series of papers have appeared dealing with the relationship between delayed-type hypersensitivity reactions and blood coagulation (5, 10, 2, 9). Dvorak et al. (7) published a scheme of the supposed pathogenesis of delayed-type hypersensitivity reactions in the skin and the hypothetical participation of the coagulation system in the production of local inflammation. In this scheme the authors emphasized the importance of lymphokines.

Maillard et al. (12) elucidated important details about the action of skin reactive factor (a lymphokine) synthesized by guinea pig lymphocytes in the presence of antigen or mitogen. They found that skin reactive factor could shorten the kaolin clotting time and, to a certain extent, activate fibrinolysis, and that certain inhibitors of the kinin and coagulation systems had depressing effects on skin reactive factor activity.

More recently, Coeugnet & Bendixen found that lymphokines from Con-A-stimulated human lymphocytes had direct thrombin-like procoagulant activity (3) and could aggregate the thrombocytes (4).

In our experiments we tested lymphokines from Con-A-stimulated lymphocytes for their effect on fibrinolysis and kinin generation in human plasma. On the basis of these results a new mechanism for lymphokine activity is suggested.

MATERIAL AND METHODS

Adult guinea pigs of both sexes were used for skin reactive factor testing. The animals were kept under standard conditions, fed on standard diet and allowed to drink water *ad libitum*. Guinea pigs used for kinin assays were fed with vegetables only, and in the last 24 h were given no food.

Material

Concanavalin-A (Sigma), lysozyme (Worthington), ellagic acid (Koch-Light), Gordox® (R. G. Budapest, identical with Trasylol® Bayer), atropine sulphate (Sandoz), N-2-hydroxyethyl piperazine (HEPES) (Calbiochem), polyethyleneglycol (Fluka), chlorpyraminum hydrochloricum (Suprastin® R.G. Budapest), Ficoll® 400 (Pharmacia Fine Chemicals), Uromiro® 75 per cent solution (Bracco Industria Chimica S.p.A. Milan).

Glassware: Siliconized glassware or plastic dishes and tubes were used throughout the experiments, except for the partial thromboplastin time determinations.

Preparation of Lymphokines

Normal human blood anticoagulated with citrate was used. The preparation of lymphocytes was carried out in Uromiro-Ficoll gradient in Parker's 199 medium at room temperature. No serum was added. The preparations were centrifuged at 800 g for 15 min and washed twice; they contained 90-95 per cent mononuclear cells.

10^7 Lymphocytes were cultured at 37°C in 5 per cent carbon dioxide and 95 per cent air milieu in the presence of 200 µg Concanavalin-A (Con-A) in Parker's 199 medium completed with 100 IU/ml of penicillin and streptomycin for 36 h. The cell-free supernatants of the lymphokine-containing and control cell cultures were chromatographed on a Sephadex® G-75 column and eluted with 0.01 M phosphate buffer pH 7.4. In the control preparations Con-A was added at the end of cultivation. The protein-containing fractions were collected and concentrated. Protein concentrations of the preparations used were generally about 1 mg/ml.

We named the leukocyte migration inhibitory factor and skin reactive factor containing preparations of Con-A-stimulated lymphocyte supernatants as lymphokines, or active supernatants (AS), and the supernatants of non-stimulated

lymphocytes as controls (CS). Leukocyte migration inhibitory factor was tested with human leukocytes according to Szabó's capillary method (11). Skin reactive factor testing was carried out on guinea pig skin on the basis of the measurement of the blue areas on the inner surface of the skin 4 h after an intravenous injection of 1 per cent Evans blue (12). AS resulted in migration inhibition and greater skin permeability than CS.

AS had also procoagulant activity which shortened the partial thromboplastin time (4).

The measurement of fibrinolytic activity was carried out using the fibrin tube technique, a one-dimensional diffusion method (8). The activity was expressed in mm of dissolved fibrin column during 18 h in 37° C.

0.8 ml of human non-contact plasma (kept in siliconized or plastic dishes separated from glass surfaces) were incubated at 37° C for 30 min with 0.2 ml of the AS, CS, ellagic acid (1 mg/ml) and phosphate buffer.

Lysozyme-treated plasmas were prepared by adding 5 mg lysozyme to 1 ml plasma.

In vitro Assay of Kinins

0.2 ml of AS, CS, ellagic acid (0.5 mg/ml), phosphate buffer, lysozyme (5 mg/ml) and Gordox (10 IU/ml) were incubated with 0.8 ml of non-contact human plasmas for 15 min. The mixtures were tested on isolated pieces of terminal ileum from guinea pigs for the presence of kinins. The pieces of ileum were suspended in a 9-ml plastic organ bath containing oxygenated Tyrode's solution, 1 µg/ml of atropine sulphate and 5 µg of the antihistaminic agent, chlorpyraminum hydrochloricum (Suprastin), at 37° C.

Contractions of the smooth muscle pieces were registered by a kymograph and the amounts of generated kinins were estimated using standard curves produced by synthetic bradykinin.

All the results are the averages of at least three experiments.

TABLE 1
The Effect of Culture Supernatants of Human Lymphocytes on the Fibrinolytic Activity of Human Plasma.

Samples	Fibrinolytic activity (mm) mean $\pm$ s.d.	
	Normal plasma	Lysozyme plasma
Buffer	12 $\pm$ 1	14 $\pm$ 1
Control (CS)	15 $\pm$ 1	14 $\pm$ 1
Active supernatants (AS)	20 $\pm$ 1	14 $\pm$ 1
Ellagic acid	22 $\pm$ 1	14 $\pm$ 1

RESULTS

Table 1 shows that AS increased the fibrinolytic activity of the plasma as did ellagic acid, which is a potent activator of factor XII (Table 1). In lysozyme-treated plasma both the AS and ellagic acid effects were blocked.

Both AS and ellagic acid generated kinins in the plasma (Table 2). In lysozyme-treated plasma the kinin generation was inhibited. Gordox (kallikrein inactivator) decreased the kinin generating capability of the AS.

TABLE 2
*The Effect of Culture Supernatants of Human Lymphocytes on
Kinin Generation in Human Plasma.*

Samples	Kinin generation ng/ml mean $\pm$ s.d.
Normal plasma	240 $\pm$ 20
Plasma + Gordox®	80 $\pm$ 10
Plasma + lysozyme	—

DISCUSSION

The supernatants of Con-A-stimulated lymphocytes containing leukocyte migration inhibitory factor, skin reactive factor and procoagulant activities had fibrinolysis-activating and kinin-generating effects on human plasma.

The activation of fibrinolysis can be manifested either via factor XII (1) or independently from the Hageman factor (15). The fact that lysozyme (an inhibitor of factor XII) in our experiments prevented AS from activating fibrinolysis showed that factor XII might also be participating in the reaction.

Our new finding demonstrates that lymphokines are capable of generating kinins from normal non-contact plasma. Mail-lard et al. (12) were not able to detect the generation of kinin by lymphokines from heated sera. In our experiments we always worked with whole fractions of Sephadex-purified

lymphokines and we found the kinin-generating capability of the preparations to be especially susceptible to freezing, concentration procedures and storing manipulation. The kinin-generating effect of lymphokines can be based either on Hageman-dependent kallikrein activation or on the independent, direct production of kinins from human kininogens (15, 14).

To summarize, we confirmed the procoagulant and fibrinolysis-activating effects of lymphokines. We also demonstrated the generation of kinin from non-contact plasma by the influence of lymphokines. This gives a new possibility for elucidating the pathomechanism of delayed-type hypersensitivity reactions of the skin, where bradykinin plays an important role in the symptoms of inflammation (7, 12, 14).

Even though the coagulation results prove that *in vitro*-produced lymphokines directly produce thrombin (4) we cannot exclude the role of factor XII activation in the local regulation of delayed-type hypersensitivity reactions through kinin generation, fibrinolysis activation and production of fibrin deposits.

SUMMARY

Supernatants from Con-A-stimulated human lymphocytes containing leukocyte migration inhibitory factor, skin reactive factor and procoagulant activities were tested for their effect on fibrinolysis and kinin generation. The supernatants of stimulated lymphocytes activated the fibrinolysis and generated kinin in human plasma. These results are discussed from the aspect of cell-mediated (type IV) immune inflammation.

ACKNOWLEDGEMENT

We thank I. Tőkés for the technical assistance.

REFERENCES

1. Austen, K. F. (1974): Hageman-factor dependent coagulation, fibrinolysis and kinin generation. *Transplant. Proc.* 6, 39-45.

2. Busch, G. J., Kobayashi, K., Hollenberg, N. K., Birtch, A. G. & Colman, R. W. (1975): Hyperacute renal allograft rejection in primate. *Amer. J. Path.* 80, 1-20.
3. Coeugnet, E. & Bendixen, G. (1976): Lymphokines and thrombosis. II. *Acta allergol.* 31, 107-115.
4. Coeugnet, E. & Bendixen, G. (1976): Lymphokines and thrombosis. I. *Acta allergol.* 31, 94-106.
5. Colvin, R. B. & Dvorak, H. F. (1975): Role of the clotting system in cell-mediated hypersensitivity. *J. Immunol.* 114, 377-387.
6. Dumonde, D. C., Kelly, R. H., Preston, P. M. & Wolstencroft, R. A. (1975): Lymphokines and macrophage function in the immunological response. In Ralph von Furth (ed.): *Mononuclear phagocytes in immunity, infection and pathology*, p. 675. Blackwell Scientific Publications, Oxford, London, Edinburgh, Melbourne.
7. Dvorak, H. F., Mihm, M. C., Dvorak, A. M., Johnson, R. A., Mansen, E. J., Morgan, E. & Colvin, R. B. (1974): Morphology of delayed type hypersensitivity reactions in man. *Lab. Invest.* 31, 111-130.
8. Fésüs, L. & Ölveti, E. (1975): Measurement of fibrinolysis in rat system by one-dimensional diffusion method. *Experientia* 31, 256-257.
9. Hard, R. C. & Still, W. J. S. (1975): Intravascular fibrin deposits, hepatic infarcts and thrombocytopenia in parent (F₁) mouse chimeras with host-versus-graft syndrome. *Amer. J. Path.* 70, 137-146.
10. Hattler, B. G., Rocklin, R. E., Ward, P. A. & Ricklen, F. R. (1973): Functional features of lymphocytes recovered from human renal allograft. *Cell. Immunol.* 9, 289-296.
11. Kincses, É. & Szabó, G. (1974): Cell-mediated and humoral immune response after lens injury. *Proc. 1st Symp. of Immunology and Immunopathology of the Eye, Strassbourg*. In Böke, W. & Luntz, M. H. (eds.): *Mod. Prob. Ophthalmol.* vol. 16, p. 95-101. Karger, Basel (1976).
12. Maillard, J. L., Pick, E. & Turk, J. L. (1972): Interaction between sensitized lymphocytes and antigen *in vitro*. *Int. Arch. Allergy* 42, 50-68.
13. Pick, E. & Turk, J. L. (1972): The biological activities of soluble lymphocyte products. *Clin. exp. Immunol.* 10, 1-23.
14. Rocha e Silva, M. (1974): Present trends in kinin research. *Life Sci.* 15, 7-22.
15. Schreiber, A. D. & Austen, K. F. (1974): Hageman factor independent fibrinolytic pathway. *Clin. exp. Immunol.* 17, 584-600.
16. Wilhelm, D. L. (1973): Mechanisms responsible for increased vascular permeability in acute inflammation. *Agents and Actions* 3, 297-306.

Authors' address:

Sándor Sipka
 Pathophysiological Institute
 University Medical School
 4012 Debrecen, Pf. 23.
 Hungary

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.