Concanavalin A Inhibition of Tissue Factor (Thromboplastin) Activity

By L. R. Zacharski, R. Rosenstein, and P. G. Phillips

Studies with the jackbean lectin, concanavalin A, were undertaken to clarify the relationship between tissue factor and the cell membrane of cultured fibroblasts. Concanavalin A blocks the coagulant activity of tissue factor of cultured human skin fibroblasts and also rabbit brain. The inhibitory effect, which is dose-dependent and reversed by alpha-methyl-D-mannopyranoside, cannot be explained by an effect on other reactants necessary for clot formation. These results support the contention that tissue factor is located on the cell surface. Since concanavalin A does not affect adhesion-related phenomena in cultured cells, these results also suggest that tissue factor produced by trypsinized fibroblasts is a consequence rather than the cause of cell adhesion and that tissue factor is not synonymous with the adhesive site on the cell surface.

Tissue Factor (T.F., tissue thromboplastin) is a coagulant whose pathophysiologic significance is unknown. The limitations inherent in assaying a tissue-bound rather than circulating coagulation factor have restricted study of T.F. However, identification of T.F. in cultured cells permitted studies which indicate that T.F. is a surface-related substance. For example, T.F. activity in cultured fibroblasts is reduced by trypsinization or by repeated washing of intact monolayers. Furthermore, T.F. has been demonstrated immunohistochemically on cell membranes and has been related to the adhesive properties of cultured fibroblasts. The reduction in T.F. activity which accompanies trypsinization is associated with detachment of cells from the flask floor. When trypsinized cells are cultured in fresh medium, regeneration of T.F. activity follows adhesion and spreading of the cells on the floor of the flask. Regeneration of T.F. activity can be prevented by not allowing the cells to adhere or not allowing adherent cells to spread. T.F. observed in cultured cells is entirely cell bound. None can be identified in the medium overlaying the cells despite repeated washing.

The jackbean lectin, concanavalin A (con A), which possesses the capacity to bind to alpha-D-glucopyranosyl or alpha-D-mannopyranosyl residues, has been of singular value in the study of cell membranes. This suggested that con A might prove useful in studying the relationship between T.F. and the cell surface.

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MATERIALS AND METHODS

Human skin fibroblasts were cultured and their T.F. activity assayed by methods described previously. Fibroblast monolayers in 25 cm² plastic flasks (Falcon) were studied 24 hr after passage at which time they possess potent T.F. activity. The medium overlying the monolayers was removed and the monolayers washed twice with phosphate-buffered saline (PBS, Grand Island Biological Co.) at pH 7.3. Two milliliters of PBS containing various concentrations of crystalline con A (Pharmacia) were then added to the monolayers and incubated at room temperature for 1 hr. The PBS-con A was removed, the monolayers washed twice more with PBS, and finally 5 ml (the volume of the original medium) of PBS were added to the flasks. The flasks were opened and the monolayers mechanically dislodged from the flask floor. An aliquot was then withdrawn and frozen and thawed to disrupt the cells and provide a more uniform suspension of cell fragments prior to assay for T.F. activity. T.F. was assayed by a one-stage technique patterned after the prothrombin time in which 0.01 ml of cell lysate was added to 0.1 ml of buffered saline and 0.1 ml of either normal plasma or plasma congenitally deficient in factor VIII procoagulant activity. To this mixture 0.1 ml of 0.01 M CaCl₂ was added and the clotting time determined.

The effect of con A on the coagulant activity of rabbit brain T.F. (thromboplastin, Ortho) was determined by mixing equal volumes of a 1:10 dilution of stock thromboplastin (prepared according to manufacturer's specifications) in PBS with various concentrations of con A in PBS. Mixtures were incubated at room temperature for 1 hr. As a control, PBS without con A was used. 0.1 ml of each mixture was then added to 0.1 ml of 0.02 M CaCl₂ and the clotting time determined upon addition of 0.1 ml of either normal or factor VIII-deficient plasma. In another experiment, equal volumes of thromboplastin prepared in serial two-fold dilutions and con A at a concentration of 50 μg/ml of PBS were mixed and incubated at room temperature for 1 hr. Coagulant activity was then determined as in the preceding experiment.

Inhibition and reversal of the effects of con A were studied with alpha-methyl-D-mannopyranoside (AMM, Sigma).

![Fig. 1. Con A inhibition of T.F. coagulant activity of cultured fibroblasts observed in assays with normal (----) and factor VIII-deficient (×-----×) plasmas.](image-url)
Results

Addition of con A to fibroblast monolayers which possess potent T.F. activity or to rabbit brain results in dose-related inhibition of T.F. activity. The results obtained in typical experiments are presented in Figs. 1 and 2. The inhibitory effect of con A on T.F. activity of fibroblast monolayers occurs within 5 min after contact with intact cells and is maximal within ½ hr. Addition of con A to monolayers does not result in premature lifting of cells from the floor of the flask. Inhibition observed when 2 ml of con A (200 μg/ml of PBS) are added to washed monolayers is reversed by subsequent addition of 1 ml of 5 x 10⁻² M AMM. Inhibition of T.F. activity by con A does not occur if the AMM is mixed with the con A prior to application to the monolayers. AMM alone has no effect on the clotting time. Based upon previous studies of the cell-binding of isotopically-labeled con A, 0.16 μg of con A was calculated to be 10 times the minimum present on the cells incorporated into the assays in the above experiments. When this amount of con A is added to fibroblast T.F. immediately upon assay with normal or factor VIII-deficient plasma, no effect on the clotting time is observed. Furthermore, when 0.1 ml of con A solution in various concentrations up to 800 μg/ml is added to 0.1 ml of 0.01 M CaCl₂ and 0.1 ml normal plasma, no prolongation of the clotting time is observed.

Discussion

Con A blocks the coagulant activity of preformed T.F. of cultured human skin fibroblasts or rabbit brain. Since inhibition of activity can be prevented by prior addition of AMM, the interaction between T.F. and con A possesses sugar specificity. Since activity can be recovered by addition of AMM subse-
quent to con A, activity is simply masked but not destroyed by con·A. Con A has no effect on the plasma used in the assay system and therefore must be blocking T.F. However, it cannot be determined from these experiments whether con A blocks T.F. coagulant activity by binding directly to T.F. on the cell surface or by binding to an adjacent site, thereby blocking T.F. indirectly. The former alternative is most likely, since Pitlick has recently established that con A binds to and inhibits the coagulant activity of purified T.F.

Zacharski et al. demonstrated that T.F. production by cultured fibroblasts is related to adhesion and spreading of these cells. However, the cause and effect relationship between T.F. and cell spreading remained uncertain. It is significant that con A has a variety of effects on cell surfaces, but adhesion-related phenomena are unaffected. For example, con A does not inhibit membrane transport of nonelectrolytes in polymorphonuclear leukocytes (PMN) or adhesion of polyvinyltoluene beads to PMN surface membranes, but phagocytosis of such particles is inhibited. Lutton demonstrated that spreading of macrophages on glass did not affect con A binding by these cells. In an attempt to relate con A combining sites to cell adhesion, Steinberg and Gepner studied the effect of this agent on the reaggregation of dissociated embryonic cells, the sorting-out and rearrangement of histotypically dissimilar cells within multicellular aggregates, and the spreading of one cell over another. Using a monovalent form of con A which itself did not cause cell aggregation but which prevented aggregation upon addition of polyvalent con A, these workers found no inhibition of spontaneous cell aggregation, sorting or spreading. They concluded that con A binding sites on cell surfaces did not play a major role in adhesion-dependent processes. In the light of these findings, our results suggest that T.F. produced by trypsinized fibroblasts is a consequence rather than the cause of cell adhesion and that T.F. is not synonymous with the adhesive site on the cell surface.

Con A not only blocks T.F. but also stimulates lymphocyte blastogenesis. It is of interest, therefore, that Rickles et al. demonstrated T.F. production by lymphocytes stimulated to undergo blastogenesis with phytohemagglutinin or purified protein derivative of tubercle bacilli. Furthermore, con A inhibits cell migration and phagocytosis. In this regard, Lerner et al. observed that leukocytes which migrate into experimental thrombi synthesize T.F. after several hours. Although a function for T.F. other than its coagulant activity has not been defined, it is conceivable that T.F. may be related, perhaps indirectly, to a site on the cell surface responsible for communication with the cell interior or a site responsible for triggering certain specialized cell functions.

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REFERENCES

2. Zacharski LR, McIntyre OR: Membrane
to plasma membranes by peroxidase-conjugated antibodies. Science 175:766, 1972
6. Pitlick FA: Personal communication
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