CONTAMINATION OF THROMBOSPONDIN WITH VITRONECTIN

To the Editor:

We read the recent report showing interaction of platelet integrins, especially the αβ₃ vitronectin receptor, with thrombospondin-agarose with interest. This finding is in agreement with previous reports of adhesion of cells to substrate adsorbed thrombospondin by a mechanism that is inhibited by Arg-Gly-Asp-containing peptides. However, in our hands the major interaction of cells with thrombospondin is with heparan sulfate proteoglycan and adhesion of cells to substrate-adsorbed thrombospondin is sensitive to heparin but not to Arg-Gly-Asp peptides.

Considerable amounts of vitronectin were recently demonstrated in platelet releasates, and vitronectin was shown to be uniquely able to bind to surfaces in the presence of large amounts of competing proteins. Inasmuch as thrombospondin is usually purified from platelet releasate on the basis of its affinity for heparin and its large size, we wondered whether thrombospondin purified from platelet releasate is contaminated with vitronectin. Western blotting of approximately 70 μg of purified thrombospondin showed approximately 0.17 μg of vitronectin (Fig. 1). Thus, our thrombospondin contains approximately 0.25% vitronectin. Culture plates have been prepared for cell adhesion assays by coating with solution containing 20 to 40 μg/mL thrombospondin. In contrast, half maximal adhesion is achieved on plates coated with solution containing 0.1 μg/mL vitronectin. Thus, the trace contamination with vitronectin at the level found in our preparations could explain Arg-Gly-Asp-sensitive adhesion to plates coated with thrombospondin at concentrations greater than 20 μg/mL. It is impossible to calculate whether the contamination could account for binding of vitronectin receptor to thrombospondin affinity columns.

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Fig 1. Vitronectin content of typical thrombospondin preparation. Purified thrombospondin (TSP) (lanes 1 and 5, 10 μg; lanes 2 and 6, 70 μg) and vitronectin (VN) (lanes 3 and 7, 0.1 μg; lanes 4 and 8, 0.7 μg) were analyzed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate gel under reduced condition, electrophoretically transferred to nitrocellulose paper, either probed with a monoclonal antibody against VN (lanes 1 through 4), or stained the proteins with Comassie Blue (lanes 5 through 8). The immunoblots were then quantified by densitometry.

REFERENCES

7. Preissner KT, Holzüchter S, Justus C, Müller-Berghaus G:


RESPONSE

The letter of Drs Sun and Mosher shows that thrombospondin preparations can be contaminated with 0.25% vitronectin. We have recently detected comparable levels of vitronectin in our preparations and would concur with this observation. However, the proposal that these trace amounts of vitronectin could account for the Arg-Gly-Asp-sensitive adhesion to thrombospondin we regard as unlikely for the following qualitative and quantitative reasons.

Qualitatively, the properties of cells on thrombospondin substrates are different from those on vitronectin substrates.1-9 Endothelial cells attached to thrombospondin but did not spread and form focal contacts.1-7 If thrombospondin is adsorbed to the substrate in the absence of calcium, adhesion is markedly inhibited.9 By contrast, endothelial cells attach, spread, and form focal contacts on vitronectin, which is adsorbed to plastic in phosphate-buffered saline.9 Several groups have reported that attachment to thrombospondin can be inhibited by antibodies to thrombospondin.1-5 Tuszyński et al16 have shown that human melanoma cells attach to thrombospondin that has been purified by a protocol that is less likely to lead to vitronectin contamination. These investigators have, in fact, reported that they were unable to detect vitronectin in their preparations by direct enzyme-linked immunosorbent assay.9 Their results are consistent with ours in that they observed that Arg-Gly-Asp-containing peptides and antibodies directed against the vitronectin receptor inhibited attachment of cells to thrombospondin.8

Quantitatively, it seems unlikely that there is enough vitronectin present in the samples to give the results we obtained.1,10 Drs Sun and Mosher reference the report of Preissner et al10 as stating that half maximal adhesion is achieved on plates coated with 0.1 μg/mL of vitronectin. This value would appear to apply to the human omental tissue microvascular endothelial cells (HOTMEC), which seem to be quite sensitive to vitronectin.9 Maximal response is reached at approximately 6 to 10 μg/mL of vitronectin with human umbilical vein endothelial cells (HUVEC), which is one of the cell types that we and others have used. If the attachment which we observed was due to the 0.25% vitronectin contamination, then one would expect to have to use much higher levels of thrombospondin than we and others have used to reach maximal response.1,12 A similar argument can be made for the column data. We used approximately 2 mg of thrombospondin per milliliter of settled gel to prepare the columns. This would correspond to 5 μg of vitronectin per milliliter of settled gel. Pytel et al13 reported that 2 mg of vitronectin per milliliter of settled gel resulted in “low” yields of the vitronectin receptor. It therefore seems unlikely that trace amounts of vitronectin on the column would be sufficient to produce a detectable effect.

While we agree that thrombospondin can interact with cells via heparan sulfate proteoglycans as noted by Sun et al12 there is considerable data to suggest that other macromolecules, including the αβ integrin, platelet glycoprotein IV, and sulfatides can mediate the interaction of thrombospondin with cell surfaces.1,6,13,14

There is ample precedent for interactions of other extracellular matrix proteins, such as fibronectin and laminin, to cells via multiple receptors, including both integrins and heparan sulfate proteoglycans.15,16 Which receptor-ligand interaction is dominant in a particular experiment depends on experimental conditions and on the spectrum of the different receptors expressed by the cell. For instance, the cells which we have reported to attach to thrombospondin (endothelial and smooth muscle cell) express integrin in significant amounts, and their adhesion to thrombospondin is blocked by antibodies to this integrin as well as by Arg-Gly-Asp-containing peptides. In our experiments, the cells were trypsin-treated, which would be expected to remove surface heparan sulfate proteoglycans but not integrins. Under other conditions or in different cells, heparin-thrombospondin interactions may well be important. We are currently performing tissue localization studies in an effort to determine if thrombospondin colocalizes with different receptors in different tissues.

Several groups have addressed the importance of different portions of the molecule in interactions with cells. Antibodies to both the NH₂ and COOH-terminal portions of the molecule inhibit platelet aggregation.17,18 Haveystick et al19 have shown that the hemagglutinating activity of thrombospondin is retained in proteolytic fragments that lack the heparin-binding domain. A similar proteolytic fragment also supports attachment of keratinocytes, melanoma cells, and malaria-parasitized erythrocytes.17,20 Taraboletti et al17 have shown that removal of the heparin binding domain did not affect the ability of thrombospondin to support heptatosis. In contrast, chemotaxis was blocked by removal of the heparin-binding domain or by antibodies to the heparin-binding domain. These data indicate that the interactions of cells with thrombospondin, like those of other adhesive proteins, are complex and involve multiple domains on the molecule and multiple receptors on the cell surface.

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REFERENCES


Contamination of thrombospondin with vitronectin [letter; comment]

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