Reply to Commentary

Reply to comment on 'Effect of purified, soluble urokinase receptor on the plasminogen-prourokinase activation system' (A. A-R. Higazi)

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Our article [1] documents two major issues. Firstly, the soluble uPAR preparation has no stimulatory effect, but actually a weak attenuating effect, on the plasminogen-pro-uPA activation system as long as precautions are taken to avoid the problems of adsorption and denaturation that occur in very dilute protein solutions. Secondly, the stimulatory effect that can actually be measured in solution in dilute samples (i.e. when these precautions have not been taken) cannot be ascribed to the binding between uPAR and pro-uPA but rather reflects cascade initiation, catalyzed by proteolytic contaminants. Together, these findings indicate that the conditions used by Higazi and coworkers [2] are not suited for this type of study and that the conclusion of the recombinant, soluble uPAR stimulating the proteolytic cascade system is based on an artifact.

The comment by Higazi [3] on our article completely fails to discuss the major point that measurements in the absence of carrier proteins or detergents are subject to serious problems in interpretation, and that the inclusion of such carriers abolishes any stimulatory effect. Instead, the comment limits the discussion to measurements done in the absence of carriers, questioning our interpretation of two properties of the system: (1) the dependence on the concentration of the soluble uPAR and (2) the independence on blocking of uPAR with ATF. In neither case, however, do the results favor the alternative interpretation suggested in the comment, as will become clear in the following.

1. The concentration dependence

The observation that the slopes of the curves of Fig. 3B [1] converge toward a certain maximum value, referred to as saturation in the comment [3], is actually in complete agreement with our interpretation. The statement in the comment, i.e. that this observation counts against a proteolytic effect, thus reflects a misunderstanding of our graphic representation of the data. The ordinate of Fig. 3B represents plasmin activity and therefore, at any time point, the slope of each curve represents the generation of plasmin activity per time unit, which is a measure of the plasminogen activator activity at the time point in question. A maximum value for the slope therefore exists which simply represents the situation where maximum conversion of pro-uPA into two-chain uPA has occurred (although this was not stated explicitly in the article). Addition of increasing amounts of a protease contaminant, contained within the soluble uPAR preparation, would lead to a situation where this maximum slope is reached almost immediately, exactly as observed. It should be noted, however, that at the later time points, surface adsorption phenomena complicate the analysis in these carrier-devoid samples, as discussed in our article [1].

2. The ATF dependence

From Fig. 2B of our article [1], it is absolutely clear that ATF is unable to prevent the activating effect of the soluble uPAR preparation. This finding again favors our interpretation that the activating effect is caused by a protease contaminant since the amount of ATF used is sufficient to block uPAR completely against binding to pro-uPA. The latter statement is documented by the successful blocking of the soluble uPAR, obtained with the same concentration of ATF, in the carrier-containing samples; see Fig. 2A of the article. In the comment, rather than evaluating the whole activation profile, attention is focused on just two points of measurement (10 and 15 min, respectively). In the light of the very small delta values in question as well as the unavoidable baseline fluctuations (see Fig. 2B), no conclusions can be drawn from single time point measurements. (Furthermore, the data in question are not replotted correctly in the comment, compare Fig. 1B of the comment [3] with Fig. 2B of our article [1]; at 15 min the activity value in the presence of ATF should not be 50% but actually 75% of that found in the absence of ATF.) Any true effect of ATF should, however, persist throughout the assay. The feed-back activation of pro-uPA, caused by the active plasmin generated, cannot account for the lack of effect of ATF at the later time points as is suggested in the comment. The successful blocking of an effect of the soluble uPAR protein would result in a sample with the same feed-back activation profile as that found in a sample where no suPAR had been added. As seen in Fig. 2B [1], this is clearly not the case.

Altogether, we reemphasize the conclusion that all of our results count against any stimulatory function of the uPAR protein *in solution*. We agree completely that the present proteolytic system is complicated and that some of the reactions involved may be sensitive to changes in experimental conditions. However, we are not aware of any study, irrespective of conditions, where a stimulatory effect of the soluble uPAR molecule on this system has been rigorously demonstrated. It should be stressed that our present results and conclusions are in complete accordance with our previous findings [4–6] as well as the recent results of Ellis [7], even though the opposite is claimed in the comment. In this connection, the suggestion that the use of different plasmin substrates can explain the discrepancy between the results of Ellis [7] and Higazi et al. [2] is disfavoured by the fact that we have used the same plasmin substrate as the latter group [1,2].

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Finally, it is important to point out that the lack of stimulation by recombinant, soluble uPAR, studied in a purified system, fits completely with the requirements known for acceleration of plasminogen activation at the cell surface. The latter process requires the simultaneous binding of pro-uPA to the glycolipid-anchored membrane protein uPAR, and of plasminogen to other cellular binding sites [6] whereas in the absence of plasminogen binding, uPAR-expressing cells do not stimulate plasminogen activation; see [4] for a detailed discussion.

References

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