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LETTER TO THE EDITOR

Involvement of annexin A2 in anti-β_2GPI/β_2GPI-induced tissue factor expression on monocytes

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Dear Editor:

Growing evidence demonstrates that β_2-glycoprotein I (β_2GPI) is the key target for antiphospholipid antibodies which are closely associated with thrombotic events in antiphospholipid syndrome (APS) [1]. Anti-β_2GPI/β_2GPI complex can bind to the surface membrane of monocytes and endothelial cells, promoting tissue factor (TF) activity on these cells, and thus increasing the risk of thrombosis. However, the mechanisms underlying the actions of anti-β_2GPI/β_2GPI are not well understood. It was proposed that the effects of anti-β_2GPI/β_2GPI require the transduction of a signal into the cell and, therefore, involve a cell surface receptor. Since the effects of anti-β_2GPI/β_2GPI do not appear to be mediated by Fc receptors, nor by negatively-charged phospholipids of the cell membrane, investigators posit the existence of a distinct cell surface receptor for β_2GPI.

In previous work, McCrae and colleagues demonstrated annexin A2 (ANX2, formerly called annexin II) as a high-affinity receptor for β_2GPI on endothelial cells (Ma *et al*) [2]. Furthermore, they showed that ANX2 mediates anti-β_2GPI/β_2GPI complex binding to endothelial cell surface, stimulating the activation of endothelium and increasing the levels of TF, VCAM-1 (vascular cell adhesion molecule 1) and other inflammatory molecules in circulation [3]. Their findings establish an important point that a cell surface receptor is critical in mediating the effects of autoantiphospholipid antibodies (APL) on endothelial cells. Our previous study showed that anti-β_2GPI could induce monocyte TF activity in APS [4]. Finding the mediator for anti-β_2GPI/β_2GPI in monocytes would be a key step in further understanding APL associated pathology in APS. McCrae's observation on endothelial cells led us to ask similar questions for monocytes. Is ANX2 expressed on monocytes? Does ANX2 mediate anti-β_2GPI/β_2GPI binding to monocytes, leading to stimulation of TF expression?

Using the specific anti-ANX2 antibody, we have demonstrated by western blotting that both peripheral blood monocytes and the monocytic cell line, MM6 cells, contained ANX2 protein (36 kDa) in their membrane lysates (Figure 1A). The surface ANX2 expression on blood monocytes (Figure 1B a-c) and MM6 cells (Figure 1B d-f) was also detected by flow cytometry analysis. The mean fluorescence intensity of cells treated with the calcium chelator EGTA was obviously weaker than that of cells without the EGTA treatment, suggesting that ANX2 is a calcium-dependent protein, which is consistent with previously published results [5].

We next investigated whether ANX2 can mediate anti-β_2GPI/β_2GPI binding to monocytes, and thus stimulating TF expression on these cells. The results showed that TF activity on monocytes induced by anti-β_2GPI/β_2GPI was decreased when cells were pre-incubated with the monoclonal anti-ANX2 antibody, while the control antibody had no such inhibitory effects. Importantly, anti-ANX2 antibody did not affect the effect of LPS on TF expression (Figure 1C). This result suggests that ANX2 on the cell surface is important for mediating the binding of anti-β_2GPI/β_2GPI and the subsequent stimulation of TF activity. We also examined the effect of exogenous β_2GPI on monocytes. Interestingly, incubation with exogenous β_2GPI enhanced the reaction of anti-ANX2 and anti-β_2GPI antibodies with these cells (Figure 1D), suggesting a stabilization effect by formation of the ternary complex consisting of ANX2, β_2GPI, and a corresponding antibody (anti-ANX2 or anti-β_2GPI).

In summary, this study shows that ANX2 is expressed on monocytes and is involved in monocyte activation and

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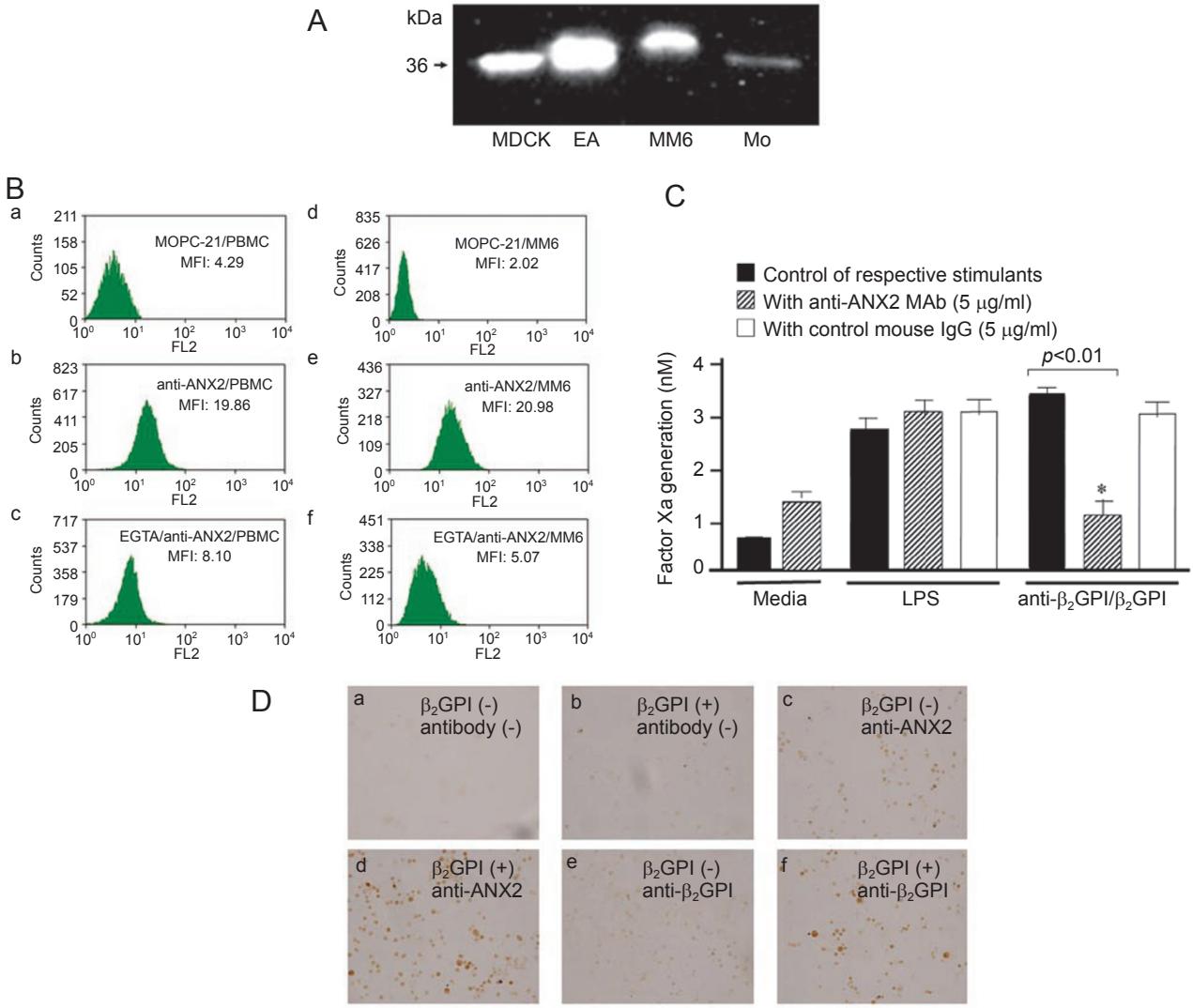

Figure 1 (A) ANX2 expression on different cells detected by western-blotting with anti-ANX2 antibody (a monoclonal antibody from ZYMED Laboratories, South San Francisco, CA, USA, cat # 03-4400). MDCK lysate was a positive control supplied with the anti-ANX2 antibody kit; EA was from EAHY 926 cell lysate (also as a positive control); MM6 was the monocytic cell line lysate; Mo was from blood monocyte lysate. **(B)** Cell surface ANX2 expression measured by flow cytometry. Blood monocytes (a, b, c) and MM6 cells (d, e, f) were treated with anti-ANX2 antibody (same as in A) in the presence (c, f) or absence (b, e) of EGTA (10 mM) and labeled with secondary antibody goat-anti-mouse IgG1-PE. PI was used to stain the nuclei. The isotype-matched antibody (MOPC-21) was used as a negative control (a, d). **(C)** The inhibitory effect of anti-ANX2 antibody (same as in A) on monocyte TF activity induced by anti-β_2GPI/β_2GPI. **(D)** Exogenous β_2GPI enhanced anti-ANX2 antibody binding to the surface of monocytes. Monocytes were incubated with (b, d, f) or without (a, c, e) β_2GPI (100 mg/ml) and analyzed by immunocytochemistry with anti-ANX2 (same as in A) (c, d) or anti-β_2GPI (RP-1, a monoclonal anti-β_2GPI antibody, from Roubey's lab) (e, f) antibodies. The negative control was also shown (a, b, with no first antibodies).

TF expression induced by anti-β_2GPI/β_2GPI complex. Additional studies are needed to further elucidate how ANX2 binds to β_2GPI or anti-β_2GPI/β_2GPI, and how the binding leads to transmembrane and downstream signaling responses.

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