Response to: 'Blood plasma versus serum: which is right for sampling circulating membrane microvesicles in human subjects?' by Liu *et al*

The correspondence on our study is very much appreciated.[1] We have shown that the microvesicles (MVs) in the serum from patients with systemic lupus erythematisus *(SLE) contain higher concentration of double strand DNA (dsDNA) and have higher interferon-stimulated gene (ISG)-inducing activity than healthy controls. In the letter from Liu *et al*, authors addressed several concerns regarding the use of serum-derived MVs in experiments since these may not represent the physiological MVs present in the circulation of patients with SLE.[2] We agree that plasma is preferable for the preparation of MVs. However, reporter activity could not be evaluated using blood plasma due to the formation of gel-like material in supernatant during cell-based reporter assay. For this reason, we decided to collect serum for our study, making it difficult to simultaneously collect enough plasma samples for this study.

Liu *et al* first pointed out that phosphatidylserine-positive MVs, including apoptosis-derived membrane vesicles, may be involved in clot formation in the sampling tube. If so, larger quantities of MVs may be isolated from plasma compared with those from serum, and we expect that our experimental results will become more prominent.

Second, it is possible that 'artificial' MVs may have formed from platelets during clotting process. With regard to this concern, Dieker and colleagues have shown that plasma-derived MVs are able to induce type I interferon (IFN),[3] suggesting that non-platelet-derived MVs possess ISG-inducing activity. Since platelets are normally anucleated, it is unlikely that platelet-derived MVs contain genomic dsDNA. As for mitochondrial DNA (mtDNA), serum of healthy donors and patients with SLE contained similar amounts of mtDNA, further indicating that platelet-derived MVs were not the source of ISG-inducing activity that we observed. However, the contribution of MVs formed by other cells during serum preparation needed to be addressed.

As for the third point, surface proteins on MVs may be cleaved during coagulation process and lose their original properties. If certain features of MVs are possibly lost during serum preparation, it may be more appropriate to use plasma-derived MVs. We are certainly interested in the contribution of surface molecules on MVs in ISG-inducing activity as it may affect the efficiency of internalisation by IFN-producing cells. We appreciate the suggestion and will investigate further to clarify this concern.

Certainly, we share the concerns that coagulation process during serum preparation may affect the population and property of collected MVs, and admit that there were insufficient points in our study. However, since others have demonstrated type I IFN induction with MVs derived from SLE plasma,[3] we believe that our results are reproducible even when plasma samples are used. There are only few reports that have evaluated MVs in the blood from patients with SLE. Thus, we need further studies to clarify the differences of apoptosis-derived membrane vesicles and other MVs when they are isolated from serum or plasma.

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