The reply

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LETTER

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Reply

To the Editor:

We thank Chepy et al[1] and Akbarzadeh et al[2] for their interest in our recent publication, "Absence of functional autoantibodies targeting angiotensin II receptor type 1 and endothelin 1 type A receptor in circulation and purified IgG from patients with systemic sclerosis",[3] and appreciate the opportunity to respond to their comments.

In our study, we investigated whether total IgG from patients with systemic sclerosis (SSc) (n = 5–18) could induce signaling and cell activation through angiotensin II receptor type 1 (AT_1) and endothelin 1 type A receptor (ET_AR) using various cellular readouts, including endothelial cells (ECs) with endogenous receptor expression and Chinese hamster ovary (CHO) cells overexpressing AT_1 or ET_AR. Because neither approach detected functional AT_1- or ET_AR-activating autoantibodies in purified IgG from patients with SSc (SSc-IgG), we developed a novel protein capture assay to detect circulating anti-AT_1 antibodies in isolated SSc-IgG (n = 28). Using this approach, we found no evidence of specific AT_1-binding IgG. These findings align with observations from Chepy et al, who did not find a correlation between anti-AT_1 or anti-ET_AR seropositivity and changes in EC proteome on incubation with SSc-IgG.[4] Interestingly, Chepy et al described an association between antinuclear antibody (ANA) positivity and distinct EC proteomic signatures.

We agree with Akbarzadeh et al that the functional effects observed using SSc-IgG may relate to experimental design and assay conditions. According to their comments, both the CHO cell lines and ECs used may not accurately detect antibody–receptor interactions. However, we are convinced that robust antibody-mediated effects should be reproducible across laboratories and model systems. We specifically selected CHO cells overexpressing AT_1 or ET_AR to replicate the cellular background of the original studies first reporting these autoantibodies.[5] Our data confirmed that these overexpressing CHO cell lines exhibited normal receptor signaling on stimulation with their natural agonists, and this signaling could be blocked by receptor-specific antagonists, indicating proper receptor function and downstream signaling. As noted by Akbarzadeh et al, varying functional readouts may lead to different interpretations. Therefore, we also applied two additional assays to analyze SSc-IgG reactivity to AT_1 and ET_AR. We observed no differences in results between primary human dermal microvascular ECs and immortalized human umbilical vein ECs. Notably, the reported protein captur eassay employed solubilized membrane proteins from nontransfected cells as a negative control, an essential step in confirming assay specificity, which is not always included in the development of an anti-AT_1 enzyme-linked immunosorbent assay (ELISA).[5]

Chepy et al also referred to a murine monoclonal antibody (mAb) reported to recognize human AT_1, which might help validate AT_1 signaling or binding.[6] Unfortunately, we did not have access to this mAb for inclusion in our experimental models. This mAb was generated by immunizing mice with membrane extracts from CHO cells overexpressing AT_1, followed by screening hybridoma supernatant against the same membrane preparations in ELISA. Because the target recognized by the mAb was not molecularly defined, nonspecific binding to unrelated components cannot be excluded. Thus, although interesting, these results should be interpreted cautiously, especially because confirmatory data using validated models and molecularly defined readouts, such as testing on transfected versus parental cells, were not available.

The authors noted that patient-specific factors, such as genetic variations in AT_1, immune activation status, or individual IgG reactivity, might influence outcomes of SSc-IgG binding to ET_AR or AT_1. We agree and emphasize that a deeper understanding of autoantibody roles in SSc is likely better achieved by focusing on ANAs and/or the B cells producing them. ANAs—particularly against anti–topoisomerase I (ATA) and anti-centromere protein B—are disease specific and strongly associated with distinct clinical phenotypes, and their presence and levels correlate with disease development.[7] The recent success of CD19 chimeric antigen receptor T cell therapy in SSc, in which treatment led to decreased ATA levels, supports this concept.[8–10]

In conclusion, we emphasize the importance of reproducibility to confirm antibody-mediated effects in validated, molecularly defined assays. Given conflicting reports on AT_1- and ET_AR-targeting autoantibodies in SSc, focusing on ANAs and/or ANA-producing B cells may provide key insights into autoreactive B cell responses in SSc.

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