HHS Public Access

Author manuscript

Sci Immunol. Author manuscript; available in PMC 2020 July 31.

Published in final edited form as:

Sci Immunol. 2020 January 31; 5(43): . doi:10.1126/sciimmunol.aaz9319.

Overline: Rheumatoid Arthritis

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Following the publication of our paper (1), we were contacted by an investigator who voiced some technical concerns about our mass spectrometric analyses to identify and characterize citrullinated proteins present in neutrophil extracellular traps (NETs)[1]. Given the magnitude of the mass shift expected between a citrulline and an arginine residue (+0.984 Da), misannotation of citrulline-containing peptides can occur because of fragmentation of misassigned monoisotopic mass peaks. Additionally, the +0.984 Da mass increase upon citrullination is identical to the mass increase observed upon protein deamination of asparagine and glutamine residues within a peptide, thereby confounding the correct assignment of citrullinated species. Lastly, most of the previously identified peptides contained a C-terminal citrulline. While several endogenous proteases, including cathepsin B, cleave after citrulline, trypsin, the protease used in sample preparation, does not (2,3). These discrepancies prompted us to reevaluate the initial proteomic data more stringently. Upon reevaluation, the spectra were either not consistent with citrullination or not definitive enough to be consistent with a citrullinated residue. Therefore, we generated new NET

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samples by treating human neutrophils from peripheral blood with rheumatoid factor and calcium ionophore. NETs were isolated and subject to mass spectrometric analyses and the datasets are now reported in the technical comment by Salinger et al [4]. Upon reanalysis, we found that 15 peptides were citrullinated in NETs generated using rheumatoid factor and that these peptides are different than the peptides originally described in the manuscript (1). Based on this, we have made changes to Figures 1E and 1F, added a section to the supplementary methods explaining the technique now used for mass spectrometric analyses and deleted supplementary table 1 (as this would have been redundant with data presented in Figure 1F). We have also modified the second paragraph of the results section to highlight citrullinated proteins found in NETs using the updated mass spectrometric analyses. We apologize for the error and note that the new datasets do not change our findings that NETs are a key source of citrullinated autoantigens and that RA autoantigens can be presented by synovial fibroblasts to the adaptive immune system and generate pathogenic immune responses (1).

References and notes.

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