MS. MINZHI YU (Orcid ID : 0000-0002-3141-7285)

MR. SANG YEOP KIM (Orcid ID : 0000-0002-5781-9039)

DR. ANNA SCHWENDEMAN (Orcid ID : 0000-0002-8023-8080)

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Reply

Minzhi Yu MS[1,2], Sang Y. Kim PhD[1,2], Emily E. Morin PhD[1,2], Anna Schwendeman PhD[1,2,*]

[1] Department of Pharmaceutical Sciences, University of Michigan, Ann Arbor, MI 48109, USA

[2] Biointerfaces Institute, University of Michigan, Ann Arbor, MI 48109, USA

* To whom correspondence should be addressed

North Campus Research Center, 2800 Plymouth Road, Ann Arbor, MI 48109, USA annaschw@med.umich.edu

To the Editor:

We thank Dr. Vuilleumier for his interest in our review paper on high-density lipoproteins (HDLs) in systemic lupus erythematosus (SLE)[1] and his comments on anti-apolipoprotein A-1 (apoA-1) autoantibodies. Dr. Vuilleumier raises an important point that the prevalence of high anti-apoA-1 IgG levels is not specific to SLE, which we fully agree with. The prevalence of anti-apoA-1 autoantibodies in

different disease settings has been summarized in Table 1. Inconsistent results could be attributed to the assay-to-assay differences introduced by different enzyme-linked immunosorbent assay (ELISA) assays and different positivity cut-off values used [2].

Questions were also raised concerning whether anti-apoA-1 IgG is an independent predictor of cardiovascular (CV) events in SLE patients. Indeed, anti-apoA-1 IgG has not been found to be independently associated with CV events in SLE [3, 4]. As pointed out by Dr. Vuilleumier, anti-apoA-1 IgG has been found to be associated with CV events in a broad array of clinical settings [5]. Such inconsistency certainly deserves further investigation.

Dr. Vuilleumier also raised questions about our hypothesis that oxidized apoA-1 may play a role in inducing anti-apoA-1 autoantibodies. We acknowledge that the formation of anti-apoA-1 autoantibodies could be attributed to various genetic and environmental factors, as argued by Dr. Vuilleumier. At the same time, our hypothesis is based on our experience in the biopharmaceutics field, where oxidation and misfolding are recognized immunogenic factors for therapeutic proteins and peptides [6, 7]. In the case of apoA-1, oxidation at Met-112 and Met-148 has been suggested to cause conformational change of apoA-1 by disrupting the α-helix structure [8, 9]. Methionine oxidation on apoA-1 has also been shown to induce the formation of amyloid fibril, which could be immunogenic [10]. Studies by Vinditto *et al.* showed that apoA-1 peptide with oxidized Met-148 could bind with goat polyclonal antibodies against human apoA-1 [11], and oxidized apoA-1 appeared to be more immunogenic in mouse models [12]. In addition to methionine oxidation, apoA-1 could undergo other oxidative modifications such as 3-chlorotyrosine and 3-nitrotyrosine in autoimmune settings [13, 14]. Particularly, 3-nitrotyrosine modification has been proven to be immunogenic for other proteins and was suggested to be responsible for impaired immunological tolerance [15]. Thus, further studies on 3-chlorotyrosine and 3-nitrotyrosine modified apoA-1 species would be helpful to investigate the underlying mechanisms of anti-apoA-1 autoantibodies in autoimmune settings.

Table 1. Prevalence of anti-apoA-1 autoantibodies in different populations.

Reference	Population	Prevalence	Note
Antiochos et al. 2017 [16]	5,220 participants with mean age 52.6 years	19.9%	Positivity cut-off corresponds to 97.5% of the reference distribution from 140 healthy blood donors.
Satta et al. 2018 [17]	237 HIV positive patients with no current lipid-lowering therapy	58%	Positivity cut-off corresponds to 97.5% of the reference distribution from 140 healthy blood donors.
Pruijm et al. 2012 [18]	71 patients on maintenance hemodialysis	20%	Positivity cut-off corresponds to 97.5% of the reference distribution from healthy blood donors.
Vuilleumier et al. 2010 [19]	69 rheumatoid arthritis (RA) patients and 46 matched controls	17% in RA patients and 2% in healthy control	Positivity cut-off corresponds to 97.5% of the reference distribution from healthy blood donors.
Nigolian et al. 2019 [4]	76 individuals satisfying at least four ACR SLE criteria	43%	Positivity cut-off corresponds to 97.5% of the reference distribution from 48 healthy donors.
Butuca et al. 2007 [20]	55 SLE patients and 150 age- and sex‑matched healthy subjects	36% in SLE patients 0.7% in healthy control	Positivity cut-off was above 5 SD of the mean of healthy controls.

O'Neill et al. 2010 [21]	39 SLE patients with high disease activity, 42 SLE patients with low disease activity and 34 healthy controls	35.9% in high activity group; 12% in the low activity group	Positivity cut-off was above 3 SD of the mean of healthy controls.
El-Lebedy et al. 2016 [22]	102 patients with type 2 diabetes (T2D), 112 patients with both T2D and cardiovascular diseases (CVD), and 88 healthy controls.	8.8% in patients with T2D, 35.7% in patients with both T2D and CVD, 6.1% in healthy controls	Positivity is set at absorbance value≥2.1 OD using Human anti-apoA-1antibody ELISA Kit MyBioSource Inc. #MBS7034.

SD: standard deviation, OD: optical density.

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