JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 5, NO. 4, 2020

EDITORIAL COMMENT

Targeting Phosphorylcholine in Established Atherosclerosis?*

Dimitrios Tsiantoulas, PhD

Atherosclerosis is a lipid-driven chronic inflammatory disease that results in the formation of atherosclerotic plaques, which on rupture or erosion lead to myocardial infarction and stroke. The recent clinical trial CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcome Study) has demonstrated the therapeutic value of immunomodulation in atherosclerotic cardiovascular disease (CVD) in humans and has paved the way toward the development of additional therapeutic strategies against the maladaptive immune response underlying atherosclerotic plaque formation (1).

A crucial step in the initiation and progression of atherosclerosis is the oxidation of low-density lipoprotein (OxLDL) in the subendothelial space of arteries. OxLDL displays oxidation-specific epitopes (adducts generated on oxidative modification) that are immunogenic and are recognized by different cell types including endothelial cells, T cells, monocytes, and macrophages, resulting in the triggering of proinflammatory responses (2). Oxidation-specific epitopes include phosphorylcholine (PC)-containing oxidized phospholipids that are also present on OxLDL and apoptotic cells and exhibit a strong proinflammatory and proatherogenic effect in vivo (3). Natural immunoglobulin M (IgM) antibodies (preexisting germline-encoded products) are an important arm of humoral immunity and have the capacity to recognize oxidation-specific epitopes in both mice and humans. Mice lacking soluble IgM antibodies display dramatically accelerated atherosclerosis (4). Moreover, whereas the association of OxLDL-specific IgG levels in plasma with cardiovascular disease risk in humans requires further investigation, several epidemiological studies have demonstrated that anti-PC IgM levels in plasma are inversely associated with CVD (4). Thus, immunomodulatory strategies targeting PC may be beneficial in mitigating atherosclerosis.

In this issue of *JACC: Basic to Translational Science*, Ståhle et al. (5) investigated the effect of a monoclonal IgG1 anti-PC antibody (X19-mu) on inflammation and vascular function in established atherosclerosis in $Ldlr^{-/-}ApoB^{100/100}$ mice that were first fed an atherogenic diet for 12 weeks and then switched to regular chow diet for 6 weeks while they were treated weekly with the X19-mu antibody or saline. Treatment with the X19-mu antibody did not alter LDL, high-density lipoprotein, and blood glucose levels in plasma. Furthermore, the lesion size in mice treated with the X19-mu antibody was similar to lesions in the mice that received saline. In addition, X19-mu treatment did not alter the collagen deposition and total macrophage area as well as the proportions of M1- and M2-type macrophages in lesions. Despite similar macrophage and phagocytic cell content, mice that were treated with the X19-mu antibody displayed a modest reduction in interleukin (IL)-1β content in the lesions. Notably, compared with cells that were collected from control mice, peritoneal macrophages isolated from transgenic mice overexpressing a single chain variant of the anti-PC E06 antibody that had been injected with the oxidized phospholipid 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (oxPAPC), displayed strongly reduced IL-1β expression (3). Thus, it is conceivable that X19-mu treatment may contribute to reducing specific proinflammatory

*Editorials published in *JACC: Basic to Translational Science* reflect the views of the authors and do not necessarily represent the views of *JACC: Basic to Translational Science* or the American College of Cardiology.

From the Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria. Dr. Tsiantoulas has reported that he has no relationships relevant to the contents of this paper to disclose.

The author attests they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2020.03.005

responses in macrophages and/or phagocytic cells in atherosclerotic lesions. Along this line, Ståhle et al. (5) showed reduced ^{18}F-fluorodeoxyglucose uptake in lesions of X19-mu-treated mice compared with in lesions from control mice.

It is important to point out a significant limitation of the study by Ståhle et al. (5): their study lacks of a group of mice treated with an isotype control antibody. This is particularly important because only the Fc portion of the X19-mu is of murine origin, and thus an immune reaction against X19-mu clone during the 6-week treatment cannot be excluded, which could also potentially limit the effect of the antibody. Furthermore, the different effector functions of the Fc portion of IgG antibodies that display different affinity to Fcγ receptors may also determine the effect of such a therapeutic approach. Fcγ receptors are divided into 2 main categories—the activating and inhibitory receptors—which confer both proatherogenic and atheroprotective effects (6). In addition, the capacity to activate the complement system differs among the different IgG classes and thus these properties could be of importance with respect to the efficacy of an anti-PC IgG-based therapeutic strategy (6).

Previous studies have shown the atheroprotective effect of PC-based immunization or anti-PC IgM passive infusion strategies in the aortic root and vein-graft atherosclerosis (4). Ståhle et al. (5) showed that in contrast to saline-treated mice, treatment with the X19-mu antibody preserved the coronary flow reserve (on adenosine stress) in the left coronary artery before and after 6 weeks of treatment in mice with established atherosclerosis. These data show that anti-PC targeting has the capacity to improve coronary vascular function, which is highly relevant for human disease. To explore the underlying mechanism of the effect of X19-mu antibody intervention on coronary vascular function, Ståhle et al. (5) treated human aortic endothelial cells with lipoprotein(a). Treatment with lipoprotein(a) led to a decreased intracellular nitrate concentration in endothelial cells treated with the isotype control antibody, whereas cells treated with the fully human PC-specific monoclonal antibody X19-A05, which has comparable binding affinity to PC as the X19-mu antibody, preserved intracellular nitrate concentration. These data are consistent with the protective effect of the PC-specific IgM E06 antibody in inhibiting IL-8 production by endothelial cells stimulated with apoptotic cells (6).

In summary, the study by Ståhle et al. (5) provides new insights into the therapeutic effect of anti-PC immunotherapy in atherosclerosis, particularly with respect to the homeostasis of the coronary vascular function.

ADDRESS FOR CORRESPONDENCE: Dr. Dimitrios Tsiantoulas, Medical University of Vienna, Lazarettgasse 14, AKH BT 25.2, Vienna 1090, Austria. E-mail: dimitris.tsiantoulas@meduniwien.ac.at.

REFERENCES

1. Ridker PM, Everett BM, Thuren T, et al., for the CANTOS Trial Group. Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med 2017;377:1119–31.

2. Binder CJ, Papac-Milicevic N, Witztum JL. Innate sensing of oxidation-specific epitopes in health and disease. Nat Rev Immunol 2016;16:485–97.

3. Que X, Hung MY, Yeang C, et al. Oxidized phospholipids are proinflammatory and proatherogenic in hypercholesterolaemic mice. Nature 2018;558:301–6.

4. Sage AP, Tsiantoulas D, Binder CJ, Mallat Z. The role of B cells in atherosclerosis. Nat Rev Cardiol 2019;16:180–96.

5. Ståhle M, Silvola JMU, Hellberg S, et al. Therapeutic antibody against phosphorylcholine preserves coronary function and attenuates Vascular ^{18}F-FDG uptake in atherosclerotic mice. J Am Coll Cardiol Basic Trans Science 2020;5:360–73.

6. Tsiantoulas D, Diehl CJ, Witztum JL, Binder CJ. B cells and humoral immunity in atherosclerosis. Circ Res 2014;114:1743–56.

KEY WORDS atherosclerosis, coronary flow reserve, inflammation, ^{18}F-fluorodeoxyglucose positron emission tomography, phosphorylcholine