Deadly tricks to combat atherosclerosis

L. Temmerman and E.A.L. Biessen*

Experimental Vascular Pathology, Cardiovascular Research Institute Maastricht (CARIM), University of Maastricht, Debyelaan 25, Maastricht 6229 HX, The Netherlands

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This editorial refers to ‘Phosphatidylserine liposomes mimic apoptotic cells to attenuate atherosclerosis by expanding polyreactive IgM producing B1a lymphocytes’ by H. Hosseini et al., pp. 443–452.

Cardiovascular disease is still the largest morbidity and mortality issue in Western society. Although current medication has proved effective in reducing cardiovascular disease risk, the need for additional therapeutic options remains. In this issue, Hosseini et al. present an elegant new concept with real bench-to-bedside potential: administration of phosphatidylserine (PS) liposomes to dampen atherosclerosis-related immune responses by mobilizing and expanding atheroprotective B1a cells (Figure 1).1

B cells were long believed to act anti-atherogenic. This notion was recently refined (as reviewed by Perry et al.2), when the groups of Mallat and Kyaw independently demonstrated clear detrimental effects of the classical IgG producing B2 cell subset on plaque progression,3,4 whereas Binder et al. showed B1a cells to inhibit atherosclerosis.5 The latter finding was further expanded by Kyaw et al., imputing B1a cell-mediated protective effects to natural IgM antibodies produced by these cells.6 Expanding on their previous work the same group has, in this study, explored new strategies to harness B1a cell’s therapeutic potential to the potential of atherosclerosis. They argued that apoptotic cells expressing double-stranded (ds) DNA and phosphatidyl serine (PS) can activate TLR9 and PS-receptors TIM1/4, respectively, on B1a cells and unleash their protective functions. To this end, they used apoptotic thymocytes and by extension, synthetic PS liposomes (previously shown to mimick apoptotic cell-induced anti-inflammatory effects7), to mobilize (peritoneal) B1a cells in atherosclerotic ApoE-/- mice.

Hosseini et al. make a strong case: their work sets PS liposomes on the map as promising therapeutic candidates in the treatment of atherosclerosis.8 Moreover, they are the first to unveil B1a cell stimulatory capacity of PS liposomes. For proof-of-concept they demonstrate that biweekly intraperitoneal injections with apoptotic thymocytes result in substantial reductions in atherosclerotic plaque size, necrotic core, plaque macrophage, and T-cell content and cytokine expression pattern. In addition, only B1a cells were significantly increased in the peritoneum of treated mice, while plasma levels of natural IgM antibodies reactive against oxidized LDL, leucocytes, and T cells were almost doubled. The authors thus succeed in their primary goal to recruit atheroprotective B1a cells and increase IgM antibody levels. The beauty of the study lies however in the results obtained with PS liposomes, which can be produced in a more controlled manner and at much larger scale and do not impart the health risks associated with human primary or immortalized cells. Phosphatidylserine liposomes gave a near-perfect phenocopy of apoptotic cell associated effects. Neither apoptotic cells nor PS liposomes were able to exert their protective roles in splenectomized atherosclerotic mice, which show depletion of the peritoneal B1a cell pool6, illustrating the critical involvement of B1a cells in PS-mediated protection.

The authors propose TIM-1, a PS recognizing molecule, as a mediator of apoptotic cell or PS liposome-induced effects on B1a cells.1 However, the actual involvement of TIM-1, and more in general the molecular mechanism of the profound effects remains somewhat obscure. The moderate increase in IL-5 plasma levels, a known B1a cell mitogen, in treated mice suggests that indirect B1a cell induction by PS-activated phagocytes may also takes place. Moreover, it is still unclear whether PS liposomes act by activating B1a cells in peritoneum and how such interaction implicates the spleen. B1a cells are rather scarce in spleen, and although Rothstein et al. have identified a splenic CD138+ B1a subset particularly effective in elaborating IgM,9 they are generally thought to play a modest role in IgM production. This raises the question to what extent CD169+ marginal zone macrophages (MZM) may contribute to the PS-liposome effects as well. As shown by Ravishankar et al. they can release tolerogenic IDO10 and regulatory T-cell recruiting CCL22 in response to apoptotic cell-exposed dsDNA, leading to tolerance induction.10 In line, MZM depletion led to increased autoimmunity and proinflammatory cytokine production, echoing the PS liposome phenotype. Further studies will be required to conclusively establish the precise mode of action of PS liposomes in atherosclerosis.

In summary, Hosseini et al. present a fresh and new therapeutic angle to tackle atherosclerosis, and by extension many other chronic inflammatory disorders. In essence, they managed to hijack a protective mechanism the body has created to avoid excess inflammation upon normal cell death. Their paper leaves us with a number of exciting questions. Firstly, will B1a cells—the key players in PS liposome-induced atheroprotection—turn out to be equally potent and PS liposome targetable in other chronic inflammatory or autoimmune diseases? Iv injected PS liposomes have been used in other animal models of arthritis11 and myocardial infarction,12 though their beneficial actions were in those cases entirely attributed to PS-activated phagocytic effects.

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* Corresponding author. Tel: +31 43 3874635, Email: erik.biesen@maastrichtuniversity.nl

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Phosphatidylserine-induced increases in IgM production were not described, though based on the data presented here they should definitely be looked into. Secondly, the authors report a PS liposome B1a cell-mediated effect on several polyreactive IgM subtypes, ensuring a broad tolerogenic response. While this offers clear opportunities for targeting other inflammatory diseases, given that natural IgMs are currently attracting considerable interest as potent immune suppressive agents, such tolerogenic state could impact on host defense against tumour-associated (neo)epitopes or pathogens. Secondly, is this methodology directly translatable to a human setting? In this regard it is worth noting that IgM memory B cells have recently been proposed as the human antipode of B1a cells. Whether this subset is equally capable of mediating atheroprotective responses to PS liposome exposure will be one of the outstanding questions for the near future. Another issue relates to PS-liposome administration. Obviously ip injection is not fit for human application, but may well be critical for B1a dependent atheroprotection. Moving forward, researchers will have to find an effective way to keep the B1a cell targeting both specific as well as efficient enough, also when translated into more clinical applications. Thirdly, Hosseini et al. chose to study effects on atherosclerosis onset; studies are awaited on PS liposome effects in more advanced stages of disease. Based on the presented pilot work, a positive outcome is to

**Figure 1** Apoptotic cells expose phosphatidyl serine (PS) residues on the membrane. Artificial PS-coated liposomes are able to serve as apoptotic-cell-mimics. Administration of apoptotic cells or PS liposomes will promote B1a-cell activity and expansion in the peritoneum, possibly through interaction of PS with TIM-1 receptors. Activated B1a cells will produce increased levels of natural IgM antibodies, which are secreted into the blood where they confer protection against atherosclerotic plaque formation by (i) sequestering oxidized LDL, (ii) inducing elimination of apoptotic cells, and (iii) reducing autoantibody formation, in part by interaction of IgM/OxLDL immune complexes with Fc(micro) receptors on other immune cells. Apoptotic cell and PS liposome dependent B1a cell activation implicates the spleen by a still unknown mechanism; although a role of the recently identified splenic CD138+ B1a cells, representing a major source of IgM, cannot be excluded, it seems more likely that this involves IgM/Fc(micro)R induced tuning of T/B cell status in spleen or the participation of (splenic) CD169+ macrophages, which are primarily responsible for PS-marked cells/liposomes engulfment and subsequent release of the B1a mitogen IL-5.
be expected, and would greatly uplift the therapeutic value of their methodology. All in all, the impressive body of data presented here lays a thorough foundation for further studies into the therapeutic use of PS liposomes in the prevention of cardiovascular disease.

References