



## Commentary

## Can Circulating Regulatory T Cells Predict Cardiovascular Disease?



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## ARTICLE INFO

## Article history:

Received 28 August 2016

Accepted 28 August 2016

Available online 30 August 2016

Adaptive immune responses against self-molecules cause autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and type 1 diabetes. Adaptive immune responses are coordinated by T helper cells expressing CD4. Normally, T cells are tightly controlled not to react with self-molecules, but autoimmunity can be triggered when T cells escape the control mechanisms that maintain self-tolerance or when self-molecules are altered or modified beyond what the immune system is trained to recognize as self. Regulatory T cells (Tregs) are a subpopulation of CD4<sup>+</sup> T cells involved in maintaining immune homeostasis and peripheral tolerance by counteracting autoimmune responses. Defining characteristics of Tregs have been the expression of CD25 and the forkhead box P3 transcription factor (FOXP3), even though these proteins can also be expressed by activated conventional CD4<sup>+</sup> T cells. More recently, demethylation of a specific region in the FOXP3 gene has been associated with stable FOXP3 expression in Tregs, but not in activated conventional T cells (Floess et al., 2007).

The disease process in atherosclerosis is driven at least in part by auto-immune reactions to self-proteins such as apolipoprotein B and heat shock proteins and thus a role for Tregs could be anticipated (Nilsson et al., 2015; Wick et al., 2014). In fact, there is plenty of evidence that is consistent with Tregs playing a role in atherosclerosis, myocardial infarction and a number of other cardiovascular diseases (Meng et al., 2016). Many studies have found that the number of Tregs are reduced in patients with unstable coronary artery disease whereas others have found an increase in Tregs in ST-elevation myocardial infarction patients (Ammirati et al., 2010; Cheng et al., 2008; Han et al., 2007; Mor et al., 2006). A large prospective population based cohort study (n = 700) has also found an association between increased risk of myocardial infarction and reduced Tregs in the circulation (Wigren et al., 2012).

DOI of original article: <http://dx.doi.org/10.1016/j.ebiom.2016.07.035>.

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In the present issue of *EBioMedicine*, Barth and coworkers investigate associations between baseline levels of Tregs and cardiovascular disease in a large prospective case-cohort sample, embedded in the European Prospective Investigation into Cancer and Nutrition (EPIC) Heidelberg cohort, followed-up for 7 years and including 276 myocardial infarction cases and 778 controls (Barth et al., 2016–in this issue). In this study the DNA demethylation of the Treg-specific demethylated region (TSDR) of the FOXP3 gene was used to quantify Tregs as ratio of total T cells (tTL), also determined by an epigenetic signature in the CD3 gene. The authors found that individuals with a low Treg/tTL ratio suffered less myocardial infarctions during follow-up independently of sex. The significant association was, however, lost when the model was supplemented with additional cardiovascular risk factors, such as smoking, hyperlipidemia, and hypertension, and dietary factors including energy-adjusted dietary intakes of red and processed meat (Barth et al., 2016–in this issue). There are many differences between this study and the previous large prospective study by Wigren et al. (2012). First, Tregs were quantified differently, not only in terms of method used, but also the Treg ratio was reported as percentage of total T cells by Barth et al., whereas Wigren et al. reports the Treg ratio as a percentage of CD4<sup>+</sup> T cells. Also, the cohorts under study differed, mainly by the fact that Barth et al. excluded diabetics. In addition, the EPIC cohort was a bit younger and had less hypertension than the cohort studied by Wigren et al. Notably, none of the two prospective studies found any associations between circulating Tregs and stroke. Furthermore, the study by Wigren et al. found no association between Tregs and the extent of subclinical atherosclerosis measured as carotid intima media thickness (IMT) with ultrasound. The lack of association between Tregs and subclinical atherosclerosis has been confirmed by Ammirati et al. who also did not find any association between carotid IMT at baseline, nor did they find any difference in Tregs between slow and rapid carotid IMT progressors during 6 years of follow-up (Ammirati et al., 2010).

Assuming that quantification of total Tregs using demethylation of the FOXP3 gene or flow cytometry are correlates of true Tregs, these studies suggest that circulating Tregs may not be a strong predictor of myocardial infarction that could translate into a usable predictor in the clinic. The studies do not, however, speak against the hypothesis that Tregs play a role in cardiovascular disease as in other autoimmune diseases. One possibility for the lack of a stronger association between Tregs and cardiovascular disease could be that total Tregs have a diverse set of antigen specificities and perhaps Tregs with a specificity for antigens relevant in atherosclerosis and cardiovascular disease would be better predictors of cardiovascular disease. Interestingly, relevant auto-antigens such as apolipoprotein B and heat shock proteins have

been defined and evaluated as therapeutic vaccines in animal models (Nilsson et al., 2015; Wick et al., 2014). It remains to be determined if antigen-specific Tregs can be quantified in human blood and if they could be used for disease prediction that affects clinical decision making. It also remains to be determined if monitoring Tregs could be used to evaluate the efficacy of novel immunomodulatory therapies currently in development.

In conclusion, regulatory T cells play a protective role in atherosclerosis and cardiovascular disease and they could be important therapeutic targets. Recent data, however, indicates that the fraction of regulatory T cells in the total T cell compartment has limited use for clinically relevant disease prediction.

## Disclosures

None.

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