Tregs and human atherothrombotic diseases: toward a clinical application?

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Immunology has recently penetrated the field of atherothrombosis. A number of human and experimental studies have documented that both cellular and molecular immune effectors are involved at various stages of the pathological process 1 . This has directed our attention towards the use in atherothrombosis of immunomodulatory strategies, commonly employed for the prevention and treatment of other chronic inflammatory diseases. The administration of immunosuppressive drugs is the most common approach for treating immune-mediated diseases. However, the long-term administration of non-antigen-specific agents that cannot distinguish between beneficial and destructive immune responses is a major drawback of this strategy.

Interesting alternatives consist of strategies aimed at potentiating the physiological regulatory mechanisms, if defective. In the field of atherothrombosis research, experimental studies have clearly shown that a defect in regulatory CD4^{+} T cells (Tregs) favors atherogenesis (reviewed in ref. 2). Based on their potent immunosuppressive activity, the expansion of the Treg compartment by cell transfer or stimulating molecules can be envisaged as a new therapeutic strategy.

These findings have provided a strong incentive to perform clinical studies with the aim of assessing whether a defective Treg compartment is associated with the progression of atherosclerotic disease and the occurrence of atherothrombotic manifestations in humans. Three previous clinical studies, in a limited number of patients with coronary artery disease, first reported that patients with acute coronary syndromes have lower numbers of and/or inefficient Tregs in their blood 3 –5 . A larger clinical study reported in this issue 6 interestingly challenges these findings and a second study 7 explores the role of Tregs in stenotic and dilative pathological remodeling of atherosclerotic aortas.

Prior to opening the debate on the role of Tregs in clinical atherothrombosis, it is important to reach a consensus on the phenotypic markers of Tregs. Indeed, the definition of these suppressive T cells can be equivocal due to their heterogeneous origin and to consistent differences in their characteristic markers between humans and mice. Initially, Tregs were defined as CD4^{+} CD25[+/high] . Because this population may also include activated effector T cells, it is not surprising that the number of Tregs reported is highly variable and does not differ between healthy individuals and patients suffering from coronary artery disease 5 , 6 or abdominal aortic aneurysm 8 . The demonstration that Tregs require the forkhead box P3 transcription repressor 9 , 10 has allowed discrimination of Tregs among CD4^{+} CD25^{+} T cells. However, this requires a complex (intracellular) staining technique which is suitable for experimental studies but difficult to use routinely in longitudinal clinical studies. To overcome this technical hurdle, one can use the (low) CD127 expression as a surrogate marker of Tregs 11 .

By using this identification strategy, Ammirati et al.6 have identified circulating Tregs as CD3^{+} CD4^{+} CD25^{high} CD127^{low} in patients with carotid or coronary atherosclerosis and have evaluated the diagnostic/pronostic value of measuring their circulating levels. While the previous studies reported a significant decrease in the percentage of blood Treg levels in patients suffering from myocardial infarction 3 –5 , Ammirati et al. 5 found that these levels were, on the contrary, significantly increased (Table) in the blood of these patients.

Even though the methodology employed to identify Tregs was different, the patient population and the time of sampling was similar in the four studies and therefore one cannot advocate methodological issues to explain the different results. This means that the evidence for a role for Tregs in myocardial infarction requires further clarification. To this aim, it is mandatory to firstly establish the biological significance of circulating Tregs. Indeed, a variation in the number of Tregs in the blood may either reflect a global change of the pool or that, at the time of sampling, they have been redistributed between the blood and the sites of inflammation. Recent experimental work suggests that Tregs in the vascular compartment infiltrate the inflamed tissue, migrate to draining lymphoid organs where they suppress the generation of immune effectors, and then recirculate back to the inflamed tissue, where they contribute to the downregulation of local immune responses 12 . Therefore, the dynamics of Treg trafficking hampers the interpretation of their peripheral blood count at a given time-point.

In unstable angina, however, the four clinical studies consistently found that the percentage of circulating Tregs is decreased, suggesting that this characterizes patients with acute coronary syndromes without evidence of myocardial necrosis. How can we interpret this finding? Like the authors, we could hypothesize that these patients suffer from a global Treg defect, possibly favoring the

destabilization of coronary atherosclerosis. However, we can also envisage that Tregs of these patients are reduced in the bloodstream because they have been massively recruited at the sites of unstable coronary arteries where they might attempt to control the inflammatory reaction. If we extrapolate this concept to the whole acute coronary syndrome cohort, this could explain the difference with patients suffering from myocardial infarction. The relative increase in Tregs in the blood of the latter, reported by Ammirati et al. 6 , may indeed reflect an insufficient recruitment of these cells in the inflamed arterial and/or myocardial tissue. Consequently, unrestrained local inflammation would precipitate the transition from unstable angina towards myocardial infarction. If this hypothesis is correct, Treg-based therapeutic strategies will have to overcome the mechanisms that prevent Treg from entering the target tissue in these patients.

The two articles published in this issue also point to another interesting aspect: The role of Treg in chronic manifestations of atherosclerosis may depend upon the type of vascular remodeling. Indeed, both studies show that in the case of inward remodeling, leading to stenosis of either coronary 6 or carotid 6 arteries or the aorta 7 , the blood Treg count is normal. The clinical value of peripheral Tregs in patients with stable, occlusive atherosclerotic disease thus appears rather limited, both in diagnosis and as a prognostic marker. Indeed, the blood Treg level was not able to predict either the presence or the progression of carotid atherosclerosis in the six-year follow-up study of Ammirati et al.6 . In a pathophysiological perspective, the fact that peripheral Tregs are normal in these patients can be interpreted in at least two ways: either human atherogenesis does not involve a Treg defect or the Treg compartment is efficient in maintaining tolerance and preventing the inflammatory events that precipitate the occurrence of atherothrombotic events.

In contrast, in patients with dilative vascular remodeling of atherosclerotic aortas (AAA), Yin et al. 7 found that blood Treg levels are reduced and display a defective suppressive function in vitro . Taken together, the results of these two studies suggest that Tregs may be particularly important for the control of the immune effectors that are involved in arterial wall dilation and thrombosis, two pathological features common to AAA and acute coronary syndromes.

Future experimental and clinical studies are warranted to establish the clinical value of quantifying Tregs in atherothrombosis. Interventional experimental studies should aim at elucidating the causes underlying the Treg defects in atherosclerotic diseases. In addition, large prognostic clinical studies should serve to evaluate the Treg threshold that can identify patients at risk of developing life-threatening atherothrombotic diseases.

As for the therapeutic perspective, despite the data generated in preclinical animal models that successfully show that Tregs can prevent or cure several T cell-mediated diseases, many questions remain to be addressed for the translation of this approach to the clinic. A major obstacle is technical and relates to cell manipulation. The phenotype used by Ammirati et al. 6 (CD4$^+$ CD25$^+$ CD127^{low}) allows for the isolation and purification of viable autologous Tregs from the blood, but because of their limited number, they need to be further expanded in vitro . However, Tregs are poorly proliferating cells, and the ex vivo expansion of Tregs remains an unresolved issue, especially, when antigen-specific Tregs are desired. In fact, current expansion protocols generate polyspecific Tregs that may cause " pan-immunosuppression" if transferred in vivo. At present, the only clinical trials of immunotherapy based on Tregs are performed in bone marrow transplantation for the prevention or cure of graft-versus host disease (reviewed in ref. 13).

Finally, although it appears certain that Tregs critically control atherosclerotic diseases, the translation of this knowledge into clinical practice will require extensive future work.

References:

- 1 . Hansson GK . Inflammation, atherosclerosis, and coronary artery disease . N Engl J Med . 2005 ; 352 : 1685 - 1695
- 2 . Mallat Z , Ait-Oufella H , Tedgui A . Regulatory t-cell immunity in atherosclerosis . Trends Cardiovasc Med . 2007 ; 17 : 113 - 118
- 3 . Cheng X , Yu X , Ding YJ , Fu QQ , Xie JJ , Tang TT , Yao R , Chen Y , Liao YH . The th17/treg imbalance in patients with acute coronary syndrome . Clin Immunol . 2008 ; 127 : 89 - 97
- 4 . Han SF , Liu P , Zhang W , Bu L , Shen M , Li H , Fan YH , Cheng K , Cheng HX , Li CX , Jia GL . The opposite-direction modulation of cd4+cd25+ tregs and t helper 1 cells in acute coronary syndromes . Clin Immunol . 2007 ; 124 : 90 - 97
- 5 . Mor A , Luboshits G , Planer D , Keren G , George J . Altered status of cd4(+)cd25(+) regulatory t cells in patients with acute coronary syndromes . Eur Heart J . 2006 ; 27 : 2530 - 2537
- 6 . Ammirati E , Cianflone D , Banfi M , Vecchio V , Palini A , De Metrio M , Marenzi G , Panciroli C , Tumminello G , Anzuini A , Palloshi A , Grigore L , Garlaschelli K , Tramontana S , Tavano D , Airoldi F , Manfredi AA , Catapano AL , Norata GD . Circulating cd4+cd25hicd127lo regulatory t-cell levels do not reflect the extent or severity of carotid and coronary atherosclerosis . In press
- 7 . Yin M , Zhang J , Wang Y , Wang S , Böckler D , Duan Z , Xin S . Deficient cd4+cd25+ t regulatory cell function in patients with abdominal aortic aneurysms . In press
- 8 . Caligiuri G , Rossignol P , Julia P , Groyer E , Mouradian D , Urbain D , Misra N , Ollivier V , Sapoval M , Boutouyrie P , Kaveri SV , Nicoletti A , Lafont A . Reduced immunoregulatory cd31+ t cells in patients with atherosclerotic abdominal aortic aneurysm . Arteriosclerosis, thrombosis, and vascular biology . 2006 ; 26 : 618 - 623
- 9 . Khattri R , Cox T , Yasayko SA , Ramsdell F . An essential role for scurfin in cd4+cd25+ t regulatory cells . Nat Immunol . 2003 ; 4 : 337 - 342
- 10 . Bacchetta R , Passerini L , Gambineri E , Dai M , Allan SE , Perroni L , Dagna-Bricarelli F , Sartirana C , Matthes-Martin S , Lawitschka A , Azzari C , Ziegler SF , Levings MK , Roncarolo MG . Defective regulatory and effector t cell functions in patients with foxp3 mutations . J Clin Invest . 2006 ; 116 : 1713 - 1722
- 11 . Liu W , Putnam AL , Xu-Yu Z , Szot GL , Lee MR , Zhu S , Gottlieb PA , Kapranov P , Gingeras TR , Fazekas de St Groth B , Clayberger C , Soper DM , Ziegler SF , Bluestone JA . Cd127 expression inversely correlates with foxp3 and suppressive function of human cd4+ t reg cells . J Exp Med . 2006 ; 203 : 1701 - 1711
- 12 . Tomura M , Honda T , Tanizaki H , Otsuka A , Egawa G , Tokura Y , Waldmann H , Hori S , Cyster JG , Watanabe T , Miyachi Y , Kanagawa O , Kabashima K . Activated regulatory t cells are the major t cell type emigrating from the skin during a cutaneous immune response in mice . J Clin Invest . 2010 ; 120 : 883 - 893
- 13 . Roncarolo MG , Battaglia M . Regulatory t-cell immunotherapy for tolerance to self antigens and alloantigens in humans . Nat Rev Immunol . 2007 ; 7 : 585 - 598

Table

Treg count variations in the blood of patients with CAD

Study	NCA	CSA		UA		MI	
	n	n	Variation	n	Variation	n	Variation
Mor et al.[5]	28	28	=	nd	nd	32	↓
Han et al.[4]	12	18	↓	16	↓↓	24	↓↓↓
Cheng et al.[3]	20	22	↓	17	↓↓	26	↓↓↓
Ammirati et al.[6]	75	36	=	39	↓	50	↑↑

NCA: Normal coronary arteries; CSA: Chronic stable angina; UA: Unstable angina; MI: Myocardial infarction; nd: not determined.