

Artery Research

ISSN (Online): 1876-4401

ISSN (Print): 1872-9312

Journal Home Page: <https://www.atlantis-press.com/journals/artres>

IL-17 producing CD4+CD45RO+ T-cells in atherosclerosis express GTR molecule

Atefe Ghamar Talepoor, Negar Behnamfar, Mohammad Javad Zibaenezhad, Mehrnoosh Doroudchi

To cite this article: Atefe Ghamar Talepoor, Negar Behnamfar, Mohammad Javad Zibaenezhad, Mehrnoosh Doroudchi (2017) IL-17 producing CD4+CD45RO+ T-cells in atherosclerosis express GTR molecule, Artery Research 21:C, 20–28, DOI: <https://doi.org/10.1016/j.artres.2017.12.004>

To link to this article: <https://doi.org/10.1016/j.artres.2017.12.004>

Published online: 3 December 2019



IL-17 producing CD4+CD45RO+ T-cells in atherosclerosis express GITR molecule



Atefe Ghamar Talepoor ^{a,c}, Negar Behnamfar ^{a,c},
Mohammad Javad Zibaenezhad ^b, Mehrnoosh Doroudchi ^{a,*}

^a Memory T-cell Laboratory, Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

^b Cardiovascular Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Received 29 October 2017; received in revised form 6 December 2017; accepted 11 December 2017

Available online 27 December 2017

KEYWORDS

Atherosclerosis;
Immunopathology;
T cell;
Inflammation

Abstract *Background:* Atherosclerosis (AS) is a chronic inflammatory disease of vessel walls associated with infiltration of immune cells which their function is controlled by different co-stimulatory and co-inhibitory receptors. We investigated the expression of co-inhibitory molecules on the memory and effector T-cells in patients with Atherosclerosis.

Methods: Patients included 9 hypertensive, dyslipidemic, non-diabetic, non-smoker individuals with the diagnosis of coronary artery disease and controls were 8 normotensive, normolipemic, non-diabetic, non-smoker individuals with normal coronary angiography/insignificant coronary artery disease. PBMCs were separated from the blood and memory T-cell subsets as well as the expression of Glucocorticoid-induced tumor necrosis factor receptor (GITR), Programmed Death-1 (PD-1), IL-17A and IFN- γ were quantified by flowcytometry.

Results: CD4+CD45RO+ memory T-cells and CD4+CD45RO- effector T-cells in patients expressed the highest level of GITR molecule. The IL-17 producing memory CD4+CD45RO+ T-cells were enriched in GITR molecule in the patients group ($P = 0.03$). The increased population of GITR+effector CD4+CD45RO- T-cells in patients, however, did not produce IL-17 ($P = 0.03$). PD-1 expression on memory T-cells of the patients was higher than the controls and was concomitant with the lack of IFN- γ expression ($P = 0.05$). IFN- γ production by effector T-cells was only seen in the PD-1- population in both groups.

Conclusions: We provide data on the expression of GITR molecule on IL-17 producing memory T-cells in patients with CAD. A population of memory T-cells, which expressed PD-1 and were not producing IFN- γ , also increased in patients' blood. These data suggest the modified phenotype/function of T-cell subsets in the atherosclerotic inflammation.

© 2017 Association for Research into Arterial Structure and Physiology. Published by Elsevier B.V. All rights reserved.

* Corresponding author. Memory T-cell Laboratory, Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, P.O. Box: 71345-3119, 71348-45794 Shiraz, Iran. Fax: +98 (71) 32351575.

E-mail address: mdoroud@sums.ac.ir (M. Doroudchi).

^c These authors contributed equally in this manuscript.

Introduction

Activated T-cells play a key function in the defense and protection of the organisms in the hostile living environment. However, inflammatory responses associated with T-cell activation are known to be involved in the pathogenesis of several diseases including atherosclerosis.¹

Various studies have shown the persistence of different subsets of CD4⁺ T-cells in the atherosclerotic plaque, where they play pro-atherogenic and/or anti-atherogenic roles during the progression of atherosclerosis. In this regard, Th1 and Th17 cells exert pro-atherogenic actions whereas Treg cells play athero-protective roles.²

T-cell function is controlled by different co-stimulatory and co-inhibitory receptors.³ The balance between these receptors is critical for the prevention of inflammatory and autoimmune diseases.^{4,5} Co-inhibitory T-cell receptors such as Program Death-1 (PD-1) and Cytotoxic T Lymphocyte Antigen-4 (CTLA-4), exert their inhibitory actions by recruitment of protein phosphatases to block activation signaling, up-regulation of proteins that prevent autoimmune reactions and down-regulation of the ligands of co-stimulatory receptors.⁶

PD-1 (CD279) belongs to the CD28 family of proteins and is expressed on the peripheral CD4⁺ and CD8⁺ T-cells, B cells, T regulatory (Treg) and Natural Killer T (NKT) cells.⁷ PD-1 ligand 1, PD-L1 (B7-H1), is expressed on T-cells, B cells, mast cells, monocytes, epithelial and vascular endothelial cells (ECs) while PD-L2 (B7-DC) is mainly expressed on innate immune cells such as macrophages and dendritic cells.⁸ PD-1 ligation on Tregs leads to inhibition of cell proliferation, decreased TNF- α and IFN- γ secretion and reduced activity of PI3K signaling pathway.⁹ In healthy individuals, resting T-cells express low levels of PD-1, which upon their engagement, the initial events in T-cell activation are blocked.¹⁰ Increased expression of PD-1 after repeated antigen exposure leads to reduced effector function of exhausted T-cells, as well.¹¹

Other than strict co-inhibitory or co-stimulatory functions, there are molecules in the immune system which exert either functions depending on the cellular milieu. A member of TNF receptor superfamily; i.e. Glucocorticoid-induced tumor necrosis factor receptor (GITR) is known to differentially function depending on the cell on which it is expressed. In general, GITR is expressed on activated CD4⁺ and CD8⁺ T-cells, B cells, Natural Killer (NK) cells, and macrophages.¹² Because GITR agonistic antibody and recombinant GITRL lead to increased CD4⁺CD25⁻ T-cells proliferation, GITR is introduced as a co-stimulatory receptor.¹³ GITRL expressed on ECs, B cells and monocytes, induces NF-KB activation, increases CD4⁺CD25⁻ conventional T-cells expansion, survival, and enhances cytokine secretion.¹⁴ GITR expression on recently activated CD4⁺ and CD8⁺ T-cells leads to their increased expansion and cytokine secretion.¹⁵ Moreover, GITR expression is necessary for virus-specific clonal expansion and persistence of memory CD8⁺ T-cells in vivo.¹⁶

GITR interaction with its ligand, affects both conventional CD4⁺CD25⁻T-cells and Treg cells.¹⁷ Previous studies have shown that CD4⁺CD25⁺ Treg cells express high levels of GITR on their surface.^{9,12} Co-culture of conventional T-

cells with CD4⁺CD25⁺ Treg cells in the presence of anti-GITR antibody, abrogates suppressor activity of Treg cells.¹³ Also injection of the same antibody to adult mice leads to autoimmune gastritis due to Treg cells depletion.^{14,18} In spite of originally suggested GITR immunomodulatory role,¹⁹ later studies suggest that GITR ligation with GITRL causes decreased Treg cell suppressor function and their expansion.²⁰ However, there is still much controversy regarding GITR function on Treg cells. As a more accepted view, GITR engagement activates effector as well as Treg cells, however, does not participate in the mechanisms of Treg suppression.⁶ In addition, GITR-KO Treg cells are shown to maintain their suppressive function.²¹

In a previous study, we showed that CD4⁺CD45RO⁺ memory T-cells of patients with atherosclerosis produce IL-17 both ex-vivo and in-vitro.²² Here we report a high level of GITR expression on the CD4⁺CD45RO⁺ memory and CD4⁺CD45RO⁻ effector T-cells of the patients with atherosclerosis as compared to the controls. Moreover, a notable percentage of the memory CD4⁺GITR⁺ T-cells produced IL-17 in the patients group.

Methods

Subjects

This study was approved by the ethics committee of Shiraz University of Medical Science (SUMS). The participants were informed about the aim of this study as well as safety and security measures and then a written informed consent was obtained. After informed consent, 20 ml heparinized blood was obtained from each of the 9 non-diabetic, non-smoker patients (5 men and 4 women aged 50–60 yrs, mean = 57.13 \pm 3.7 yrs) who were diagnosed with coronary artery disease confirmed by carotid angiography and had hypertension [systolic blood pressure (SBP) \geq 140 mmHg and/or diastolic blood pressure (DBP) \geq 90 mmHg] and dyslipidemia according to the established guidelines.²³

Control group consisted of 8 non-diabetic, non-smoker, normotensive, normolipemic individuals with normal/insignificant coronary artery disease confirmed by carotid angiography (4 men, 4 women aged 50–60 yrs, mean = 44.5 \pm 5.16 yrs) from each of whom 20 ml blood was also obtained. None of the patients and controls was on the Statin therapy.

Peripheral blood mononuclear cells (PBMCs) isolation

PBMCs were isolated by density gradient centrifugation (Ficoll–Paque PLUS, GE Healthcare Europe, GmbH, Germany) and cryopreserved in 10% dimethylsulfoxide (DMSO; Sigma–Aldrich) in fetal bovine serum (FBS Biosera, UK).

Flowcytometric analysis of memory T-cell subsets

For enumeration of Th17 and Th1 cells as well as the expression of co-inhibitory molecules, PBMCs (5×10^5 cells) were washed and stimulated with plate coated purified

anti-CD3 (BD Pharmingen, 62.5 $\mu\text{g/ml}$) and soluble purified anti-CD28 (BD Pharmingen, 0.5 mg/ml) or phorbol myristate acetate (PMA, 50 ng/ml, Sigma) plus ionomycin (ION, 250 ng/ml, Sigma) at 37 °C and 5% CO₂. Golgi-stop was added after 1 h and the cells were incubated for another 15 h at 37 °C and 5% CO₂. Then the cells were washed and stained using conjugated antibodies: anti-CD45RO-FITC (BD Pharmingen), anti-CD4-PerCP (BD Pharmingen), and either anti-IFN- γ -APC (BD Pharmingen) plus anti-PD-1-PE (BD Pharmingen) or anti-GITR-APC (BD Pharmingen) plus anti-IL-17-PE (BD Pharmingen), and were incubated in 4 °C for 30 min. The cells were subsequently washed and resuspended in PBS containing 10% FBS. For each sample, 1×10^5 cells were required by FACScalibur flowcytometer. Live lymphocytes were gated on forward and side scatter and further analyzed for surface marker and cytokine expression. Flowcytometry analysis was carried out by flowjo software (version 7.6.2).

Flowcytometric analysis of GITR expression

For the analysis of GITR expression on memory T-cell subsets, PBMCs (5×10^5 cells) were washed and stimulated with plate-coated purified anti-CD3 (BD Pharming, 62.5 $\mu\text{g/ml}$) antibody and soluble purified anti CD28 (BD Pharmingen, 0.5 $\mu\text{g/ml}$) at 37 °C and 5% CO₂. After 1 h Golgi-stop was added and cells were incubated for another 15 h at 37 °C and 5% CO₂. Then the cells were washed and stained for surface markers using conjugated antibodies: anti-CD45RO-FITC (BD Pharmingen), anti-CD4- PerCP (BD Pharmingen), anti-GITR-APC (BD Pharmingen) in 4 °C for 30 min. The cells were subsequently washed and resuspended in PBS containing 10% FBS. Live lymphocytes were gated by forward and side scatter and analyzed for surface marker expression.

Statistical analysis

The Statistical analyses were performed using SPSS software (version 15, Chicago, IL) and GraphPad prism (version 5, La Jolla, CA). Mann–Whitney U test was used for non-parametric comparison of the medians; therefore, results were demonstrated as median and range. *P*-values less than 0.05 were considered significant. The subpopulations with less than 100 events were not included in the analysis.

Results

GITR expression on the CD4+ T-cell subpopulations

The frequencies of GITR expressing CD4+ T-cell subsets were also analyzed in the non-stimulated condition and after stimulation with anti-CD3 and anti-CD28 antibodies for patient and control groups. The representative dot plots of stimulated T-cells and the gating strategy used to detect the frequency of GITR+ T-cells are shown in Fig. 1A.

The highest percentage of GITR expression was observed on the CD4+CD45RO+ memory T-cells and CD4+CD45RO– effector T-cells in non-stimulated condition in patients as compared with controls (Fig. 1B). The stimulation affected the GITR expression levels on controls

in memory CD4+ T-cells (Fig. 1B). However, there was no difference in the GITR MFI between groups before and after stimulation.

Frequencies of IL-17 and GITR expressing CD4+CD45RO+ memory T-cells

The main difference between patients and controls regarding this population was that the IL-17 producing memory T-cells were enriched in GITR molecule in the patient group. As shown in Fig. 2A and B, in patients group (but not controls) the CD4+CD45RO+GITR+IL-17+ memory T-cells, produced IL-17 even before stimulation (0.22, 1.32 vs. 0.05, 1.88, *P* = 0.03). The frequency of CD4+CD45RO+GITR+IL-17– T-cell subset in non-stimulated condition in patients was non-significantly higher than controls group (1.37, 4.009 vs. 0.56, 3.143, *P* = 0.09) and had no significant change after stimulation (2.21, 5.47 vs. 1.32, 13.57, *P* = 0.08). After stimulation with anti-CD3 and anti-CD28 antibodies, the frequency of CD4+CD45RO+GITR+IL-17– T-cell subset increased in the control group (1.32, 13.57 vs. 2.21, 5.47, *P* = 0.6).

Frequencies of IL-17 and GITR expressing CD4+CD45RO– effector T-cells

As shown in Fig. 2A and C, the frequency of CD4+CD45RO–GITR+IL-17– T-cell subset in non-stimulated condition in patients was higher than in controls group (2.41, 6.44 vs. 0.57, 2.99, *P* = 0.03) and showed no significant change after stimulation (2.41, 6.44 vs. 2.56, 9.31, *P* = 0.07).

PD-1 expression on the CD4+ and CD4– T-cell subpopulations

The frequencies of PD-1 expressing CD4+ T-cell subsets were also analyzed in the non-stimulated condition and after stimulation with anti-CD3 and anti-CD28 antibodies for patient and control groups. The representative dot plots of stimulated T-cells and the gating strategy used to enumerate PD-1 T-cells are shown in Supplementary Fig. 3A. The highest percentage of PD-1 expression in patients was observed on the CD4+CD45RO+ memory T-cells as compared with controls; however, the difference was not significant (Fig. 3B). There was no difference in the PD-1 MFI between groups before and after stimulation.

Frequencies of IFN- γ and PD-1 expressing CD4+CD45RO+ memory T-cells

As shown in Fig. 4A and B, in the non-stimulated condition, the frequencies of CD4+CD45RO+IFN- γ –PD-1+ memory T-cells in patients were higher than the control group (0.26, 0.62 vs. 0.12, 0.38, *P* = 0.05). A percentage of patients CD4+CD45RO+ T-cells that did not express PD-1 were producing IFN- γ even before stimulation. The frequencies of CD4+CD45RO+PD-1–IFN- γ + memory T-cells increased after stimulation with anti-CD3 and anti-CD28 antibodies in both controls (0.68, 1.37, *P* = 0.03), and patients (1.00, 6.11, *P* = 0.06).

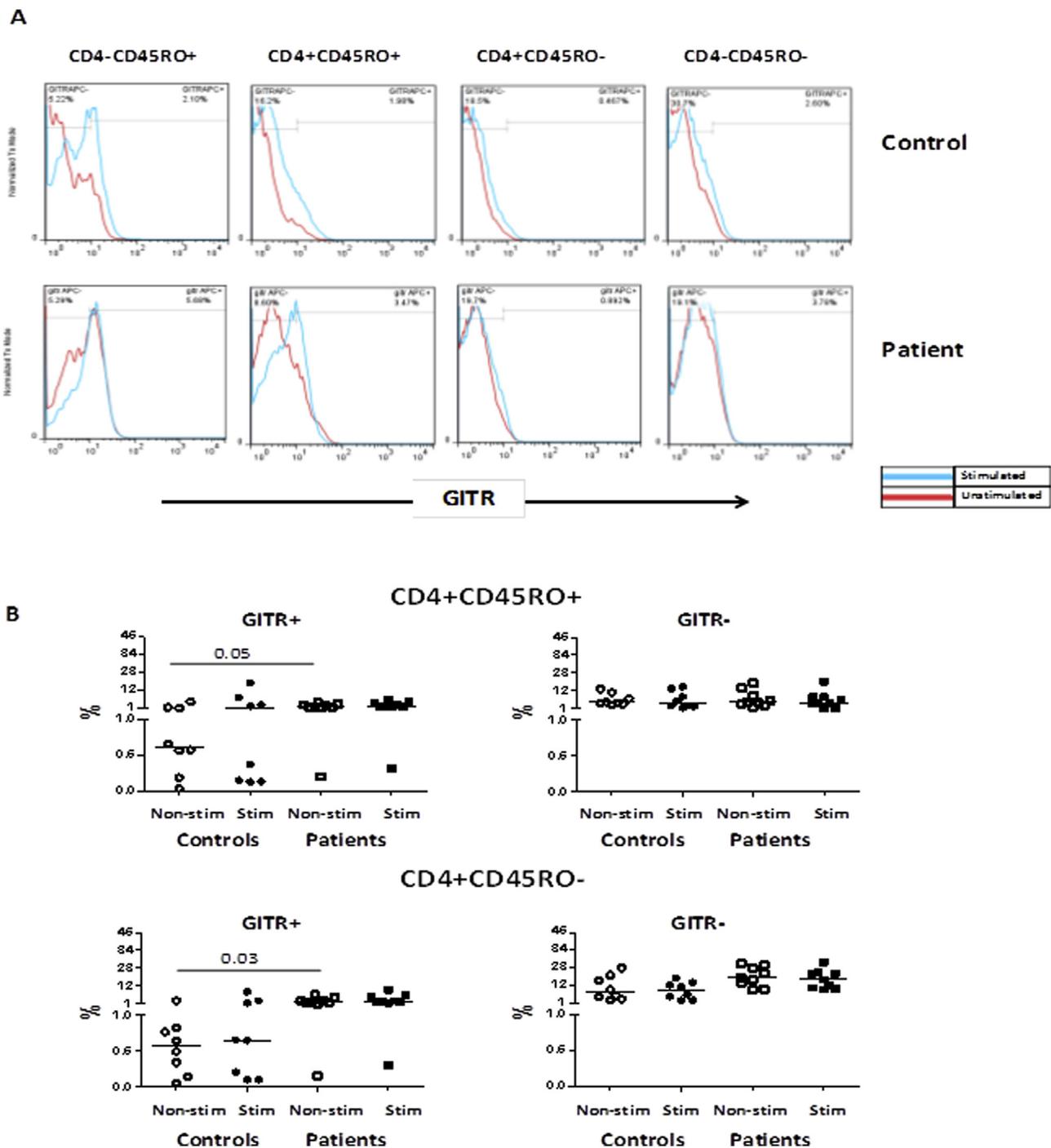


Figure 1 (A) Representative histograms indicating GITR expression in CD4⁺ T-cells and CD4⁻ T-cells subpopulations in one patient and one control individual in non-stimulated condition and after stimulation with anti-CD3 and anti-CD28 antibodies; (B) Comparison of the frequencies of memory and effector CD4⁺ T-cell based on the GITR expression between patients and controls.

Frequencies of IFN- γ and PD-1 expressing CD4+CD45RO⁻ effector T-cells

As shown in Fig. 4A and C, a small percentage of CD4⁺CD45RO⁻ effector T-cells were producing IFN- γ in patients even before stimulation. After stimulation, the frequency of CD4⁺CD45RO⁻IFN- γ +PD-1⁻ effector T-cells increased in both patients (0.46, 3.17, $P = 0.02$)

and controls (0.27, 0.85, $P = 0.008$) as compared with non-stimulated condition (0.11, 0.27 vs. 0.02, 0.83, $P = 0.20$).

Discussion

In this study, we found an increased expression of GITR molecule on memory (CD4⁺CD45RO⁺) and effector

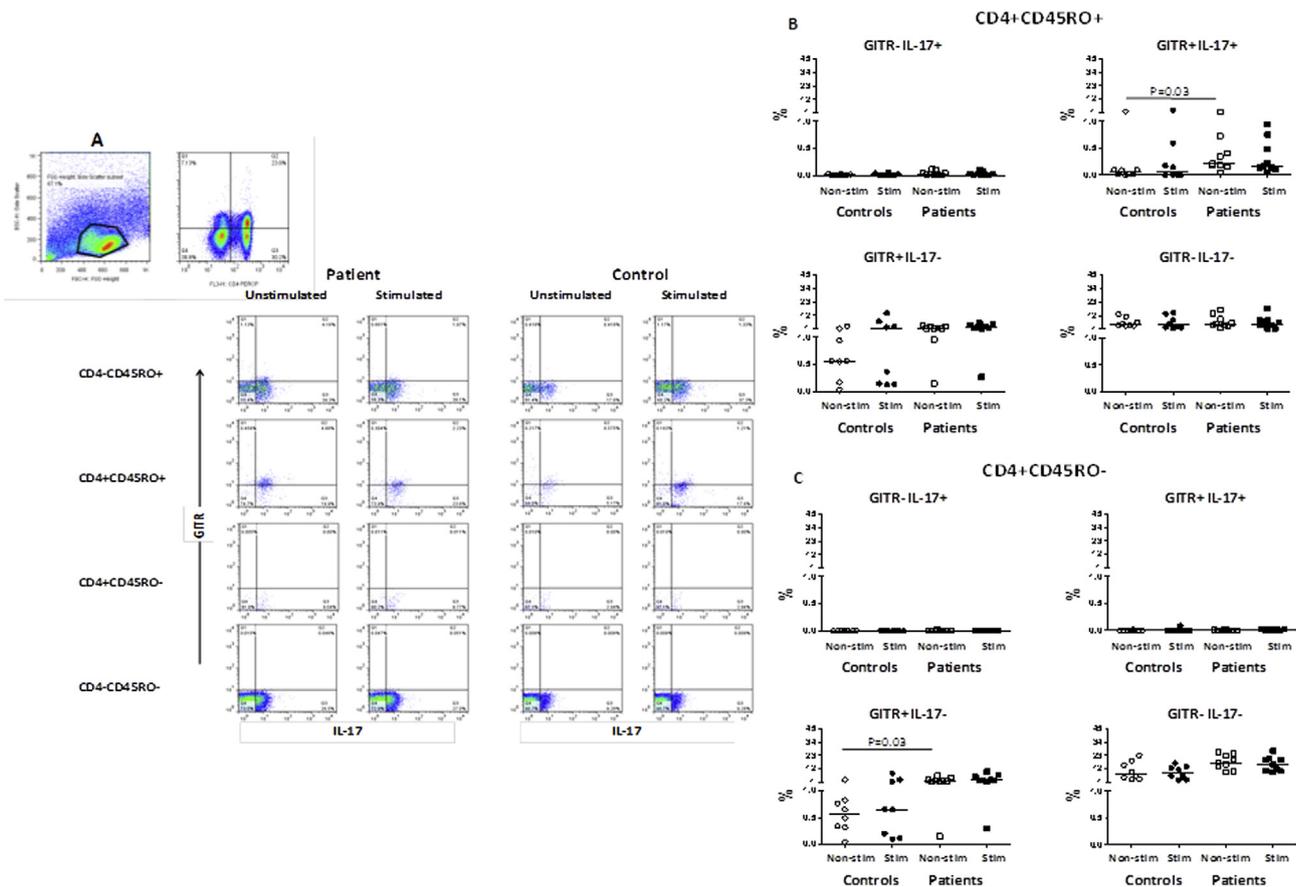


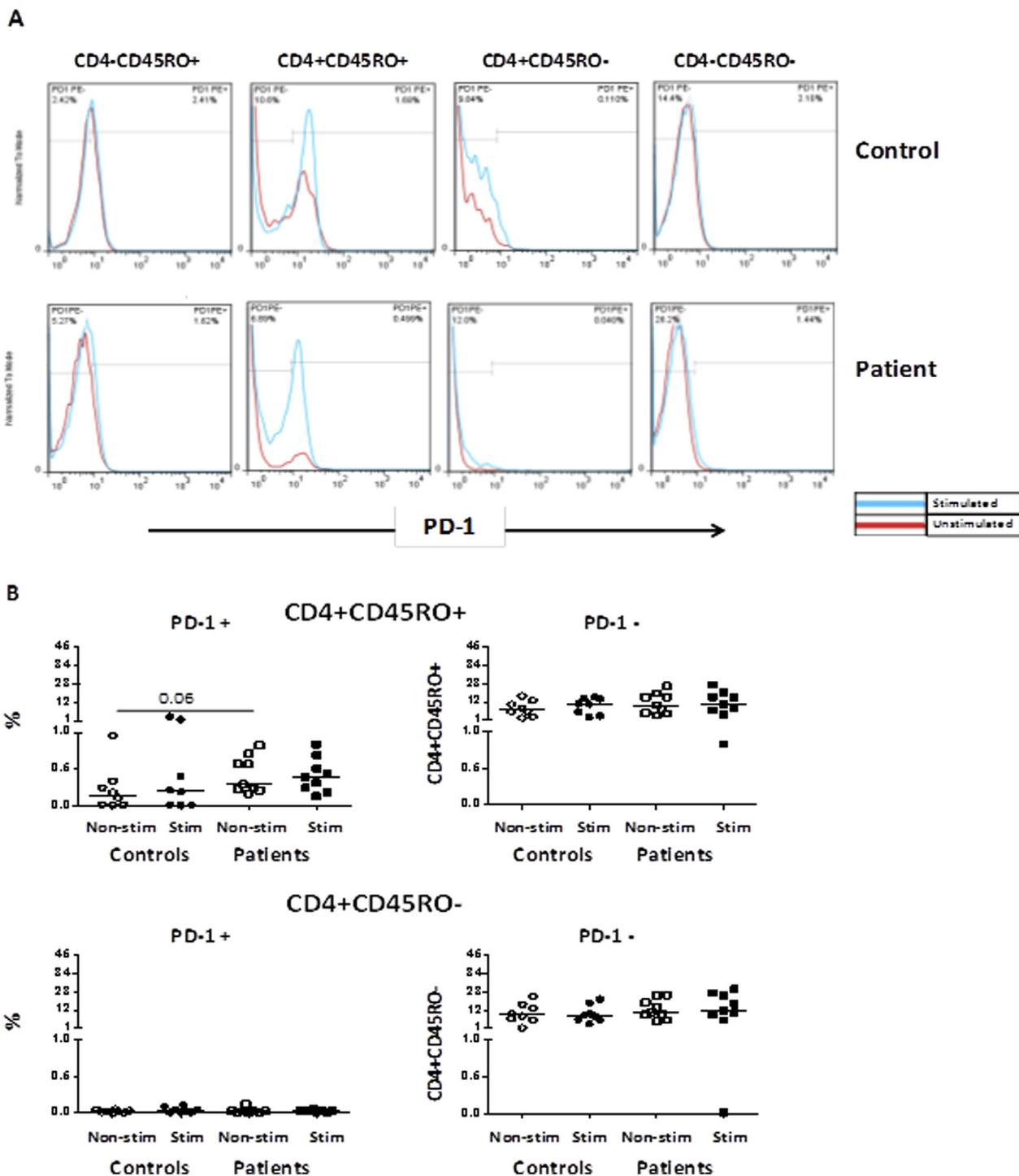
Figure 2 (A) Representative dot plots indicating GITR and IL-17 expression in CD4+CD45RO+ and CD4+CD45RO– T-cells in one patient and one control individual in non-stimulated condition and after stimulation with anti-CD3 and anti-CD28 antibodies. The gating was performed on lymphocytes followed by CD4 and CD45RO before gating for IL-17 and GITR molecules; (B) The frequencies of IL-17 and GITR expressing CD4+CD45RO+ T-cells (% total) in patients with atherosclerosis and in controls; (C) The frequencies of IL-17 and GITR expressing CD4+CD45RO– T-cells (% total) in patients with atherosclerosis and controls.

(CD4+CD45RO–) T-cells in the peripheral blood of patients with atherosclerosis compared with control subjects.

Most of previous studies have suggested that GITR is a co-stimulatory molecule which plays a major role in inflammatory conditions. Studies of lung inflammation in GITR^{-/-} mice have shown lower inflammatory response, decreased NO production and iNOS activity as well as decreased expression of adhesion molecules and infiltration of inflammatory cells in the absence of GITR molecule. In addition, GITR absence decreases lung injury and joint injury in experimental mice models.^{24,25} GITR signaling is involved in the process of inflammation and inflammatory diseases where stimulation of GITR results in the activation of matrix metalloproteinase-9 (MMP-9) and expression of TNF- α in primary macrophages.²⁶ GITR and GITRL expression are also shown to be distributed in the human atherosclerotic plaque specimens.²⁶ Thus it is not surprising that GITR expression is increased on the peripheral blood CD4+ T-cells during atherosclerotic inflammation.

In our study, analyzing the CD4+ T-cells functional subsets showed that the GITR molecule is overexpressed on IL-17+ T-cells in the CD4+CD45RO+ memory subset and IL-17– T-cells in the CD4+CD45RO– effector subset in patients. Among CD4+ T-cell subsets, mostly Th17 cells

secrete IL-17, by which they induce epithelial and myeloid cells to release chemokines and cytokines and recruit neutrophils and macrophages. In atherosclerotic inflammation, these cells can promote plaque instability and enhance pro-inflammatory and pro-atherogenic phenotype.²⁶ In addition, Th1 cells, which produce IL-12 and IFN- γ , mediate the expression of type A and B scavenger receptors that can recognize modified and oxidized-LDL (ox-LDL).²⁷ Th1 cells can induce ECs inflammation and foam cells formation in atherosclerosis.²⁸ Our findings showed that IFN- γ was produced by the PD-1– subset of CD4+CD45RO+ memory and CD4+CD45RO– effector T-cells in patients (Fig. 3b). Stimulation by anti-CD3 and anti-CD28 increased IFN- γ production in both T-cell subsets in patients and controls. These findings are in line with previous reports showing that Th1 cells employ different means to aggravate the progression of atherosclerosis and lead to the plaque instability.²⁹ A fraction of CD4+CD45RO+ memory T-cells in patients, however, expressed PD-1 and lacked the potential to produce IFN- γ after stimulation (Fig. 4b). The expression of PD-1 on memory T-cells is suggested to be associated with aging.^{30,31} It is; however, not clear if these PD1 expressing memory CD4+ T-cells have diminished TCR-mediated pro-



liferation and/or are prone to senescence as reported previously.³¹ Having said so, the scarcity of PD-1+CD4+CD45RO- effector T-cells in both groups challenges the significance of PD-1 inhibition in this subset as opposed to other diseases.^{32,33}

GITR expression plays diverse roles in different subtypes of T-cells. GITR triggering in the presence of anti-GITR antibody or soluble GITRL can inhibit the suppressor effect

of T regulatory cells.^{34,35} It is already shown that Treg cell function in atherosclerosis is diminished and depletion of peripheral Tregs increases atherosclerotic lesion size and plaque vulnerability in early stages of atherosclerosis.³⁶⁻³⁸ Therefore, an increase in the expression of GITR molecule can be an accelerating mechanism in atherogenesis and can weaken the Treg function in combating unwanted inflammation.

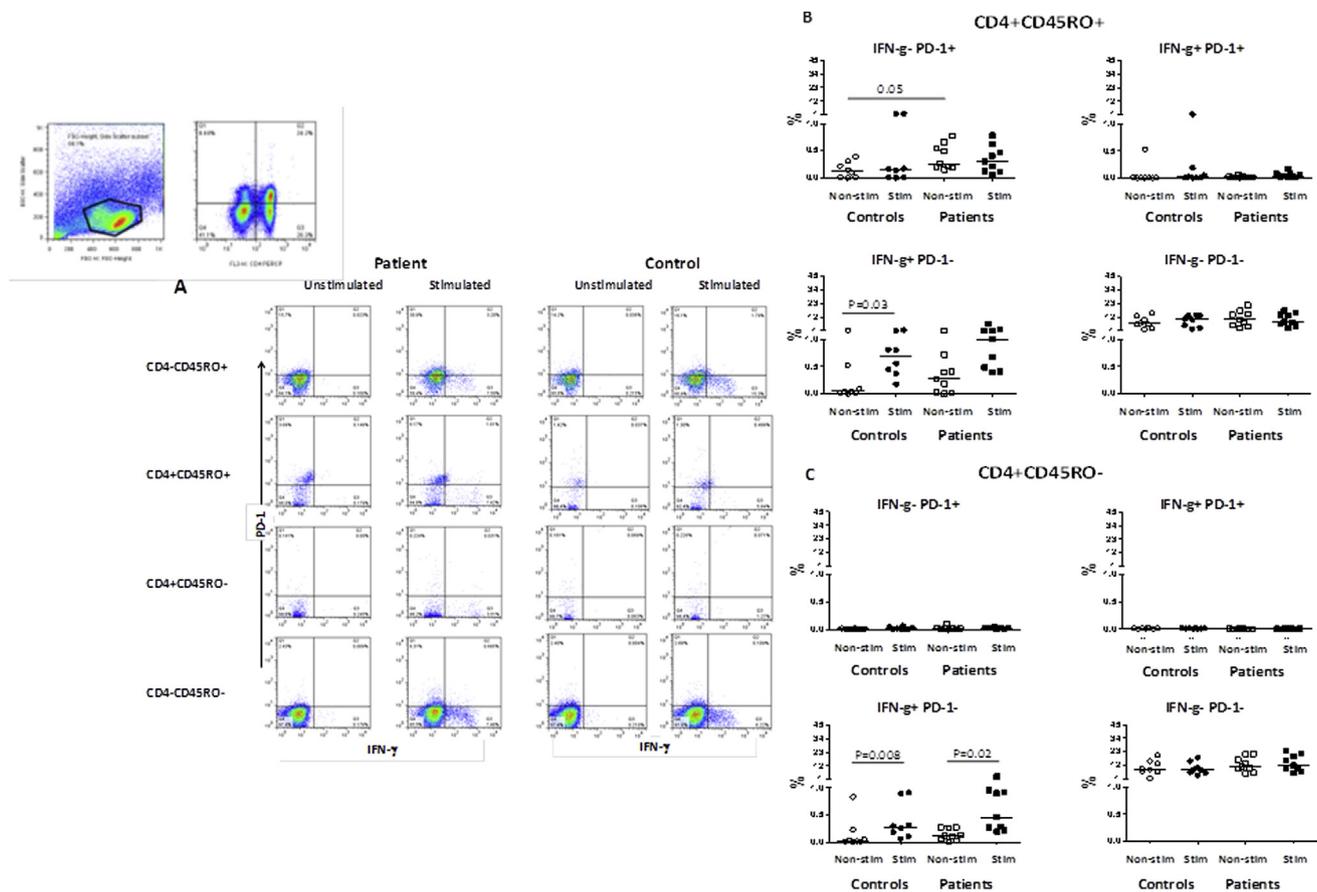


Figure 4 (A) Representative dot plots indicating IFN- γ and PD-1 expression in CD4+CD45RO+ and CD4+CD45RO- T-cells in one patient and one control individual in non-stimulated condition and after stimulation with anti-CD3 and anti-CD28 antibodies. The gating was performed on lymphocytes followed by CD4 and CD45RO before gating for IFN- γ and PD-1 molecules; (B) The frequencies of IFN- γ and PD-1 expressing CD4+CD45RO+ T-cells (% total) in patients with atherosclerosis and in controls; (C) The frequencies of IFN- γ and PD-1 expressing CD4+CD45RO- T-cells (% total) in patients with atherosclerosis and controls.

In a previous study, we demonstrated that the percentage of CD4+ T-cells producing both IL-17 and IFN- γ was increased in patients with atherosclerosis. Inflammatory environment and IL-6 can reduce the expression of FOXP3 and induce IL-17 expression in Treg cells.^{33,39} Therefore, the lower IFN- γ MFI and higher IL-17 MFI observed in patients compared to controls can be suggestive of the Th1 to Th17 shift.²² In the current study, we observed that the CD4+CD45RO+IL-17+ memory T-cells are also GITR+. While CD4+CD25+ Treg cells express high levels of GITR on their surface, it is interesting to see if the GITR+ T-cells in atherosclerosis are IL-17 producing Tregs.^{9,12} In any case, the GITR expression and IL-17 production by CD4+CD45RO+ T-cells along with the generally reduced function of Tregs, provides a favorable milieu for progressive inflammation. It is worth mentioning that our study has several limitations; one of the limitations of our study is that it only uses one methodology to evaluate the expression of GITR and PD-1. A detailed evaluation of gene expression and confirmation of protein expression of these molecules would be more appropriate. Moreover, using stringent criteria and high cost of experiments limited the number of patients and controls which are included in this study, and has decreased the power of conclusions.

Conclusion

Our study provides data on the expression of GITR molecule on IL-17 producing memory T-cells in patients with positive coronary artery disease confirmed by carotid angiography as compared to control subjects. A population among memory T-cells, which expressed PD-1 and was not producing IFN- γ , was also increased in patients' peripheral blood. These data support the involvement of IL-17 producing memory T-cells in the atherosclerosis and suggest the modified phenotype/function of T-cell subsets under chronic inflammation.

Declaration of interest

The authors declare no competing interest.

Acknowledgments

This project was financially supported by a grant (92-6593) from Shiraz University of Medical Sciences, Shiraz, Iran. The authors would like to thank all the individuals who gave their consent and participated in the study. We also

acknowledge the technical help of Mr. Hossein Golmoghaddam, Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.artres.2017.12.004>.

References

- Patel DD, Kuchroo VK. Th17 cell pathway in human immunity: lessons from genetics and therapeutic interventions. *Immunity* 2015;**43**:1040–51.
- Gong F, Liu Z, Liu J, Zhou P, Liu Y, Lu X. The paradoxical role of IL-17 in atherosclerosis. *Cell Immunol* 2015;**297**:33–9.
- Lichtman AH, Binder CJ, Tsimikas S, Witztum JL. Adaptive immunity in atherogenesis: new insights and therapeutic approaches. *J Clin Invest* 2013;**123**:27–36.
- Sinclair NS. Why so many coinhibitory receptors? *Scand J Immunol* 1999;**50**:10–3.
- Xu C, Gagnon E, Call ME, Schnell JR, Schwieters CD, Carman CV, et al. Regulation of T cell receptor activation by dynamic membrane binding of the CD3 ϵ cytoplasmic tyrosine-based motif. *Cell* 2008;**135**:702–13.
- Thaventhiran T, Sethu S, Yeang HXA, Al-Huseini L, Hamdam J, Sathish JG. T cell co-inhibitory receptors-functions and signalling mechanisms. *J Clin Cell Immunol* 2012:S12. <https://doi.org/10.4172/2155-9899.S12-004>.
- Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. *Immunol Rev* 2010;**236**:219–42.
- Watanabe N, Nakajima H. Coinhibitory molecules in autoimmune diseases. *Clin Dev Immunol* 2012;**2012**:269756.
- Soo RA. Shedding light on the molecular determinants of response to anti-PD-1 therapy. *Transl Lung Cancer Res* 2015;**4**:816.
- Riley JL. PD-1 signaling in primary T cells. *Immunol Rev* 2009;**229**:114–25.
- Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 2006;**439**:682–7.
- You S, Poulton L, Cobbold S, Liu C-P, Rosenzweig M, Ringler D, et al. Key role of the GITR/GITR ligand pathway in the development of murine autoimmune diabetes: a potential therapeutic target. *PLoS One* 2009;**4**:e7848.
- van Olffen RW, Koning N, van Gisbergen KP, Wensveen FM, Hoek RM, Boon L, et al. GITR triggering induces expansion of both effector and regulatory CD4⁺ T cells in vivo. *J Immunol* 2009;**182**:7490–500.
- Zhan Y, Gerondakis S, Coghill E, Bourges D, Xu Y, Brady JL, et al. Glucocorticoid-induced TNF receptor expression by T cells is reciprocally regulated by NF- κ B and NFAT. *J Immunol* 2008;**181**:5405–13.
- Ko K, Yamazaki S, Nakamura K, Nishioka T, Hirota K, Yamaguchi T, et al. Treatment of advanced tumors with agonistic anti-GITR mab and its effects on tumor-infiltrating foxp3⁺ CD25⁺ CD4⁺ regulatory T cells. *J Exp Med* 2005;**202**:885–91.
- Lin GH, Snell LM, Wortzman ME, Clouthier DL, Watts TH. GITR-dependent regulation of 4-1BB expression: implications for T cell memory and anti-4-1BB-induced pathology. *J Immunol* 2013;**190**:4627–39.
- Salomon B, Lenschow DJ, Rhee L, Ashourian N, Singh B, Sharpe A, et al. B7/CD28 costimulation is essential for the homeostasis of the CD4⁺ CD25⁺ immunoregulatory T cells that control autoimmune diabetes. *Immunity* 2000;**12**:431–40.
- Ermann J, Fathman CG. Costimulatory signals controlling regulatory T cells. *Proc Natl Acad Sci U S A* 2003;**100**:15292–3.
- Alunno A, Carubbi F, Bistoni O, Caterbi S, Bartoloni E, Mirabelli G, et al. T regulatory and T helper 17 cells in primary Sjögren's syndrome: facts and perspectives. *Mediat Inflamm* 2015;**2015**:243723.
- Nocentini G, Riccardi C. GITR: a modulator of immune response and inflammation. *Adv Exp Med Biol* 2009;**647**:156–73.
- Chen W-J, Hu X-F, Yan M, Zhang W-Y, Mao X-B, Shu Y-W. Human umbilical vein endothelial cells promote the inhibitory activation of CD4⁺CD25⁺Foxp3⁺ regulatory T cells via PD-L1. *Atherosclerosis* 2016;**244**:108–12.
- Behnamfar N, Zibaenezhad MJ, Golmoghaddam H, Doroudchi M. CD45RO⁺ memory T-cells produce IL-17 in patients with atherosclerosis. *Cell Mol Biol* 2015;**61**:17–23.
- Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo Jr JL, et al., National Heart, Lung, and Blood Institute, Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, National High Blood Pressure Education Program Coordinating Committee. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *J Am Med Assoc* 2003;**289**:2560–72.
- Krausz LT, Bianchini R, Ronchetti S, Fettucciari K, Nocentini G, Riccardi C. GITR-GITRL system, a novel player in shock and inflammation. *Sci World J* 2007;**7**:533–66.
- Cuzzocrea S, Nocentini G, Di Paola R, Agostini M, Mazzon E, Ronchetti S, et al. Proinflammatory role of glucocorticoid-induced TNF receptor-related gene in acute lung inflammation. *J Immunol* 2006;**177**:631–41.
- Kim WJ, Bae EM, Kang YJ, Bae HU, Hong SH, Lee JY, et al. Glucocorticoid-induced tumour necrosis factor receptor family related protein (GITR) mediates inflammatory activation of macrophages that can destabilize atherosclerotic plaques. *Immunology* 2006;**119**:421–9.
- Lusis AJ. Atherosclerosis. *Nature* 2000;**407**:233–41.
- Huo Y, Zhao L, Hyman MC, Shashkin P, Harry BL, Burcin T, et al. Critical role of macrophage 12/15-lipoxygenase for atherosclerosis in apolipoprotein E-deficient mice. *Circulation* 2004;**110**:2024–31.
- Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat Rev Immunol* 2003;**3**:133–46.
- Shimatani K, Nakashima Y, Hattori M, Hamazaki Y, Minato N. PD-1⁺ memory phenotype CD4⁺ T cells expressing C/EBP α underlie T cell immunodepression in senescence and leukemia. *Proc Natl Acad Sci U S A* 2009;**106**:15807–12.
- Norrie IC, Ohlsson E, Nielsen O, Hasemann MS, Porse BT. C/EBP α is dispensable for the ontogeny of PD-1⁺CD4⁺ memory T cells but restricts their expansion in an age-dependent manner. *PLoS One* 2014 Jan 3;**9**(1):e84728.
- Saha B, Choudhary MC, Sarin SK. Expression of inhibitory markers is increased on effector memory T cells during hepatitis C virus/HIV coinfection as compared to hepatitis C virus or HIV monoinfection. *AIDS* 2013 Sep 10;**27**(14):2191–200.
- Schnorfeil FM, Lichtenegger FS, Emmerig K, Schlueter M, Neitz JS, Draenert R, et al. T cells are functionally not impaired in AML: increased PD-1 expression is only seen at time of relapse and correlates with a shift towards the memory T cell compartment. *J Hematol Oncol* 2015;**8**:93.
- Ronchetti S, Zollo O, Bruscoli S, Agostini M, Bianchini R, Nocentini G, et al. GITR, a member of the TNF receptor superfamily, is costimulatory to mouse T lymphocyte subpopulations. *Eur J Immunol* 2004;**34**:613–22.
- Liao G, Nayak S, Regueiro JR, Berger SB, Detre C, Romero X, et al. GITR engagement preferentially enhances proliferation

- of functionally competent CD4⁺CD25⁺FoxP3⁺ regulatory T cells. *Int Immunol* 2010;**22**:259–70.
36. Chistiakov DA, Sobenin IA, Orekhov AN. Regulatory T cells in atherosclerosis and strategies to induce the endogenous atheroprotective immune response. *Immunol Lett* 2013;**151**: 10–22.
37. Spitz C, Winkels H, Bürger C, Weber C, Lutgens E, Hansson GK, et al. Regulatory T cells in atherosclerosis: critical immune regulatory function and therapeutic potential. *Cell Mol Life Sci* 2016;**73**:901–22.
38. Andersson J, Libby P, Hansson GK. Adaptive immunity and atherosclerosis. *Clin Immunol* 2010;**134**:33–46.
39. Olson NC, Sallam R, Doyle MF, Tracy RP, Huber SA. T helper cell polarization in healthy people: implications for cardiovascular disease. *J Cardiovasc Transl Res* 2013;**6**:772–86.