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

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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.

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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 ± 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 ± 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