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Upregulated TNFR Family Member Insufficient to Promote Apoptotic Cell Death in T Helper 17 Cells †

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† Presented at the 2nd International Cell Death Research Congress, Izmir, Turkey, 1–4 November 2018.

Published: 6 December 2018

Abstract: Fas is the receptor of tumor necrosis family receptors (TNFR) and involves in apoptosis. Since discovery of T helper 17 cells (Th17) in 2005, which are defined as a new type of helper T cells, it has become clear that the dysregulated function Th17 cells and their cytokines could contribute to pathology of diseases including autoimmune diseases and cancer. There is not much known about apoptotic and survival mechanisms of Th17 cells in the literature. Therefore, the players of apoptotic cell death in Th17 cells were investigated in the study. To carry out designed experiments, venous blood were drawn from the healthy volunteers with approval from the Noninvasive Ethics Committee. Peripheral blood mononuclear cells (PBMCs) were isolated from blood with Ficoll separation method. The naïve $CD4^+$ T cells were sorted from the PBMC. Sorted naive T cells were cultured under Th17 polarizing conditions. The activation, differentiation and apoptosis related molecules of cultured cells were monitored by Flow cytometry. Data showed that naive $CD4^+$ T cells were activated and differentiated into Th17 cells. Activated Th17 cells were Fas positive. Activated, Fas positive Th17 cells did not underwent significant plasma membrane changes. Furthermore, it was also observed that there was not much change in the Bcl-2 protein level. Bcl-2 protein is belongs to B-cell-lymphoma-2 (Bcl-2) family proteins and is major regulator of intrinsic apoptotic pathway as promoting cell survival. In addition to that the expression of Bclx-L, is an anti-apoptotic protein, were increased in these cells. Data indicates that Th17 cells (under Th17 polarization condition) were increased expression of anti-apoptotic Bcl-2 family members.

Keywords: T helper 17 cells (Th17); apoptosis; Fas receptor and Bcl-2

1. Introduction

Since discovery of T helper 17 cells (Th17), which are defined as a new type of helper T cells [1], it has become clear that the dysregulated function Th17 cells and their cytokines could contribute to pathology of diseases including autoimmune diseases and cancer. Based on existing data in the literature that there is not much known about apoptotic and survival mechanisms of Th17 cells in different diseases. Fas (CD95) is a type I transmembrane protein that is a member of tumor necrosis factor receptor superfamily. It contains a characteristic 80–100 amino acid-long death domain (DD) on its cytosolic tail. Fas is expressed widely on various types of cells such as dendritic cells, B cells and activated T cells. The interaction of Fas with FasL (CD95L) is essential for the initiation of apoptosis. Studies showed that the conditional deletion of FAS in B and T cells have led to defective lymphocyte homeostasis and tissue damage [2]. Dysregulated signaling of tumor necrosis family receptor (TNFR) is associated with many inflammatory disorders including Arthritis, Inflammatory Bowel Disease and Multiple Sclerosis [3]. It was also recently published that Fas is involved in non-apoptotic signaling pathways [4]. For example, anti-Fas antibodies shown to affect T cell co-

stimulation and secretion of cytokines like IL-2 and IFN-γ. To trigger receptor mediated apoptosis, the Fas receptor is activated by binding of FasL. This binding leads to caspase 8 activation and activated caspase-8 activates procaspase 3 into its active form [5]. Activated-caspase-3 activates various inactive apoptotic substrates and cells move into apoptotic cell death. In this study, expression of Fas and Bcl-2 family members were investigated during human T helper 17 cells differentiation.

2. Materials and Methods

Ethics approval for this study was obtained from the Noninvasive Ethics Committee of Dokuz Eylül University, İzmir, Turkey. The venous blood were drawn from the healthy volunteers by health professionals at the Dokuz Eylül University Blood Bank. Peripheral Blood Mononuclear Cells (PBMCs) were isolated by Ficoll separation method. Then, cells were dyed with Trypan Blue and counted under a microscope with a hemocytometer. After the isolation of PBMCs, magnetic bead antibody cocktail was added on the PBMC and incubated for 5 min in room temperature. After the incubation, the naïve $CD4^+$ T cells tagged with magnetic antibodies were sorted with Vario MACS. The sorting efficiency of naïve $CD4^+$ T cells was verified with Flow Cytometry. The sorted naïve $CD4^+$ T cells were cultured under Th17 polarizing conditions at 3–7 days. The activation and differentiation of cultured cells were monitored by Flow Cytometry with staining of CD25, CD69, IL-17 and CCR6) markers. To detect intracellular cytokines, cells were incubated with BFA for 4 h. Fas, 7AAD, Annexin V and Bcl-2 molecules were also measured by using flow cytometry. Flow cytometric data analysis was carried out with Guava software EasySoft™. Three biological replicates of both stimulated and negative culture were used in MS-Excell and Student's *t*-Test.

3. Results

Data showed that naive $CD4^+$ T cells were differentiated into Th17 cells. Activated Th17 cells were Fas positive. Activated Fas positive Th17 cells did not underwent significant plasma membrane changes. Furthermore, it was also observed that there was not much change in the Bcl-2 protein level. Bcl-2 protein is belongs to B-cell-lymphoma-2 (Bcl-2) family proteins and is major regulator of intrinsic apoptotic pathway as promoting cell survival. In addition to that the expression of Bclx-L, is an anti-apoptotic protein, were increased in these cells.

4. Discussion and Conclusions

Previously published data showed that IFN-γ producing Th1 cells are more sensitive to activation induced cell death compared to IL-17 producing Th17 cells and IFN-γ and IL-17 producing Th1/Th17 cells [6]. In recent studies, the soluble form of FasL, which cleaved from the membrane with metalloproteases, was reported to interact with Fas but it failed to trigger apoptosis. It was also reported that the HIF-1α also plays role in apoptosis resistance and its interactions with the Notch pathway and Bcl-2 family protein might be an important mechanism in apoptosis resistance in Th17 cells [7]. Our data indicates that Th17 cells are resistant to apoptosis under Th17 polarization conditions. One of the reasons for this outcome might be the increased expression of Bcl-xL which was observed in Th17 cells. There are also other Bcl-2 family members that need to be investigated in Th17 cells to understand the regulation of apoptosis network in Th17 cells.

Acknowledgments: This work was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK) (Grant number: 215Z127 to Ayten Nalbant). The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

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