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Does Cyclosporine Down Regulate IL-17 in Cardiac Allograft Vasculopathy?*

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ABSTRACT

Background: Cardiac allograft vasculopathy (CAV) is characterized by vascular inflammation and intimal proliferation which results in luminal stenosis and myocardial infarction. During vascular inflammation elaboration of several cytokines and differential expression of growth factors have been noted. CAV remains the major threat to long-term graft survival. CD4 and CD8 T-cell subsets play a significant role in the development of transplant rejection. Chronic transplant rejection often leads to development of CAV. A new CD4 effector cell subset that produces IL-17 (Th17) has been shown to be up-regulated in the murine system in the setting of CAV. This study assesses the level of IL-17 in cardiac transplant patients with and without CAV as compared to nontransplanted controls. **Methods:** Levels of IL-17, IL-6, MCP-1 were measured by ELISA in plasma of four nontransplanted controls, nine cardiac allograft recipients with CAV (HT-GVD) and eight post transplant subjects without a diagnosis of CAV (HT-No GVD). All post transplant patients were immune suppressed with cyclosporine. HT-GVD patients were 1 - 15 years post transplant while HT-No GVD subjects were 1 - 10 years post transplant. **Results:** IL-17, MCP-1 and IL-6 were significantly down regulated in HT-GVD subjects compared to the HT-No GVD subjects ($p < 0.001$) but not significant between controls and HT-No GVD (p = ns). **Conclusions:** A decrease in IL-17 in HT-GVD subjects as compared to HT-No GVD in the presence of cyclosporine treatment could be a consequence of down regulation of IL-6. It is likely that cyclosporine differentially regulates pro inflammatory molecules in the setting of graft vascular disease.

Keywords: Allograft; CD4 and CD8 T-Cells; Cardiac Allograft Vasculopathy; IL-17; Luminal Stenosis

1. Introduction

Cardiac allograft vasculopathy (CAV) is a complex disease which stems from a range of immunological and non-immunological insults to the allograft. CAV is characterized by vascular inflammation and intimal proliferation which ultimately results in luminal stenosis of epicardial branches, occlusion of smaller vessels, and myocardial infarction [1-7]. During vascular inflammation elaboration of several cytokines and differential expression of growth factors have been noted. CAV still remains the major threat to long term graft survival. CD4 and CD8 T-cell subsets play an important role in the development of transplant rejection [6]. A new CD4 effector cell subset that produces IL-17 (Th17) has emerged in importance. Th17 cells promote autoimmunity in mice and have been implicated in the pathogenesis of human inflammatory diseases. IL-17 is a pro-inflammatory cytokine that activates T-cells and ultimately results in the production of a variety of other cytokines, chemokines, and cell adhesion molecules. IL-17 has been shown to be up regulated in the murine system in the setting of CAV [7] hence, this study was undertaken to assess the level of IL-17 in CAV as compared to human cardiac allograft recipients without CAV as well as nontransplanted subjects.

2. Materials and Methods

Patient samples: Blood was obtained as per IRB approved protocols at Scott & White Hospital, Temple, TX (four nontransplanted controls) and University of Bologna, Italy (nine transplant patients with CAV (HT-GVD) and eight post-transplant subjects without diagnosis of CAV (HT-No GVD). All post transplant patients were immunesuppressed with cyclosporine. The levels of IL-17, IL-6, MCP-1 were measured by ELISA in plasma of

*This study is limited by the small sample size and should be validated in larger number of subjects.

four nontransplanted controls, seven CAV (HT-GVD), and seven CAV (HT-No GVD) patients. The CAV positive patients ranged from ages 56 - 61 years and CAV negative subjects ranged from 26 - 61 years (**Table 1**).

Cytokine ELISA: The level of human IL-17 in serum samples were measured using a Single Analyte ELISArray kit (SABiosciences, Frederick, MD) following the manufacturer's instructions. The Single-Analyte ELISArray Kits are designed to quantitatively measure the amount of an individual protein analyte using a standard sandwich enzyme-linked immunosorbent assay (ELISA) technique. A target-specific capture antibody has been coated onto eight well strips placed into a 96-well plate format. Serum samples were added to the wells and incubated at room temperature for three hours, after which the biotinylated secondary antibody was added and incubated for one hour at room temperature. After washing with wash buffer, complementary HRP-conjugated Ab was added and incubated for 30 minutes at room temperature. The plates were washed and developed with the substrate and the reaction was stopped with stop buffer and the OD was read at 450 nm on a microplate reader (Spectramax 250 microplate reader Molecular Devices, Sunnyvale, CA). The ELISA sensitivity for all the three cytokines assayed IL 6, IL 17 and MCP1 was 25 pg/mL. All analyses were carried out as per manufacture's protocol.

Data interpretation and statistical analyses: The changes observed in the cytokine levels were expressed as pg/mL. All experiments were performed in triplicate for each determination. Data are expressed as means ± standard error and analyzed using the Kruskal-Wallis one-way analysis of variance for significance with post tests using Prism 5.0 GraphPad software (GraphPad, San Diego, CA, USA). A p value less than 0.05 was considered statistically significant.

3. Results

IL-17 and MCP-1 (**Figures 1** and **2**) are significantly down regulated in HT-GVD subjects compared to the control subjects ($p < 0.001$). IL-6 did not show any statistically significant changes between controls and HT-GVD (p = ns) (**Figure 3**). IL-17 and MCP-1 did not show any changes that were statistically significant between controls and HT-No GVD (p = ns). IL-17, MCP-1 and IL-6 were significantly down regulated in HT-GVD subjects compared to the HT-No GVD subjects ($p < 0.001$) but not significant between controls and HT-No GVD (p = ns). HT-GVD patients were 1 - 15 years post transplant while HT-No GVD subjects were 1 - 10 years post transplant.

Table 1. Baseline characteristics of cardiac transplant patients.

Gender	Age	Years after transplant	Cardiac allograft vasculopathy	Reason for heart transplant
M	61	1	Positive	Ischemic Cardiomyopathy
F	56	2	Positive	Non ischemic Cardiomyopathy
M	71	10	Positive	Non ischemic Cardiomyopathy
M	63	1	Positive	Ischemic Cardiomyopathy
M	61	10	Positive	Non ischemic Cardiomyopathy
F	57	1	Positive	Non ischemic Cardiomyopathy
M	61	15	Positive	Non ischemic Cardiomyopathy
M	61	15	Positive	Non ischemic Cardiomyopathy
M	61	15	Positive	Non ischemic Cardiomyopathy
F	33	1	Negative	Non ischemic Cardiomyopathy
M	34	10	Negative	Non ischemic Cardiomyopathy
M	33	5	Negative	Non ischemic Cardiomyopathy
M	50	5	Negative	Non ischemic Cardiomyopathy
M	32	1	Negative	Non ischemic Cardiomyopathy
M	61	5	Negative	Ischemic Cardiomyopathy
F	54	1	Negative	Non ischemic Cardiomyopathy
M	26	1	Negative	Non ischemic Cardiomyopathy

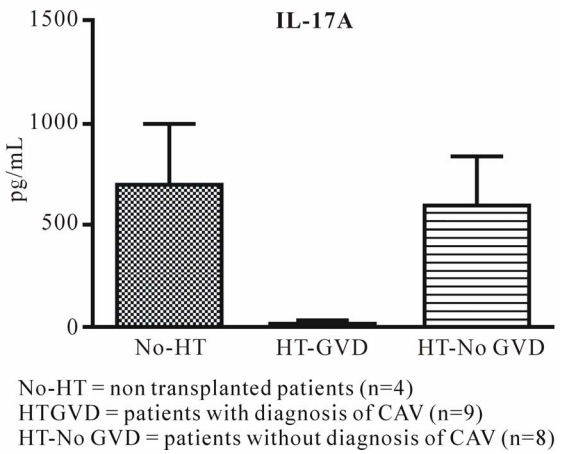

Figure 1. IL-17A expression in CAV versus control non-transplanted patients.

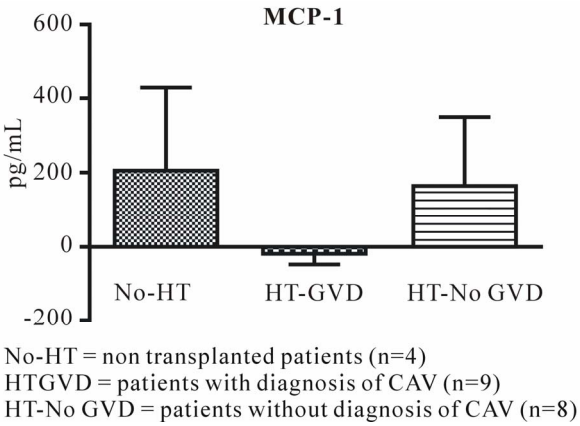

Figure 2. MCP-1 expression in CAV versus control non transplanted patients.

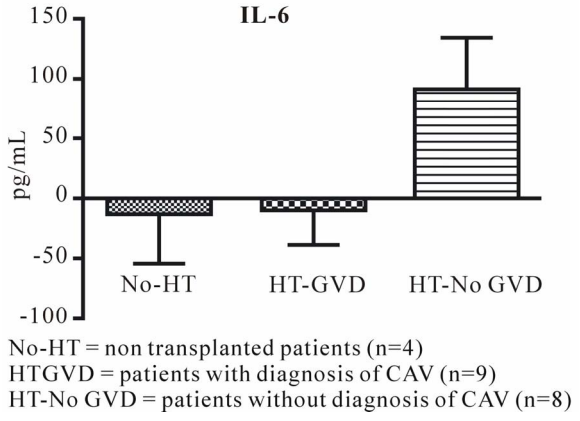

Figure 3. IL-6 expression in CAV versus control non transplanted patients.

4. Discussion

IL-17 plays a critical role in the pathogenesis of autoimmune and chronic inflammatory disorders and stimulates the induction of various pro inflammatory cytokines and chemokines. In a murine model of chronic allograft vasculopathy, IL-17 mediates an aggressive proinflammatory response culminating in severe accelerated allograft rejection and vasculopathy [7]. A variety of inflammatory and autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, Crohn's disease, psoriasis, Behcet's disease and uveitis, show IL-17 overexpression and production [8-15]. Cyclosporine is known to inhibit production of several inflammatory cytokines, such as IL-17, IL-12, IL-18, and tumor necrosis factor-α [16,17]. Several reports have shown that CsA could inhibit IL-17 production in certain autoimmune diseases, such as Vogt-Koyanagi-Harada (VKH) syndrome [18-20]. It is possible that CsA exerts its function via inhibiting IL-17 and interferon gamma production in Behcet's disease [20]. Other possible pathways include the up regulation of CD69 T-cell-surface activation marker via IL-15 and a calcium ionophore which are also noted in Behcet's disease. In rheumatoid arthritis increased IL17 levels were significantly inhibited at the transcription and translational levels by cyclosporine. This effect was explained by the fact that cyclosporine inhibited the IL-17 production by Th17 cells at the protein and at the mRNA levels. Besides, cyclosporine also markedly reduced the expression of CD69 and CD 25. Therefore cyclosporine may exert its effect through suppression of IL17 production and inhibition of TH17 cell differentiation [21].

The statistically significant down regulation of these proinflammatory cytokines in chronic CAV (HT-GVD) is interesting in the setting of calcineurin inhibition by cyclosporine. The down regulation of IL-17 in patients with CAV (HT-GVD) may reflect the Treg/Th17 balance as imbalance of Th17/Treg has been considered critical in the development of inflammation. IL-6 has a very important role in regulating the balance between IL-17 producing Th17 cells and regulatory T-cells (Treg). Th17 is involved in the pathogenesis of autoimmune responses, while Treg functions to restrain excessive effector T-cell responses. IL-6 induces the development of Th17 cells from naïve T-cells together with TGF-beta and inhibits TGF-beta-induced Treg differentiation. Thus a decrease in IL-17 in HT- GVD subjects as compared to HT- no GVD in the presence of cyclosporine treatment could be a consequence of down regulation of IL-6. It is likely that cyclosporine differentially regulates proinflammatory molecules in the setting of graft vascular disease (GVD). The decrease in MCP-1 as well as IL-6 and IL-17 differentially in the GVD patients is also suggestive of interaction of downstream effects resulting from down regulation of the individual cytokines.

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