Artery Research (2018) 21, 43–52

Available online at www.sciencedirect.com

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journal homepage: www.elsevier.com/locate/artres

Elevated levels of IL-6 and IL-9 in the sera of patients with AAA do not correspond to their production by peripheral blood mononuclear cells

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Received 2 October 2017; received in revised form 24 December 2017; accepted 30 December 2017
Available online 11 January 2018

KEYWORDS
Abdominal Aortic Aneurysm (AAA);
IL-9;
IL-10;
PBMC;
Serum

Abstract *Background:* Abdominal Aortic Aneurysm (AAA) is the stable local dilatation of abdominal aorta. AAA is an inflammatory condition in which cytokines may play a pathogenic role.
Methods: Peripheral Blood Mononuclear cells (PBMCs) were isolated from 5 men, with confirmed diagnosis of AAA and aortic dilation greater than 5.5 cm, and 5 men with normal/insignificant angiography, CT-Scan and Ultrasonography results. The supernatant of PBMCs, rested overnight in RPMI containing 10%-FBS, removed to measure IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IL-17F, IL-21, IL-22, IFN-γ and TNF-α using a commercial fluorescent-labeled bead assay.
Results: The mean serum IL-6 and IL-9 levels were significantly higher in patients than controls ($P = 0.007$ and $P = 0.007$, respectively). PBMCs from patients produced lower levels of IL-6 and IL-9 compared to controls but the differences were not significant. While serum TNF-α level was not different between groups, its production by PBMCs of patients was significantly lower than controls ($P = 0.047$). The mean serum levels of IL-10 and IFN-γ in patients were marginally higher than controls ($P = 0.055$, $P = 0.055$, respectively). Mean serum IL-2 level was not different between the groups but its production by PBMCs of patients was significantly higher than the control group ($P = 0.047$).

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https://doi.org/10.1016/j.artres.2017.12.007

Conclusions: Our study showed alteration in the levels of cytokines from inflammatory, Th1, Th2 and Th17 subtypes in the sera of patients with AAA. The production of IL-6, IL-9, IFN-γ and IL-10, however, was not solely attributed to the PBMCs. Therefore, participation of other cells in the tissue or blood should be considered in their production.

Introduction

Abdominal Aortic Aneurysm (AAA) is one of the most common forms of deadly atherosclerotic events.[1] AAA is defined as the stable local dilatation of abdominal aorta with a diameter of more than 3 cm or an increase in diameter of more than 50% relative to normal.[2] Smoking, family history, higher age and male gender are other known factors to correlate with the disease incidence.[3,4] AAA is often asymptomatic but rupture of aneurysm is a major risk of death with a mortality rate of 85–90%.[5,6] Although the prevalence of abdominal aortic aneurysms has been rising until mid 1990s, it seems that in recent years its prevalence has been decreasing. In a recent study in Sweden, ultrasonographic screening for 65-year old men showed a decrease of prevalence from 4–8% to 2.2% over the five years.[7] Abdominal aortic aneurysms cause 13,000 deaths annually in the United States.[8] Shirani et al. reported that 2.9% of CABG candidates in Iran appear to have abdominal aortic aneurysms.[9]

Smoking is a major risk factor for AAA where nicotine increases the expression of ICAM-1 and VCAM-1 and induces IL-1β and TNF-α production by macrophages in the aortic wall.[10] Also, cigarette smoke components increase the expression of MMP-2 and MMP-9.[11] AAA is an inflammatory condition in which inflammatory cells, including T lymphocytes, penetrate into various vascular layers and secrete cytokines and inflammatory chemokines. Infiltration of the vascular wall with lymphocytes and macrophages is followed by destruction of elastin and collagen in the media and adventitia layers by proteases such as matrix metalloproteinases, and smooth muscle cell loss, which decreases the thickness of media associated with new angiogenesis.[12] Currently, AAA is known as an sterile inflammatory disease, in which inflammatory response is induced by an internal stimuli such as damage associated molecular patterns (DAMP) or risk signals (such as S100A8/9 and Hmgb1), recognized by receptors on innate immune and other cells.[13] Atherosclerosis and high blood pressure are also associated with AAA.[14]

Excessive accumulation of LDL and cholesterol crystals, and secretion of post-cell death stress proteins such as S100A8/9 and Hmgb1 trigger inflammasome activation and cytokines production by aortic wall macrophages.[15,16] The accumulation of inflammatory cells, including CD4+ T cells, B cells and macrophages has been observed in the aortic lesions of AAA.[17] The secretion of cytokines can lead to the production of matrix metalloproteinases and cathepsin that through destruction of the aortic wall, inflammation, and loss of smooth muscle cells lead to aneurysm and rupture.[18]

Despite the close association between aneurysm and atherosclerosis, AAA is now known as a degenerative process that involves all layers of the vascular wall, especially media and adventitia with abundant inflammatory cells in lesions. Another important difference between these two diseases is the predominant Th1 cytokine response in atherosclerosis and Th2 cytokine response in AAA.[19] In both diseases cytokines produced by T helper cells determine the outcome of arterial inflammation. Increased levels of IL-1, IL-6, TNF-α and IFN-γ and their role in the pathogenesis of AAA have been shown.[20] On the other hand, human studies have shown that Th2 (IL-4, IL-5) cytokines and IL-10 are predominant in AAA lesions, while in the atherosclerotic lesions Th1 (IL-2, IFN-γ) cytokines are abundant.[21] Interestingly, both Th1 and Th2 cytokine genes and transcription factors are expressed in AAA[22] and both Th1 and Th2 cytokines can induce or stop expression of specific MMPs according to different conditions.[23] It is suggested that Th1 cytokines may participate in the formation of atherosclerotic lesion in early stages while Th2 cytokines participate in the further development of aneurysm.[24,25]

In addition to Th1 and Th2, other immune inflammatory cytokines are reported to play a role in either of the diseases. Previous studies have reported elevated plasma levels of IL-9 in patients with acute coronary syndrome and atherosclerosis.[26,27] In AAA, IL-17A seems to have a pathogenic role, since AAA progression significantly decreases in Il-17A−/− and- Il-23−/− rats.[28] The IL-10 immunosuppression, however, works against the progression of AAA suggested by more sensitivity of IL-10−/− mice to AngII induced AAA.[29] Interestingly, IL-10 shows a significant increase in aneurysm-affected tissue.[30]

Method and material

Patients with abdominal aortic aneurysm were diagnosed by the collaborator vascular surgeon, based on clinical and paraclinical indices. Five AAA patients were included in this study; all of them were male (100%), with an average age of 70.40 ± 6.76 years, who were listed for surgery due to their acute state. The control group was selected from among the patients referred for angiography and surveying cardiac disorders, which in addition to the normal results of echocardiography, sonography and CT scan, had normal/insignificant angiography results and no signs of AAA were observed. Control group consisted of 5 men with an average age of 71.80 ± 4.65 years. 30 ml blood was collected from both groups after informed consent. Plasma samples were isolated and stored at −80 °C until next used. Lymphodex™ (Inno-Train, Germany) concentration