ELSEVIER

Archives of Medical Research 43 (2012) 406–410

Archives of Medical Research

PRELIMINARY REPORT

Extracellular Matrix Remodeling in Takayasu's Arteritis: Role of Matrix Metalloproteinases and Adventitial Inflammation

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Received for publication May 1, 2012; accepted July 16, 2012 (ARCMED-D-12-00235).

Takayasu's arteritis (TA) is an inflammatory fibrosing arteritis affecting predominately the aorta and its main branches. Pathogenesis of this disease remains enigmatic. Despite the numerous studies, the role of adventitia in vascular lesion formation in the setting of TA has been ignored. Virtually nothing is known about the mechanism regulating inflammation in the adventitia in the setting of TA. The present study included subjects with Takayasu's arteritis and normal healthy control subjects. Isolated T cells from peripheral blood mononuclear cells (PBMCs) using nylon wool and HUT-78 (human cutaneous T lymphoma cell line) were stimulated with PHA for 24 h. Stimulated cell were fixed with paraformaldehyde and fractionated into membrane, cytosolic and nuclear fractions. These cellular fractions were co-cultured with human fibrosarcoma cell line (HT-1080) and transcriptional expression of matrix metalloproteinases (MMP-1, 3, 9 and TIMP-1) was determined using semiquantitative RT-PCR. Stimulation of MMPs-TIMP synthesis by HT-1080 cells was mimicked by a membranous fraction derived from activated T-cell isolated from TA subjects and activated HUT-78 cells, whereas cytosolic and nuclear fractions were ineffective. In conclusion, for the first time we provide evidence for the presence of a cell surface-specific antigenic moiety on T-cells of TA subjects, which is responsible for activation of fibroblasts (cells predominantly present in adventitia) to enhance MMP production and, therefore, may lead to extracellular matrix degradation.

Key Words: Takayasu's arteritis, Matrix metalloproteinase, Adventitia, T-cell, Fibroblasts.

Introduction

Takayasu's arteritis (TA) is an inflammatory fibrosing arteritis that affects predominantly the aorta and its main branches. The disease is prevalent in young women and is a significant problem in South Asian countries including India and Japan. The pathogenesis of this morbid disease is still unclear. Histopathological studies of the acute lesion revealed massive cell infiltration around the vasa vasorum and destruction of media and adventitia, along with intimal hyperplasia that leads to the formation of stenotic lesions (1,2). Adventitia is composed primarily of fibroblasts, collagen, and elastin fibers oriented longitudinally. There is considerable evidence in the literature that suggests that the adventitia is activated during the development of atherosclerosis (3,4). When destructive changes of the media and adventitia predominate, it occasionally leads to dilated lesions such as rupturing aneurysms (5).

Despite the evidence, the role of adventitia in vascular lesion formation and in the pathogenesis of Takayasu's arteritis has been controversial. Adventitial inflammation is the predominant feature in many forms of arteritis where inflammatory cells cluster in the adventitial space surrounding the afflicted artery (3–5). Matrix remodeling is a complex process and is controlled by an intricate network of cells

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0188-4409/$ - see front matter.
http://dx.doi.org/10.1016/j.arcmed.2012.07.007

and matrix interactions. The imbalance between matrix degrading enzymes, i.e., matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs), has been shown to actively participate in pathological events that lead to tissue destruction and chronic inflammation (6). In a very important study, Lacraz et al. reported that direct contact between lymphocytes and monocytes is a major pathway for induction of MMP expression (7). T-cells along with monocytes may act as major source of inflammatory mediators and can communicate with each other as well as with surrounding resident tissue cells like fibroblasts either by secretion of soluble peptides or by direct cell−cell contact (8−10).

Inflammatory cells in the adventitia are the likely source of cytokines, active chemokines, cell adhesion molecules and MMPs, which may aggravate the disease process (5). Thus, inflammatory cells may invade the media and intima through adventitia due to augmented MMP expression. Therefore, in the present study we hypothesized that direct contact of activated T-lymphocytes with fibroblasts may represent a major biological strategy for modulating the extracellular matrix (ECM) turnover.

Materials and Methods

In the present work, human cutaneous T-lymphoma cell line (HUT78) and human fibrosarcoma cell line (HT-1080) were used as cellular models. Cells were maintained in DMEM with 100 μg/mL streptomycin, 100 units/mL penicillin, 2 mM L-glutamine and heat-inactivated fetal calf serum (20% for HUT-78 and 10% for HT-1080) in a 5% CO_2 air humified atmosphere at 37°C.

Forty patients with Takayasu's arteritis were enrolled at a special clinic at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India as previously described (11−13). Anthropometric characteristics of all the study subjects are discussed elsewhere (12,13). Patients who had already undergone vascular surgeries/angioplasties for their disease and patients with hypertensive crisis were excluded from the study. At the time of enrollment of the study subjects, onset of characteristic signs and symptoms was 1 to 1½ months duration. Forty normal, healthy, unrelated subjects of both genders belonging to the same ethnic origin and socioeconomic background were included in the present study as controls. The following exclusion criteria were used: absence of any clinical history of cardiovascular disease, collagen disease, autoimmune disease and any current inflammatory disorder. Male:female ratio in the TA patient group was 8:32 as compared to 10:30 in the control subjects. The age range of TA patients was 15−53 years, whereas in controls it was 15−49 years. Body mass index (BMI) was comparable in both groups ($p > 0.05$).

Concerning clinical parameters, the most common mode of presentation was hypertension. Other common features included asymmetry of pulses, vascular bruits and breathlessness. We observed a higher blood pressure, both systolic and diastolic, in TA patients as compared to their control counterparts ($p < 0.05$). At the time of enrollment in the present study, 32 (80%) subjects were in an active phase and eight subjects (20%) were in remission. Systemic symptoms in the form of fever or arthralgia or myalgia were found in 76.3% of patients with active disease. Carotidynia, another commonly used clinical marker for disease activity, did not show a significant association with disease activity just like the presence of any claudication symptoms ($p > 0.05$). We observed no statistically significant difference between patients and control subjects as far as other biochemical parameters, i.e., lipid and lipoprotein profile, blood urea, serum creatinine and uric acid levels are concerned ($p > 0.05$; data not shown). Informed consent was obtained from all subjects prior to their participation in the study. The Medical Ethics Committee of the Institute approved the study.

Venous blood was collected from the overnight fasted individuals in the morning from an antecubital vein into vacutainers with EDTA. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll gradient method as described earlier (14). A fraction of PBMCs was removed for determination of transcriptional expression of genes (MMP-1, 3, 9 and TIMP-1) and the remaining cell suspension was incubated in plastic dishes at 37°C for 1 h. After incubation, nonadherent cells were recovered and T-cells were enriched by a nylon wool column (15). Reactivity with monoclonal anti-CD3 antibody confirmed the T-cell-rich population (data not shown).

Human T cell lines (HUT 78)/T lymphocytes isolated from study subjects were cultured at a concentration of 1×10^6 cells/mL in medium alone or were stimulated with PHA (1 μg/mL) for 24 h at 37°C. After incubation, cells were fixed in 1% paraformaldehyde (7). Different cellular fractions (cytosolic, nuclear and membranous) were prepared from the T-lymphocytes of controls, patients and T-cell lines (HUT-78) as described previously (7,16). Each cellular fraction was incubated with HT-1080 fibroblasts at a ratio of 1:8 for 24 h followed by gene expression studies. Semiquantitative RT-PCR was performed to determine the mRNA expression of MMP-1, 3, 9 and TIMP-1 in HT-1080 fibrosarcoma cells as described previously (17).

Results and Discussion

Figure 1A represents the transcriptional expression of MMPs and TIMP-1 in study subjects. We observed significantly higher mRNA expression of all the MMPs and TIMP-1 in PBMCs of TA subjects as compared to controls ($p < 0.05$). Similar to the observations as above, RT-PCR analysis showed that MMPs 1, 3 and 9 were significantly higher in subjects with active disease as compared to those

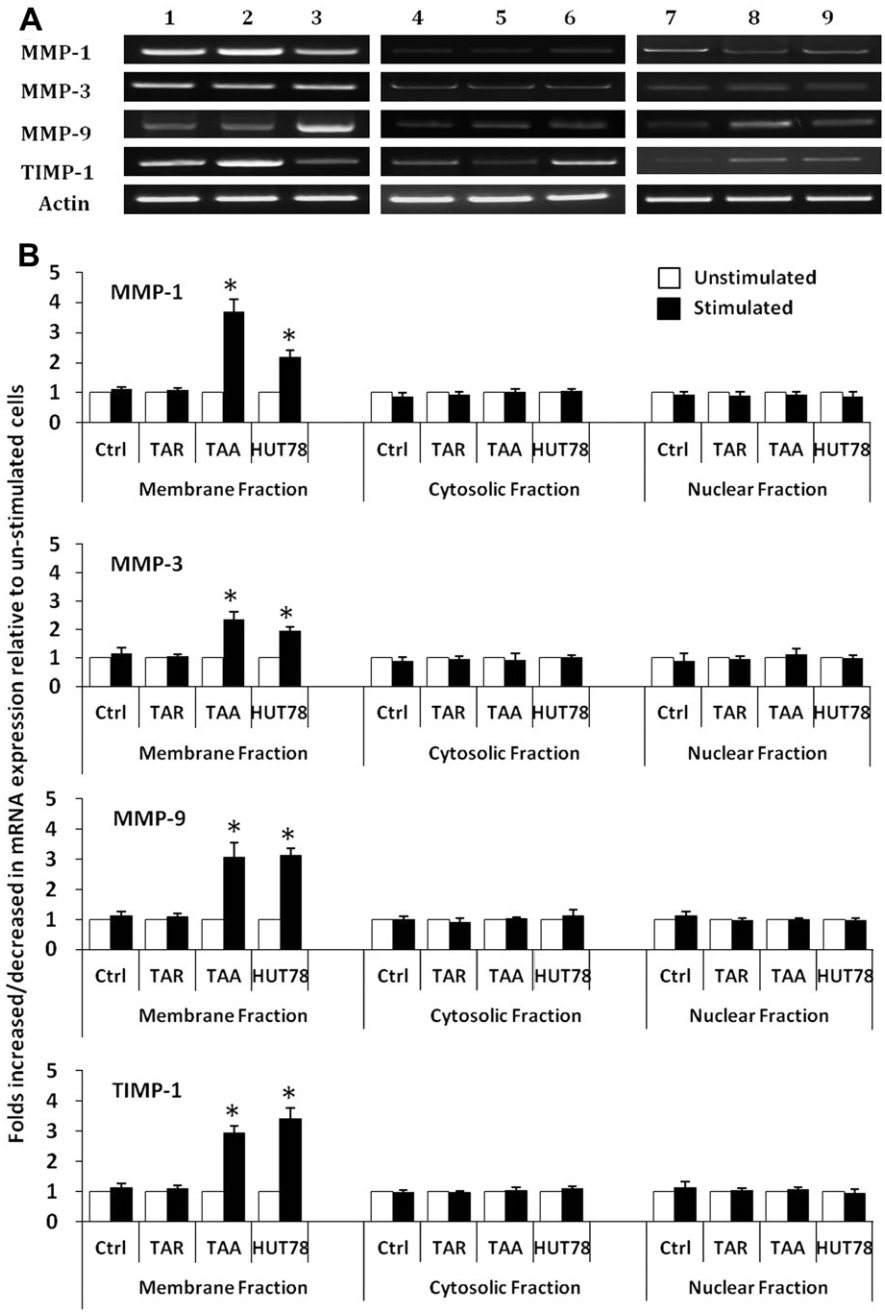

Figure 1. (A) Representative gels demonstrating mRNA expression of MMPs and TIMP-1. Lanes 1–3: TA patients with active disease; Lanes 4–6: TA patients in remission; Lanes 7–9: normal healthy controls. (B) Depicts the transcriptional expression of MMPs (MMP-1, 3 and 9) and TIMP-1 by human fibrosarcoma cell line HT-1080 when co-cultured with various cellular subfractions obtained from control subjects (Ctrl), TA subjects in remission (TAR), TA subject with active disease (TAA) and human T-cell line (HUT78). *$p < 0.05$. Unstimulated vs. stimulated.

study subjects who were in remission ($p < 0.05$), whereas TIMP-1 levels were not significantly altered ($p > 0.05$). The enhanced gene expression of MMPs in total TA subjects as well as in TA subjects with active disease as compared to normal healthy controls and patients in remission, points to the fact that extracellular matrix remodeling process is activated in active TA disease, thus making these subjects highly vulnerable to atherosclerosis and development of its related manifestations and aneurysms. Serum levels of MMPs, TIMP-1 and gelatinolytic activity of MMPs (MMP-2 and MMP-9) also demonstrated a similar trend in these subjects as previously demonstrated by our research group (12,13).

We compared the effect of three cellular subfractions, i.e., cytosolic, nuclear and membranous, isolated from T-cells on the expression of MMPs by HT-1080 cells. We

used HT-1080 cells, which have been documented as a good cellular model for fibrous tissue to decipher the role of various MMPs and TIMPs in a number of pathological conditions. We observed that it was only the membranous fraction isolated from stimulated T-cells (either derived from subjects with active TA or HUT-78 cells) that significantly enhanced mRNA expression of all three MMPs and TIMP-1, whereas no change was observed when HT-1080 fibroblasts were co-cultured with either nuclear or cytosolic subfractions. Moreover, when HT-1080 cells were incubated with different cellular fractions of stimulated T-cells isolated from control subjects or TA subjects in remission, there was no significant change in mRNA expression of genes under consideration (Figure 1B).

Our data conform to the earlier observations of Lacraz et al. (7) that indeed direct cell-to-cell contact profoundly stimulates MMP generation and enhances matrix destruction. Various cytokines and enzymes released by the resident and other cells in the adventitia may diffuse into the medial wall through vasa vasorum, thereby affecting the differentiation of medial smooth muscle cells (SMCs) or the surrounding adventitial myofibroblasts. Furthermore, breakdown of the medial wall through enhanced MMP expression may allow monocytes to enter the media and intima from the adventitial side. Also, MMP expression by adventitial cells may be important for the breakdown of the medial wall in the later stages of TA, thereby promoting aneurysms. Aortic aneurysm is commonly associated with TA, and aorta with little calcification has a greater possibility of aneurysm formation in these patients. The role of MMPs in aneurysm has been substantiated by various clinical, mouse knockout and transgenic studies (18,19). Chauhan et al. reported that the anti-aortic endothelial cell antibodies (AAECAs) present in patients with TA are directed mainly against 60−65 kDa antigen(s) and may cause vascular dysfunction by inducing expression of endothelial adhesion molecules, cytokine production and apoptosis (20). Moreover, importance of increased oxidative stress and MMPs in TA has already been established (12,13,21). In addition, characteristic fibroblast proliferation during this process may likely increase perivascular production of reactive oxygen species (ROS) and impair endothelium-dependent relaxation. Generation of angiotensin II (AngII) and cytokines by perivascular adipose tissue, as well as macrophages and mast cells present in the adventitia, could also potentiate endogenous ROS production and thus are likely to affect vascular smooth muscle responsiveness (3,4,22).

Glucocorticoids, either alone or in combination with alkylating agents, have been the mainstay of treatment for TA. Some novel treatment approaches are also being tried in TA. For example, in combination with steroids, minocycline (an inhibitor of MMPs) induced clinical remission in 9/11 cases along with a decline in inflammatory indices like C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), suggesting MMPs as a suitable drug target in TA (23). However, Matsuyama et al. demonstrated that corticosteroids do not affect MMP-3 levels in TA patients (24). A clear understanding of various molecular pathways involved in the pathophysiology of TA and effectively transferring this knowledge to the clinics remains the main thrust of current research worldwide.

The precise regulation of production and activation of MMPs is important for maintaining proper extracellular matrix (ECM) turnover. Extracellular matrix metalloproteinase inducer (EMMPRIN), a transmembrane glycoprotein, is capable of inducing MMPs through direct cell-cell interaction. However, role of EMMPRIN in inducing MMP production is not limited to only fibroblasts, it also mediates a paracrine effect on endothelial cells and cardiac myocytes, emphasizing its role in vascular remodeling. Role of EMMPRIN is well established in various pathophysiological conditions in tumor progression and vascular diseases (6,18,25). Recently, Nie et al. demonstrated that EMMPRIN expression increased in human left ventricle after acute myocardial infarction and was significantly correlated with MMP-2 and 9 levels, thus highlighting its potential role in vascular remodeling (26). Therefore, using strategies that inhibit EMMPRIN expression can serve as promising therapeutic targets. Until now there are no studies reported in the literature highlighting the significance of EMMPRIN in TA disease, thus making it worthy of study in the near future.

In conclusion, the present study provides evidence for the presence of a cell surface/membrane specific antigenic moiety on T-cells of TA subjects, which is responsible for activation of fibroblasts (cells predominantly present in adventitia) to enhance MMP production and therefore warrants further characterization in the near future.

Acknowledgments

The authors wish to thank the Indian Council of Medical Research (ICMR), New Delhi for research funding.

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