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Overexpression of interleukin-1β and interferon-γ in type I thoracic aortic dissections and ascending thoracic aortic aneurysms: possible correlation with matrix metalloproteinase-9 expression and apoptosis of aortic media cells☆

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Abstract

Objective: To examine the expression of interleukin-1β and interferon-γ and their possible roles in aortic dissections and aneurysms. **Methods:** Aortic specimens were obtained from patients with type I thoracic aortic dissection, ascending thoracic aortic aneurysms, and control organ donors. The expression of interleukin-1β, interferon-γ, matrix metalloproteinase-9, and signal transduction factors phospho-p38 and phosphorylated c-jun N-terminal kinase (phospho-JNK) were detected by real time reverse transcription-polymerase chain reaction (real time RT-PCR), Western blot, and immunohistochemistry, respectively. Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining was performed to detect apoptosis of media cells. The correlation of these factors and apoptosis was also studied. **Results:** Apoptosis in the media of thoracic aortic dissection and in ascending thoracic aortic aneurysms was dramatically higher than in the control group. The expression of interleukin-1β gradually increased from the control group, thoracic aortic dissection to ascending thoracic aortic aneurysms ($p < 0.01$, respectively). The expression of interferon-γ and matrix metalloproteinase-9 was significantly increased in the media of thoracic aortic dissection and ascending thoracic aortic aneurysms compared with the control group ($p < 0.01$, respectively). There were positive correlations between interleukin-1β versus matrix metalloproteinase-9, interleukin-1β versus phospho-p38 in thoracic aortic dissection ($p < 0.01$, respectively), and interferon-γ versus matrix metalloproteinase-9, interferon-γ versus phospho-JNK, interferon-γ versus apoptosis, and interleukin-1β versus apoptosis in ascending thoracic aortic aneurysms ($p = 0.02$, 0.02, $p < 0.01$, $p < 0.01$). **Conclusions:** Interleukin-1β and interferon-γ might effect the formation of thoracic aortic dissection and ascending thoracic aortic aneurysms possibly through the up-regulation of matrix metalloproteinase-9 and the apoptosis of media cells in humans.

Keywords: Interleukin-1β; Interferon-γ; Thoracic aortic aneurysm; Thoracic aortic dissection; Apoptosis

1. Introduction

Type I thoracic aortic dissections (TADs) and ascending thoracic aortic aneurysms (ATAAs) are the most lethal conditions encountered in vascular surgery, and are dreaded because of their rapid progression and high mortality [1,2]. Our understanding of the pathogenesis of these two diseases has increased, and it is known that hemodynamic abnormalities, such as hypertension and congenital or postnatal weakening of the aortic wall, contribute to their pathogenesis [3]. However, relatively little is known about the molecular mechanisms responsible for the genesis and progression of TAD and ATAA.

Many studies have shown that inflammation played a critical role in cardiovascular diseases, especially in the aortic dilatation diseases such as abdominal aortic aneurysms (AAAs) [4]. Inflammatory reactions generally included infiltration of inflammatory cells (such as CD4+ T cells and CD68+ macrophages) and generation of inflammatory mediators (such as the typical factors interleukin-1β (IL-1β) and interferon-γ (IFN-γ). Some studies had indicated that the inflammatory mediator IL-1β and IFN-γ may play an important role in aortic dilatation diseases [5,6]. For example, plasma levels of IL-1β have been found to be elevated in patients with AAA compared with normal individuals, or patients with coronary artery disease [5]. Keen et al. demonstrated that IL-1β, through its effect on smooth muscle cell (SMC) collagenase and collagen gene

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expression, increased the matrix turnover in aneurysms, which could lead to a change in the structure of the aortic tunica media and contributed to aneurysm formation [6]. Tang et al. found IFN-γ was up-regulated in inflammatory cells (e.g., Th1 T cells) in the aortic wall of ATAA. The elevated expression of IFN-γ and transmural inflammation was associated with increased external diameter of the aorta and decreased matrix proteins, which led to greater fragmentation of elastic fibers [7].

The changes in the aortic diameter and remodeling of the aortic wall associated with TAD and ATAA could be directly mediated by two primary mechanisms. One involved the matrix metalloproteinase (MMP) family and the tissue inhibitor of metalloproteinase (TIMP) family, which could lead to matrix degradation and increased turnover of elastin and collagen. The other involved apoptosis of media cells, which led to a change of media cell density and regression of media cell function in the aortic wall. The relationship between these two processes had not yet been established [8].

Generally speaking, the precise mechanisms of IL-1β and IFN-γ functioning in TAD and ATAA are still unknown. We hypothesized that IL-1β and IFN-γ might play a role in both processes by inducing MMPs production and apoptosis of media cells, which could destroy the media cells and the extracellular matrix, and lead to weakness of the aortic wall, or to formation of an aneurysm or dissection.

In the present study, we investigated the expression of IL-1β, IFN-γ, and MMP-9, and apoptosis of media cells in TAD and ATAA, and checked their potential relationship with the signal pathways of phospho-p38 and phosphorylated c-jun N-terminal kinase (phospho-JNK) in clinical tissue specimens from patients.

2. Materials and methods

2.1. Patients and aortic tissues

This study was approved by the Institutional Review and Ethics Committees of the Second Military Medical University and Changhai Hospital in Shanghai, China. All participants signed informed consents.

Twenty-one patients undergoing emergency repair of acute TAD and elective repair of ATAA participated in the study. The demographic and clinical characteristics of the patients and seven organ donors, who died of cause unrelated to aortic disease, are summarized in Table 1. During surgery, full-thickness aortic wall specimens were collected 3 cm above the aortic valve to consistently collect specimens from a similar site in each patient. Control specimens from normal ascending aortas were freshly obtained from the organ donors. The adventitia was carefully removed and other aortic walls were used for protein extraction for Western blot and RNA purification for real-time reverse transcription-polymerase chain reaction (real-time RT-PCR). The specimens taken from patients with TAD contained both true and false lumens. The aortas were immediately transferred to the laboratory. One part was fixed in formalin and used for histopathologic studies and immunohistochemistry; the other part was used for Western blot analysis and real-time RT-PCR.

Table 1. Demographic and clinical characteristics of the patients and control organ donors.

	Control ($n = 7$)	ATAA ($n = 10$)	TAD ($n = 11$)
Age (years)	54.6 ± 9.3	54.50 ± 11.94	45.27 ± 11.65
Men	5 (71.4%)	10 (100%)	9 (81.82%)
Maximal diameter of aorta (cm)	2.69 ± 0.25	6.61 ± 0.75†	5.80 ± 1.45†
Hypertension	3 (42.86%)	9 (90%)	8 (72.73%)
Smoking	2 (28.6%)	5 (50%)	3 (27.27%)
Debakey type I aortic dissection	—	—	11 (100.00)

Data presented as mean ± standard deviation or n (%), †$p < 0.01$ marked statistical significance with Mann–Whitney U-test or Fisher's exact test followed by Wilcoxon's rank sums test for two-by-two comparisons after adjustment compared to control group. There was no difference in age, gender, hypertension and smoking history among the three groups. The maximal diameter of aorta in TAD and ATAA was much larger than the control group ($p < 0.01$).

2.2. Protein extraction and Western blot analysis

The protein from the tissue samples (100 mg) was extracted in radioimmunoprecipitation assay (RIPA) buffer (Cell Signaling Technology, Danvers, MA, USA) and followed its protocol. The primary antibodies used included anti-IL-1β (R&D Systems, Inc., Minneapolis, MN, USA), anti-IFN-γ (R&D Systems, Inc., Minneapolis, MN, USA), and the corresponding second antibody was used (rabbit anti-goat, R&D Systems, Inc., Minneapolis, MN, USA). The detailed procedures were described in the literature [9].

2.3. Real-time quantitative PCR

RNA was extracted from 100-mg tissue samples using the conventional TRIzol method. The primer and probes for IL-1β and IFN-γ were designed with PRIMER version 5.0 software (Applied Biosystems, Foster City, CA, USA), and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal reference. An iCycler (Bio-Rad Laboratories, Hercules, CA, USA) and its system interface software were used to run samples and analyze data. The expression level of each target was normalized to the number of copies of the GAPDH transcript [9].

2.4. Immunohistochemical staining

The formalin-fixed paraffin-embedded aortic tissues were sectioned at a thickness of 5 μm. The antibodies used included those listed in the section on Western blot analysis and MMP-9 (Cell Signaling Technology, Danvers, MA, USA) plus those against the markers of the signal transduction pathways phospho-p38 mitogen-activated protein kinase (MAPK), Thr180/Tyr182 (Cell Signaling Technology, Danvers, Mass, USA), and phospho-JNK, Thr183/Tyr185 (Cell Signaling Technology, Danvers, Mass, USA). The EnVision system (Dako, Glostrup, Denmark) was used. The sections were counterstained with hematoxylin and eosin (H&E). Brown cytoplasm was characteristic of positive cells. Staining for CD4+ (Dako, Glostrup, Denmark), CD68+ (Santa Cruz, CA, USA), and α-actin (Sigma–Aldrich, St. Louis, MO, USA) was used to detect T lymphocytes, macrophages, and vessel SMCs, and confirmed the cell types around the dissected area of TAD. Motic

Images Advanced 3.2 (Motic China Group Co. Ltd., Xiamen, Fujian, China) was used to detect the positive cells (numbers and area) and grade the degree of staining (optical density unit, OD) in 10 random fields (×200), and then averaged. The product of positive cell area (μm^2) and average degree of staining was used to represent the expression of these factors.

2.5. TUNEL staining

The TUNEL reaction was performed with an *in situ* cell detection kit (Roche Applied Science, Indianapolis, IN, USA), according to the manufacturer's instruction, to detect the apoptosis of media cells. TUNEL-positive nuclei (stained brown) were counted by Motic Images Advanced 3.2 in 10 random fields (×200), and then averaged.

2.6. Statistical analysis

Continuous data were expressed as mean ± standard deviations. Data were evaluated with regard to normal distribution (Kolmogorov–Smirnov test) and homogeneity of variances (Levene's test). For analysis of differences between the groups, the one-way analysis of variance (ANOVA) was used, and, in case of differences, the Student's *t*-test was applied. In case of inhomogeneity of variances or deviation from the normal distribution, the Kruskal–Wallis ANOVA on Ranks was used. The analysis of covariance (ANCOVA) model was used to evaluate the correlation between the apoptosis of media cells and the levels of IL-1β, IFN-γ, MMP-9, phospho-p38, and phospho-JNK. Data was analyzed using the Statistical Package for Social Sciences (SPSS) V 13.0 (SPSS, Inc, Chicago, IL, USA), and a two-tailed *p* value <0.05 was considered statistically significant.

3. Results

3.1. Real-time RT-PCR and Western blot of IL-1β and IFN-γ in the thoracic aortic wall

Real-time RT-PCR was used to determine the messenger RNA (mRNA) expression of IL-1β and IFN-γ in the aortic media, and the protein level was assessed by Western blot analysis. The mRNA and protein levels of IL-1β and IFN-γ in ATAA tissue were significantly higher than in the TAD and control tissues (Fig. 1(A)–(D), $p < 0.01$, respectively). The mRNA and protein levels of IL-1β were also significantly higher in the TAD media than in the control media (Fig. 1(A) and (C), $p < 0.01$). No significant difference in IFN-γ expression in mRNA and protein levels was observed between the TAD and control tissues (Fig. 1(B) and (D), $p = 0.12$ and $p = 0.30$, respectively) (Table 2). This might indicate that IL-1β and IFN-γ were both involved in the procedure of ATAA formation. Further, IL-1β might also play some function during TAD formation.

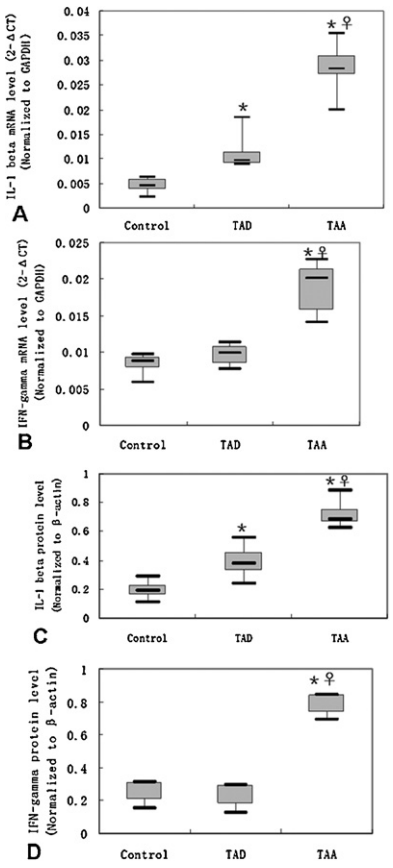

Fig. 1. Expression of interleukin-1β and interferon-γ in aortic tissues measured by real-time RT-PCR (A and B) and Western blot (C and D). (*) Significant from the control group ($p < 0.01$). (†) Significant from the TAD group ($p < 0.01$).

3.2. Immunohistochemistry of IL-1β, IFN-γ, and MMP-9 in the aortic media

Immunohistochemistry testing was conducted to explore the expression of IL-1β, IFN-γ, and MMP-9 in the media from TAD and ATAA specimens. More IL-1β, IFN-γ, and MMP-9 were expressed in both TAD and ATAA than that in the control group ($p < 0.01$, respectively, Fig. 2(A)–(I)). The expression of IL-1β was also increased in ATAA media compared with TAD ($p < 0.01$). There was no difference in the expression of IFN-

Table 2. Protein and mRNA levels of interleukin-1β and interferon-γ among three groups.

Confidence intervals (95%)	mRNA level of IL-1β	mRNA level of IFN-γ	Protein level of IL-1β	Protein level of IFN-γ
Control group	[0.0033,0.0060]	[0.0071, 0.0096]	[0.1368, 0.2518]	[0.1885, 0.3029]
TAD group	[0.0088, 0.0127]*	[0.0085, 0.0133]*	[0.3200, 0.4546]	[0.1773, 0.2536]
ATAA group	[0.0237, 0.0313]*,†	[0.0155, 0.0209]*,†	[0.6462, 0.7858]*,†	[0.6998, 0.8122]*,†

mRNA level was normalized to GAPDH and protein level was normalized to β-actin.
* Significant from the control group ($p < 0.01$).
† Significant from the TAD group ($p < 0.01$).

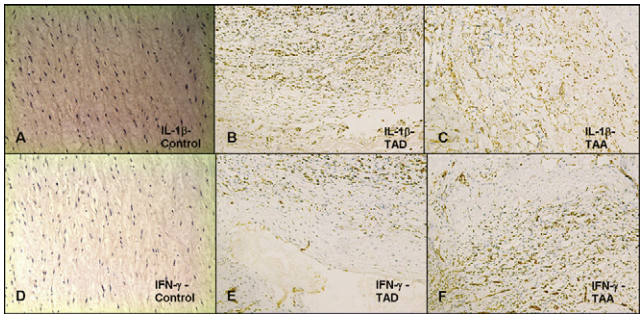

Fig. 2. Immunohistochemistry staining of interleukin-1β and interferon-γ (×200) Negative expression of interleukin-1β and interferon-γ were observed in normal aortic tissue. Interleukin-1β and interferon-γ were mainly located in the dissected part of the media in TAD and also expressed higher in the media of ATAA.

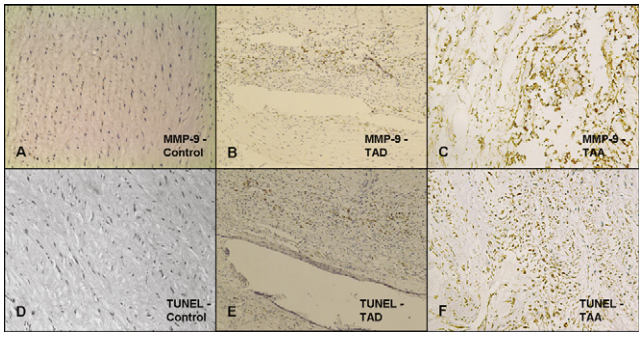

Fig. 3. Immunohistochemistry staining of matrix metalloproteinase-9 and TUNEL staining (×200) Negative expression of matrix metalloproteinase-9 and weak TUNEL staining were observed in normal aortic tissue. Matrix metalloproteinase-9 and apoptosis was mainly located in the dissected part of the media in TAD and also expressed higher in the media of ATAA.

γ and MMP-9 between ATAA and TAD ($p = 0.46$ and 0.92, respectively) (Table 3).

3.3. Apoptosis in the media of TAD and ATAA tissues

Then, we investigated whether there was significant apoptosis in the media of the thoracic aortic wall. DNA fragmentation was assessed in the aortic specimens using the TUNEL assay. TUNEL-positive cells were distributed across the entire vessel wall, and focal accumulation in the media of the aortas from the patients with TAD was seen under the microscope (Fig. 2(J)–(L)). The number of media cells with positive TUNEL staining was significantly higher in the TAD tissue than that in the ATAA tissue ($p < 0.01$). The number of TUNEL-positive cells in both the TAD and ATAA tissues was significantly higher than that in the control group ($p < 0.01$, respectively). The apoptosis in TAD and ATAA tissues might be partially induced by or related to IL-1β and IFN-γ (Figs. 2 and 3).

3.4. Immunohistochemistry of CD4, CD68, and α-SMC in the aortic media

The different inflammatory cell types and SMCs were differentiated by staining with different markers: CD4 (T lymphocyte marker), CD68 (macrophage marker), and α-SMC (smooth muscle cell marker). Many CD4- and CD68-positive cells were observed in the media of TAD, especially around the dissected area (Fig. 2(M)–(O), Fig. 4).

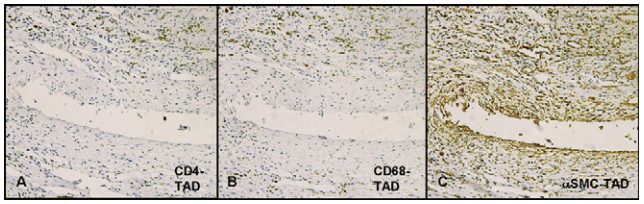

Fig. 4. Immunohistochemistry staining of CD4, CD68 and α-actin (×200). Many T lymphocytes (CD4 positive) and macrophages (CD68 positive) were observed as well as smooth muscle cells in the media of TAD especially around the dissected area.

3.5. Correlation analysis among the apoptosis and levels of IL-1β, IFN-γ, MMP-9, phospho-JNK, and phospho-p38 determined by immunohistochemistry

Analysis of the correlations among selected parameters including apoptosis and the levels of IL-1β, IFN-γ, MMP-9, phospho-JNK, and phospho-p38 was conducted. In the TAD group, it was evident that the levels of IL-1β versus MMP-9 and IL-1β versus phospho-p38 were significantly positively correlated ($p < 0.01$). Moreover, in the ATAA group, the levels of IFN-γ versus MMP-9, IFN-γ versus phospho-JNK, IFN-γ versus apoptosis, and IL-1β versus apoptosis were positively correlated with significance ($p = 0.02$, $p = 0.02$, $p < 0.01$, $p < 0.01$). Therefore, the role of IL-1β might be mediated by

Table 3. Expression of interleukin-1β, interferon-γ, matrix metalloproteinase-9 and apoptosis of media cells in immunohistochemistry among three groups..

	Interleukin-1β	Interferon-γ	Matrix metalloproteinase-9	Apoptosis
Control (μm^2 og)	0.174 ± 0.054	0.110 ± 0.042	0.216 ± 0.081	2.571 ± 1.512
Confidence intervals (95%)	[0.1238, 0.2242]	[0.0708, 0.1492]	[0.1417, 0.2909]	[1.1732, 3.9697]
TAD (μm^2 og)	$0.541 \pm 0.129^{*}$	$0.664 \pm 0.103^{*}$	$0.693 \pm 0.084^{*}$	$26.636 \pm 5.446^{*}$
Confidence intervals (95%)	[0.4826, 0.5992]	[0.5948, 0.7336]	[0.6369, 0.7498]	[22.9780, 30.2948]
ATAA (μm^2 og)	$0.773 \pm 0.084^{*,\dagger}$	$0.698 \pm 0.129^{*}$	$0.697 \pm 0.093^{*}$	$10.900 \pm 3.542^{*,\dagger}$
Confidence intervals (95%)	[0.7133, 0.8331]	[0.6059, 0.7901]	[0.6306, 0.7634]	[8.3663, 13.4337]

One-way ANOVA was used and in case of differences the Student's *t*-test was applied. In case of inhomogeneity of variances or deviation from the normal distribution the Kruskal–Wallis ANOVA on Ranks was used.

* $p < 0.01$ marked statistical significance from control group.

† $p < 0.01$ marked statistical significance from TAD group.The product of positive cell area (μm^2) and average degree of staining (OD value) was used to represent the expression of these factors. There were more interleukin-1β, interferon-γ and matrix metalloproteinase-9 expressed and apoptosis in both TAD and ATAA than that in the control group. The expression of interleukin-1β was also increased in the media of ATAA compared with TAD. Apoptosis of media cells in TAD was increased compared to ATAA group.

Table 4. Correlation analysis among expression levels of interleukin-1β, interferon-γ, matrix metalloproteinase-9, phospho-p38, phospho-JNK and apoptosis of media cells determined by immunohistochemistry.

Correlation	TAD (p value)	ATAA (p value)
Interleukin-1β versus matrix metalloproteinase-9	0.005*	0.387
Interferon-γ versus matrix metalloproteinase-9	0.368	0.024*
Interleukin-1β versus phospho-p38	0.007*	0.067
Interferon-γ versus phospho-p38	0.789	0.822
Interleukin-1β versus phospho-JNK	0.553	0.581
Interferon-γ versus phospho-JNK	0.511	0.016*
Interleukin-1β versus apoptosis of media cells	0.458	0.009*
Interferon-γ versus apoptosis of media cells	0.792	0.000*

In TAD group, the expression level of interleukin-1β versus matrix metalloproteinase-9, interleukin-1β versus phospho-p38 was positively correlated with significance. However, in the ATAA group, the level of interleukin-1β versus apoptosis, interferon-γ versus matrix metalloproteinase-9, interferon-γ versus phospho-JNK, interferon-γ versus apoptosis were significantly correlated.

* $p < 0.05$ marked statistical significance with ANCOVA model.

MMP-9 and the p38 signal pathway in TAD and by apoptosis of media cells in ATAA, while the role of IFN-γ might be mediated by apoptosis of media cells, MMP-9, and the phospho-JNK signal pathway (Table 4).

4. Discussion

There was evidence that the inflammatory processes had a critical role in the pathogenesis of TAD and ATAA. Studies have shown that a gene associated with inflammation of the aortic wall was more highly expressed in ATAA and TAD [10,11]. Kuehl et al. found that patients with ATAA or TAD, who had inflammation in the aortic wall, had a more rapid clinical progression of the disease and a worse prognosis than patients with no evidence of inflammation in the aortic wall [12]. To better clarify the molecular basis of the apparent role of inflammatory processes, we evaluated the expression of two pro-inflammatory cytokines, IL-1β and IFN-γ, as well as their possible roles in the formation of TAD and ATAA. In this study, the results showed that the expression of IL-1β was highest in the ATAA tissue, lower in the TAD tissue, and lowest in the control tissue. Further, IFN-γ was dramatically increased in the media of ATAA compared with the control group.

The *in vitro* study showed that IL-1β could induce both human or rat SMCs and macrophages to produce MMPs, especially MMP-9 [13–16] and IFN-γ could induce T lymphocytes to produce MMP-9 [17]. MMP-9 (gelatinase B) was the most critical member of the MMP family, as either type IV collagenase or elastase, which served to break down the normal structure of the arterial wall in the face of a pulsatile pressure in the aortic lumen. When these polymers were degraded and damaged, the aortic wall was weakened, which can lead to dilation and possible rupture [18]. In our study, the results of immunohistochemistry showed that MMP-9 was expressed the highest in ATAA and TAD compared with control. Furthermore, the grade of IL-1β staining had a positive correlation with MMP-9 in TAD, and the grade of IFN-γ staining had a positive correlation with MMP-9 in ATAA. These results were consistent with an effect of IL-1β and IFN-γ on MMP-9 and/or other potential MMPs in TAD and ATAA.

In addition to MMP-9, the apoptosis of SMCs may also play a role in the weakness of the aortic wall. Gene analysis showed that the gene related to apoptosis increased expression in TAD and ATAA [10,11]. In the present study, TUNEL staining showed that the degree of apoptosis in the media of TAD and ATAA was significantly higher than in the control. The correlation analysis showed that the expression of IL-1β and IFN-γ in immunohistochemistry was positively correlated with apoptosis of media cells in ATAA. This indicated that IL-1β and IFN-γ might play a role by inducing the apoptosis of media cells in ATAA. Therefore, MMPs and apoptosis of SMCs may be two independent mechanisms that act to weaken the aortic wall in response to a common stimulus such as inflammation. The co-existence of inflammatory mediators in the media of TAD and ATAA raised the possibility of extracellular matrix degradation and apoptosis of media cells.

IL-1β could induce the expression of MMP-9 through many signal transduction pathways in numerous cells [19]. The primary signal transduction pathways by which IL-1β induced MMP-9 expression in human tracheal SMCs were phospho-p38 and phospho-JNK [9]. In contrast to IL-1β, IFN-γ always induced apoptosis through the phospho-JNK and phospho-p38 signal transduction pathways [20]. In our study, the expression of phospho-p38 had a positive correlation with IL-1β in TAD tissues. IFN-γ, on the other hand, was not correlated with the phospho-p38 pathway, but with the phospho-JNK pathway in ATAA tissues. These data might suggest a potential relationship between IL-1β and phospho-p38, and IFN-γ and phospho-JNK.

However, our study had several limitations that should be mentioned. It was an observational study with a limited number of specimens of humans and lack of *in vitro* study as there were several studies focusing on the relationship between IL-1β, IFN-γ, and MMP-9 in some cell lines, such as human vascular SMCs. Nevertheless, the demonstration of the overexpression of IL-1β and INF-γ in TAA and of IL-1β in TAD, as well as their possible roles with expression of MMP-9, apoptosis of media cells, and phospho-p38 and phospho-JNK transduction pathways might provide clues to the molecular mechanisms involved in TAD and TAA, which should be pursued in future research.

In summary, we have shown that the expression of IL-1β and INF-γ was increased in TAD and ATAA tissues. Our results were consistent with the following hypothetical roles for inflammatory markers: in the media of TAD and ATAA, IL-1β might increase the degradation of the media matrix by increasing the expression of MMP-9 or apoptosis of media cells. INF-γ may increase the expression of MMP-9 and apoptosis of the media cells in ATAA but not TAD, which could lead to the extension of the aorta through the degradation of the media matrix and the pathological change of media cells. However, because of the observational study, the clear demonstration of the relationship between overexpression of IL-1β and INF-γ, and the expression of MMP-9, apoptosis of the media cells, and the phospho-p38 and phospho-JNK transduction pathways in TAD and ATAA should be pursued in future research both *in vivo* and *in vitro*.

References

[1] Ince H, Nienaber CA. Etiology, pathogenesis and management of thoracic aortic aneurysm. Nat Clin Pract Cardiovasc Med 2007;4:418—27.

[2] Meszaros I, Morocz J, Szlavi J, Schmidt J, Tornoci L, Nagy L, Szep L. Epidemiology and clinicopathology of aortic dissection. Chest 2000;117: 1271—8.

[3] Hasham SN, Guo DC, Milewicz DM. Genetic basis of thoracic aortic aneurysms and dissections. Curr Opin Cardiol 2002;17:677—83.

[4] Lindholt JS, Shi GP. Chronic inflammation, immune response, and infection in abdominal aortic aneurysms. Eur J Vasc Endovasc Surg 2006;31: 453—63.

[5] Juvonen J, Surcel HM, Satta J, Teppo AM, Bloigu A, Syrjala H, Airaksinen J, Leinonen M, Saikku P, Juvonen T. Elevated circulating levels of inflammatory cytokines in patients with abdominal aortic aneurysm. Arterioscler Thromb Vasc Biol 1997;17:2843—7.

[6] Keen RR, Nolan KD, Cipollone M, Scott E, Shively VP, Yao JS, Pearce WH. Interleukin-1 beta induces differential gene expression in aortic smooth muscle cells. J Vasc Surg 1994;20:784—6.

[7] Tang PC, Yakimov AO, Teesdale MA, Coady MA, Dardik A, Elefteriades JA, Tellides G. Transmural inflammation by interferon-gamma-producing T cells correlates with outward vascular remodeling and intimal expansion of ascending thoracic aortic aneurysms. FASEB J 2005;19: 1528—30.

[8] Lesauskaite V, Tanganelli P, Sassi C, Neri E, Diciolla F, Ivanoviene L, Epistolato MC, Lalinga AV, Alessandrini C, Spina D. Smooth muscle cells of the media in the dilatative pathology of ascending thoracic aorta: morphology, immunoreactivity for osteopontin, matrix metalloproteinases, and their inhibitors. Hum Pathol 2001;32:1003—11.

[9] Liang KC, Lee CW, Lin WN, Lin CC, Wu CB, Luo SF, Yang CM. Interleukin-1beta induces MMP-9 expression via p42/p44 MAPK, p38 MAPK, JNK, and nuclear factor-kappaB signaling pathways in human tracheal smooth muscle cells. J Cell Physiol 2007;211:759—70.

[10] Taketani T, Imai Y, Morota T, Maemura K, Morita H, Hayashi D, Yamazaki T, Nagai R, Takamoto S. Altered patterns of gene expression specific to thoracic aortic aneurysms: microarray analysis of surgically resected specimens. Int Heart J 2005;46:265—77.

[11] Weis-Muller BT, Modlich O, Drobinskaya I, Unay D, Huber R, Bojar H, Schipke JD, Feindt P, Gams E, Muller W, Goecke T, Sandmann W. Gene expression in acute Stanford type A dissection: a comparative microarray study. J Transl Med 2006;4:29.

[12] Kuehl H, Eggebrecht H, Boes T, Antoch G, Rosenbaum S, Ladd S, Bockisch A, Barkhausen J, Erbel R. Detection of inflammation in patients with acute aortic syndrome: comparison of FDG-PET/CT imaging and serological markers of inflammation. Heart 2008;94:1472—7.

[13] Gurjar MV, Deleon J, Sharma RV, Bhalla RC. Role of reactive oxygen species in IL-1 beta-stimulated sustained ERK activation and MMP-9 induction. Am J Physiol Heart Circ Physiol 2001;281:2568—74.

[14] Duftner C, Seiler R, Klein-Weigel P, Gobel H, Goldberger C, Ihling C, Fraedrich G, Schirmer M. High prevalence of circulating CD4+CD28-T—cells in patients with small abdominal aortic aneurysms. Arterioscler Thromb Vasc Biol 2005;25:1347—52.

[15] Knipp BS, Ailawadi G, Ford JW, Peterson DA, Eagleton MJ, Roelofs KJ, Hannawa KK, Deogracias MP, Ji B, Logsdon C, Graziano KD, Simeone DM, Thompson RW, Henke PK, Stanley JC, Upchurch Jr GR. Increased MMP-9 expression and activity by aortic smooth muscle cells after nitric oxide synthase inhibition is associated with increased nuclear factor-kappa B and activator protein-1 activity. J Surg Res 2004;116: 70—80.

[16] Gurjar MV, DeLeon J, Sharma RV, Bhalla RC. Mechanism of inhibition of matrix metalloproteinase-9 induction by NO in vascular smooth muscle cells. J Appl Physiol 2001;91:1380—6.

[17] Xiong W, Zhao Y, Prall A, Greiner TC, Baxter BT. Key roles of CD4+ T cells and IFN-gamma in the development of abdominal aortic aneurysms in a murine model. J Immunol 2004;172:2607—12.

[18] Longo GM, Xiong W, Greiner TC, Zhao Y, Fiotti N, Baxter BT. Matrix metalloproteinases 2 and 9 work in concert to produce aortic aneurysms. J Clin Invest 2002;110:625—32.

[19] Wu CY, Hsieh HL, Jou MJ, Yang CM. Involvement of p42/p44 MAPK, p38 MAPK, JNK and nuclear factor-kappa B in interleukin-1beta-induced matrix metalloproteinase-9 expression in rat brain astrocytes. J Neurochem 2004;90:1477—88.

[20] Chang YH, Chao Y, Hsieh SL, Hsieh SL, Lin WW. Mechanism of LIGHT/interferon-gamma-induced cell death in HT-29 cells. J Cell Biochem 2004;93:1188—202.