Cytokine 114 (2019) 61–66

Contents lists available at ScienceDirect

Cytokine

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

Short communication

High expression of S100 calgranulin genes in peripheral blood mononuclear cells from patients with Takayasu arteritis

Jayakanthan Kabeerdoss[a,1], Meera Thomas[b,1], Ruchika Goel[a], Hindumathi Mohan[a], Sumita Danda[c], Jeyaseelan L.[d], Debashish Danda[a,*]

[a] Department of Clinical Immunology and Rheumatology, Christian Medical College, Vellore, Tamil Nadu, India
[b] Department of Pathology, Christian Medical College, Vellore, Tamil Nadu, India
[c] Department of Clinical Genetics, Christian Medical College, Vellore, Tamil Nadu, India
[d] Department of Biostatistics, Christian Medical College, Vellore, Tamil Nadu, India

ARTICLE INFO

Keywords:
Takayasu arteritis
S100A12
TLR4
Calgranulins

ABSTRACT

Background: Toll-like receptors (TLR) 1 to 4 are highly expressed in aorta. Activation of TLR4 causes transmural arteritis in Human temporal artery-SCID chimera model. Neither TLR-4 nor its ligands have been studied in TA patients as yet. Aim of this study was to examine the expression of TLR4 and its endogenous ligands in peripheral blood mononuclear cells (PBMCs) of patients with Takayasu arteritis (TA).

Methods: mRNA expression of TLR4, RAGE and various endogenous TLR4 ligands were quantified in PBMCs of 24 TA patients and 19 sex and age matched healthy controls by real time PCR using specific primers and SYBR Green qPCR master mix. S100A8/A9 and S100A12 were measured in cell culture supernatant of PBMCs from TA patients and healthy controls, both in un-stimulated state as well as, after lipopolysaccharides (LPS) stimulated cultures for 4 h. Expression of S100A8/A9 in aortic tissues was assessed by immunohistochemistry.

Results: The mRNA expression of S100A8, S100A9, S100A12 and TLR4 were higher, while expression of RAGE and HSP70 were lower in TA as compared to healthy controls. Induction with LPS led to increase in secretion of both S100A8/A9 and S100A12 levels in TA as well as healthy controls. The fold of induction, measured by LPS stimulated/unstimulated control was higher in healthy controls [2.88 (1.7–3.53) fold] as compared to TA [1.345 (1–1.82) fold]; $p < 0.05$. Numerically, S100A8/A9 was also higher in healthy controls [2.04 (1.7–5.6) fold] as compared to TA [1.38 (1.09–3.6) fold], but it didn't reach statistical significance; $p = 0.129$. Mild to moderate intensity expression of S100A8/A9 protein was noted in aortic tissues from patients with TA.

Conclusion: mRNA expression of TLR4 and its ligand S100A8, S100A9, and S100A12 in PBMCs of TA patients was higher as compared to healthy controls. LPS stimulation led to higher induction of S100A12 secretion in healthy controls as compared to TA. Expression of S100A8/A9 was detected in inflamed aortic tissues from patients with TA.

1. Introduction

Takayasu arteritis (TA) is a large vessel vasculitis characterized by segmental and patchy granulomatous inflammation of aorta and its major branches. Takayasu arteritis can be divided into the following six types based on angiographic involvement: Type I - Branches of the aortic arch, Type IIa - Ascending aorta, aortic arch, and its branches, Type IIb - Type IIa region plus thoracic descending aorta, Type III - Thoracic descending aorta, abdominal aorta, renal arteries, or a combination, Type IV - Abdominal aorta, renal arteries, or both, Type V - Entire aorta and its branches [1]. The etio-pathogenesis of this condition is largely unknown, but infectious agents, and genetic factors are known to play a significant role [2]. In TA, vascular lesions are mainly due to infiltration of immune cells in the media and adventitia of aorta [2]. Both innate and adaptive immune cells constitute inflammatory cell infiltrate in TA. Mycobacterial analogue heat shock protein 65 (mHSP65) or its human analogue HSP60 have been shown to elicit both α/β and γ/δ T cells responses as well as antibody responses in earlier studies on TA; but the exact molecular mechanism of T cells proliferation and activation induced by HSP in TA is still unclear [3].

Toll-like receptors (TLR) 1, 2, 3 and 4 are highly expressed in human aorta [4]. In Human temporal artery - SCID chimeras,

* Corresponding author at: Department of Clinical Immunology & Rheumatology, Christian Medical College, Vellore, Tamil Nadu 632004, India.
E-mail address: debashisdandacmc@hotmail.com (D. Danda).
[1] Joint first authors as equal contributors

https://doi.org/10.1016/j.cyto.2018.11.033
Received 1 August 2017; Received in revised form 18 October 2018; Accepted 28 November 2018
Available online 26 December 2018

lipopolysaccharides (LPS) can induce T-cell recruitment and activation by stimulating TLR4 in vascular Dendritic cells (DC). Activation of TLR4 was shown to cause transmural panarteritis, which is a characteristic clinical feature in TA [5]. However, the ligand responsible for the activation of TLR4 in TA is unknown [5].

We hypothesised that there is an increased expression of endogenous TLR4 ligand in TA, which stimulates secretion of various pro-inflammatory cytokines. These cytokines may be responsible for inflammation and subsequent remodeling of vasculature in TA. With this background, we aimed to explore TLR-4 pathway in patients with TA by studying the expression of innate receptors viz TLR-4 and RAGE, endogenous TLR4 ligands including S100A8, S100A9, S100A12, fibronectin-1 (FN-1), High mobility group box protein (HMGB-1), HSP70 and Tenascin in PBMCs of TA patients as compared to healthy controls. We also aimed to measure S100A8/A9, a heterodimeric complex protein secreted by neutrophils as well as macrophages (which is also known as MRP 8/14) to correlate with gene expression.

2. Methods

2.1. Participants

Patients fulfilling 1990 American College of Rheumatology criteria for Takayasu arteritis with angiographically proven disease were recruited for the study [6]. Age and sex matched healthy volunteers without known history of any autoimmune disease were recruited as controls. Written consent was obtained from each subject. Venous blood was collected in heparin-coated vacutainer tubes (455051, Greiner Bio-One, Frickenhansen, Germany).

The study was approved by the Institutional review board and ethics committee of Christian Medical College, Vellore and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

2.2. RNA extraction, cDNA conversion and gene expression assays

PBMC was separated from blood by density gradient centrifugation using Ficoll-Paque™ Plus (1033378, GE Healthcare) and centrifuged at 2000 rpm for 25 min. The buffy coat obtained was taken out and washed with sterile PBS twice. PBMC was suspended in 500 µl of TRI reagent (T9424; Sigma Chemical Co) and stored at −80 °C for RNA isolation. RNA was extracted from the cells stored in TRI reagents as per manufacture's recommended protocol. RNA was quantitated by nanodrop 2000 (Thermo fisher scientific, Wilmington, DE U.S.A.). One microgram of RNA was used for cDNA conversion using ProtoScript First Strand cDNA synthesis kit (E6300S, New England Biolabs, Ipswich, MA, United States).

Quantification of mRNA expression of TLR4, RAGE, S100A8, S100A9, S100A12, Fibronectin-1 (FN-1), High mobility group box protein (HMGB-1), HSP70 and Tenascin were done using sequence specific primers with SYBR green chemistry in StepOnePlus™ Real-Time PCR Systems (Applied Biosystems). Briefly, PCR reactions were performed using 10 µl of 1X VeriQuest SYBR Green qPCR master mix (75600, USB, Affymetrix Inc, USA), 0.5 µl of 250 nM of forward primer, 0.5 µl of 250 nM of reverse primer, 2 µl of five times diluted cDNA and 7 µl of sterile water. The PCR conditions for individual gene expressions were optimized by gradient PCR and melting curve analysis was performed. PCR amplicons were verified by electrophoresis on 2% agarose gel, followed by visualization on a gel documentation system (AlphaImager® HP, ProteinSimple, CA, USA). All cDNA samples were then amplified for the genes of interest, as well as the housekeeping gene (Supplementary data 1 for list of genes and their primers). Cycle threshold (Ct) or crossing points (Cp) values obtained from triplicate runs were used for quantifying target gene expression relative to the housekeeping gene using the $2-\triangle Cp$ method [7].

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.cyto.2018.11.033.

2.3. PBMC activation

PBMCs of each subject were cultured at 10^6 cells/ml in 24 well plates (Costar, Coring NY, USA) in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum (Gibco, Thermo fischer Scientiifc Inc, USA). PBMCs were stimulated with LPS from Escherichia coli 055:B5 (L 4524, SPPigma Aldrich, Saint louis, MO, USA) in concentration of 100 ng/ml for 4 h in CO2 incubator (Forma, Thermo Scientific, USA). In a separate experimental condition, PBMCs stimulated with TAK-242(CLI-095; Invivogen, USA; 250 ng/ml), a cell-permeable inhibitor of TLR4 signalling was used along with LPS. PBMCs without LPS treatment were used as controls for each subject. At the end of 4 h, supernatants were separated and stored at −80 °C.

S100A8/A9 and S100A12 (EN-RAGE) were measured in cell culture supernatant by commercial ELISA kits (Biolegend, CA, USA and CycLex Co., Ltd. Japan respectively) as per manufacturers' instructions. LPS induced secretion was assessed by fold changes in concentration of S100A8/A9 and S100A12, which were calculated as ratio of their values in LPS stimulated serum to their corresponding levels under unstimulated conditions in the same patient. IL-6 and TNF-α were measured in cell culture supernatants by commercial ELISA kits (DY206 & DY210, R&D systems, MN, USA respectively).

2.4. Immunohistochemistry (IHC)

Aortic tissue biopsies were taken from TA patients undergoing open heart surgeries and atherosclerosis patients as controls. Formalin fixed paraffin-embedded sections were stained with mouse anti-human Calprotectin (S100A8/A9 complex) antibody (Catalog number ab22506, Abcam, USA). Visualization was based on enzymatic conversion of diaminobenzidine (DAB) into a brown-colored precipitate by horseradish peroxidase at the site of antigen localization. Staining was performed on a BenchMark Ultra automated instrument (Ventana Medical Systems). Human spleen tissues sections were used as positive controls.

2.5. Statistical analysis

The relative differences in each gene expression level were expressed as median with interquartile range, and the significance of differences between groups was assessed using unpaired *t* test with Welch correction or Mann Whitney test based on normality distribution. Wilcoxon signed rank test was used to compare S100A8/A9 and S100A12 secretion levels between LPS and non LPS treated PBMCs of the same subjects. Differential gene expression among subsets of patients with various clinical phenotypes of TA was also analysed using chi-square test. p values less than 0.05 was considered as statistically significant. Statistical analysis was done using GraphPad Prism version 5 (GraphPad Software, Inc. La Jolla, CA USA) and SPSS Statistics version 20.

3. Results

Twenty four consecutive patients with TA and 19 healthy controls [3 males and 16-females; median age: 30 (22–48) years] were recruited for the study. Demographic characteristic of patients are shown in Table 1.

3.1. mRNA expression in PBMCs of TA and healthy controls:

The expression of TLR4 was significantly higher in patients, whereas RAGE expression was lower in patients with TA than in healthy controls. Similarly, mRNA of TLR-4 ligands S100A8 (MRP8), S100A9 (MRP9), and S100A12 (MRP6) were also expressed at significantly

Table 1
Clinical Details of Patients with TA.

Parameter	n = 24
Gender (Male: Female)	6:18
Median age in years (range)	25.5 (19–58)
Median duration of symptoms in months (range)	6 (0.25–120)
Age of onset in years (range)	25 (13–57)
Angiographic types n (%)	
Type I	4 (16.67%)
Type IIb	1 (4.17%)
Type III	1 (4.17%)
Type IV	1 (4.17%)
Type V	17 (70.84%)
Median ESR in mm/1st hour (range)	38 (6–71)
Median CRP mg/dl (range)	13 (1–91)
Median ITAS (range)	2 (0–16)
Median DEI.Tak (range)	11 (4–16)
Treatment n (%)	
Treatment naïve	12 (50%)
Glucocorticoids (Prednisolone or Deflazacort)	12 (50%)
DMARDS[≠]	11 (45.84%)
Biological DMARDs (Tocilizumab)	1 (4.17%)

DMARDS[≠]: Mycophenolate mofetil + Prednisone/Deflazacort (n = 9); Methotrexate + Prednisone/Deflazacort (n = 1), Azathioprine + Prednisone/Deflazacort (n = 1).

characteristics including ITAS (Indian Takayasu activity Score) was found to be associated with expression of any of the genes studied.

3.2. S100A8/A9 and S100A12 levels in supernatants of cultured PBMCs:

PBMCs of 10 subjects each from healthy controls and treatment naïve TA were incubated with and without LPS for 4 h. Basal secretion of S100A8/A9 (without LPS) was only numerically higher in TA (692 ± 293 ng/ml) than healthy controls (435 ± 122 ng/ml), p = 0.128 (Fig. 2). LPS induced increased secretion of S100A8/A9 relative to it's basal levels, both in PBMCs of healthy controls as well as that of patients (Fig. 3).Fold changes in S100A8/A9 levels trended to be more in healthy controls as compared to patients with TA, even though statistically insignificant (P = 0.148).

Similar to results for S100A8/A9, basal secretion of S100A12 were numerically higher (not statistically significant) in patients with TA (3632 ± 1644 pg/ml) as compared to healthy controls (1193 ± 550.5 pg/ml) (p = 0.114) (Fig. 2). However, LPS stimulation led to a significant increase in S100A12 secretion in supernatant of cultured PBMCs from healthy controls, which was two times more than that for TA patients as assessed by fold changes in S100A12 levels (Fig. 3).

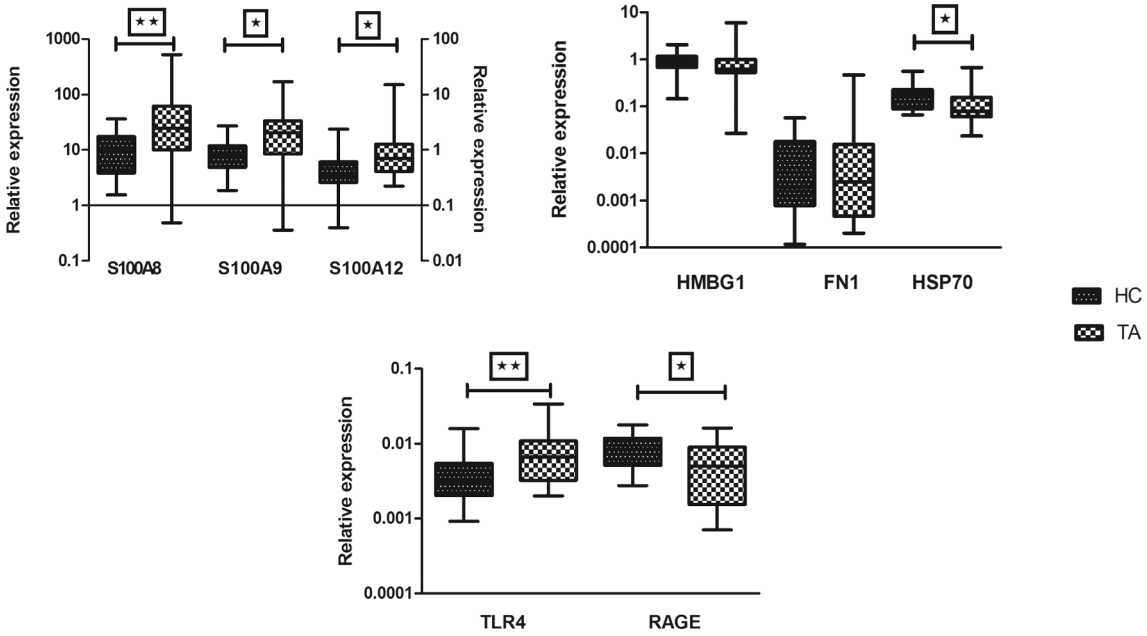

Fig. 1. Gene expression levels of endogenous TLR4 ligands, TLR4 and RAGE were quantitated in PBMC of Healthy controls (HC) (n = 19) and patients with Takayasu arteritis (TA) (n = 24). Expressions of mRNA values were shown in the box plot as median (central line), interquartile range (limit of the box) and range (whiskers). Both left and right y-axes are used; expression levels of S100A9 and S100A12 are depicted using the right y-axis. Number of * denotes strength of significance; * = p < 0.05,** = p < 0.01,*** = p < 0.005.

higher levels in PBMCs of patients with TA as compared to healthy controls (Fig. 1). On the contrary, expression of HSP70 was significantly lower in TA as compared to healthy controls. There was no difference in expression levels of HMBG1 and fibronectin, two other endogenous TLR-4 ligands.

Subset analysis on the basis of clinical phenotype and vascular area involvement showed increased S100A8 expression in 88.89% (16/18) of the patients with left subclavian artery involvement, while its expression was present only in 16.7%(1/6) of those patients without the involvement of this artery (p = 0.034). However, no significant association was found between expression of S100A8 and involvement of right subclavian artery [11/18 (61.12%) versus 2/6 (33.34%); p = 0.357]. No other clinical, angiographic or disease activity

3.3. IL6 and TNF-α secretion on TLR4 activation

We also evaluated for any difference in pro-inflammatory cytokine secretion upon activation between TA and healthy controls. We have measured these cytokines in the supernatant of PBMCs stimulated with LPS as well as, with LPS plus TAK-242 for 4 hours. In healthy controls, IL-6 secretion was increased in LPS stimulated condition, as compared to unstimulated experiments; however, the levels were lower when cultured with LPS + TAK-242 as compared to LPS alone. In TA group, though IL-6 secretion was increased by LPS, the levels were not found to be significantly different between supernatants from LPS and LPS + TAK-242 stimulated PBMCs. PBMCs of patients with TA failed to respond to TLR4 inhibitor and showed no difference in IL-6 secretion

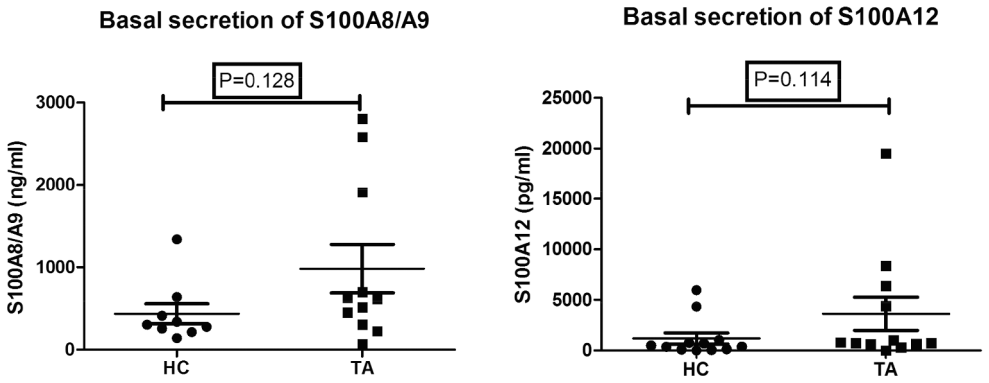

Fig. 2. Basal secretion (Unstimulated control) levels of S100A8/A9 and S100A12 in both healthy controls and TA. Values are shown in the scatter plot as median (central line) with standard error of the mean (SEM).

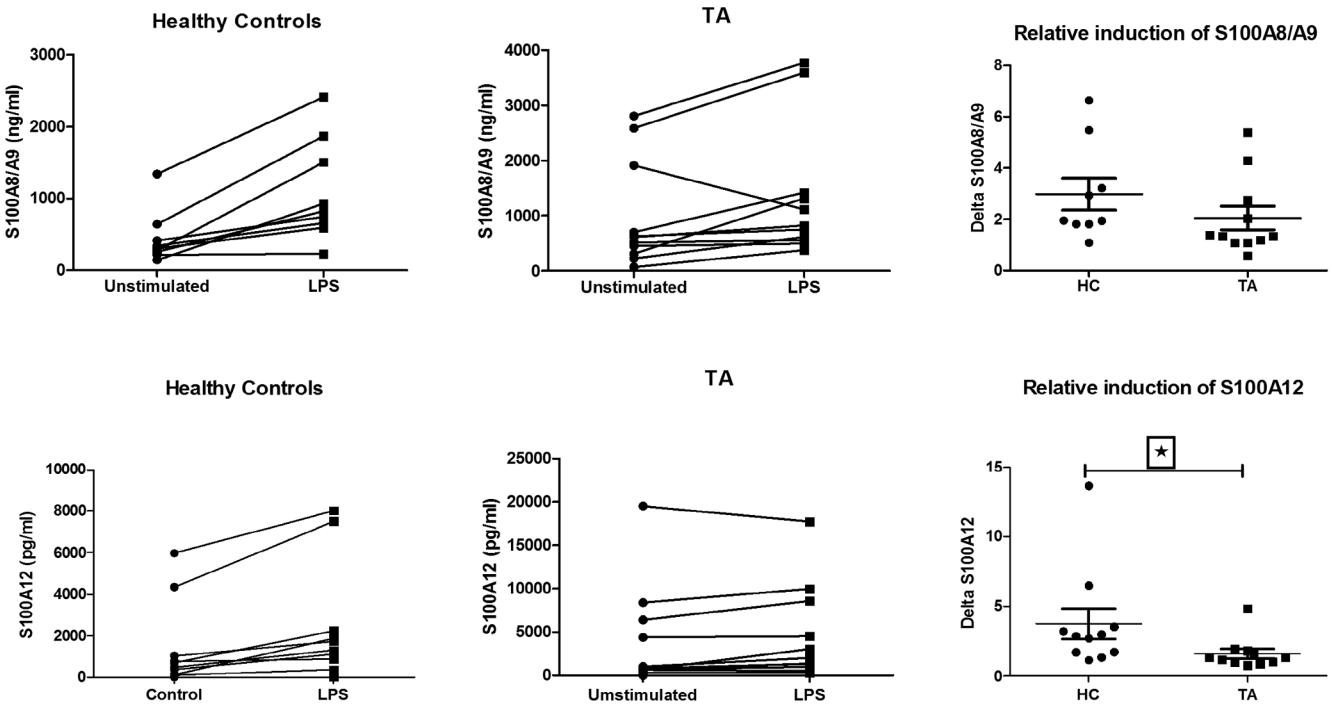

Fig. 3. S100A8/A9 and S100A12 secretion by PBMCs from TA (n = 10) and Healthy controls (n = 10), cultured with and without LPS (100 ng/ml) in RPMI medium for 4 h. Fold changes in S100A8/A9 and S100A12 values were calculated by LPS stimulated/unstimulated cells of each subject. Values are shown in the scatter plot as median (central line) with standard error of the mean (SEM); Number of * denotes strength of significance; * = $p < 0.05$. Each circle represents one subject and connecting line represents their corresponding secreted S100A8/A9 and S10012 levels in unstimulated control (without LPS) and following LPS treatment of PBMCs.

between LPS and LPS + TAK-242 stimulated PBMCs.

TNF-α levels were increased in supernatant of LPS stimulated cells as compared to controls, both in healthy subjects and TA. TNF-α levels were also increased in supernatants of LPS + TAK-242 stimulated PBMC cultures as compared to LPS alone in healthy individuals; However, no difference was observed between these two experimental conditions in patients with TA (see Fig. 4).

3.4. S100A8/A9 expression in aortic tissues

We used aortic tissues from 11 patients with TA who were already on immunosupression and two tissue samples from patients with atherosclerosis. S100A8/A9 was expressed in aortic tissues of 6 out of 11 (54.55%) patients with TA and negative in tissue samples from atherosclerosis patients. In TA, S100A8/A9 was expressed in all three layers of the vessel, i.e. intima, media and adventitia and the expression levels varied between minimal (1+) and maximum (3+) on aortic tissues (Fig. 5). All patients with moderate inflammation as assessed by H&E staining in biopsy tissue samples with infiltration of neutrophils showed evidence of S100A8/9 positivity. Persistently raised inflammatory markers were present, in-spite of immunosuppressive therapy during follow up in these patients. No staining was observed in tissue sections when there was a loss of elastic lamina and hyalinization, including in the tissue samples from 5 TA patients with inactive disease defined by ESR/CRP/ITAS values.

4. Discussion

This is the first study to test the hypothesis that TLR-4 and its ligands are involved in pathogenesis of TA. In this study, we observed higher expression of TLR4 and decreased expression of RAGE in cultured PBMCs of patients with TA, as compared to healthy controls. Among the endogenous ligands of TLR4, we observed increased expression of S100A8, S100A9 and S100A12, while lower expression of

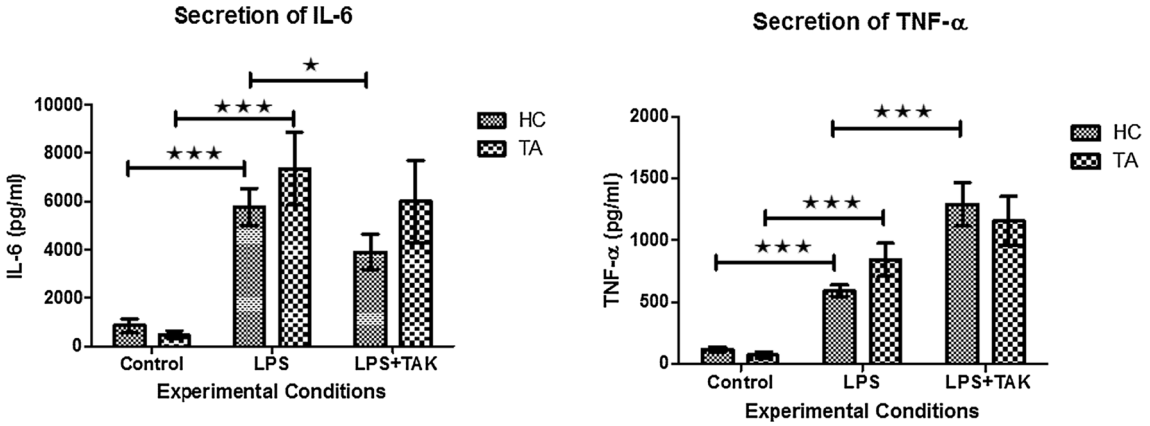

Fig. 4. Secretion of IL-6 and TNF-α by PBMCs of healthy controls (n = 8) and TA (n = 11) in different experimental conditions. Values are shown in the bar plot expressed as Mean with standard error of the mean (SEM).

HSP-70 in patients as compared to controls. Expression of HMGB1 and fibronectin, a major driver of TLR-4 activation in scleroderma, another fibrotic disease [8], did not differ between patients with TA and controls in our study. S100A8, S100A9 and S100A12 gene expression have been shown to be increased in TA in an earlier small microarray based study [9]. We also showed that S100A8/A9 was expressed in inflamed aortic tissues from patients with TA. Antibody (MAC387 clone) used in our immunohistochemistry experiment is known to recognize S100A9, S100A8/A9 complex and S100A12. Positive IHC stains in our study showed that all calgranulin proteins were expressed in active inflamed lesions of aorta in patients with TA. Our study, therefore, implicate a possible role of TLR-4-calgranulin driven pathway in pathogenesis of inflammation in TA.

Increased expression of S100 calgranulins has been shown in other

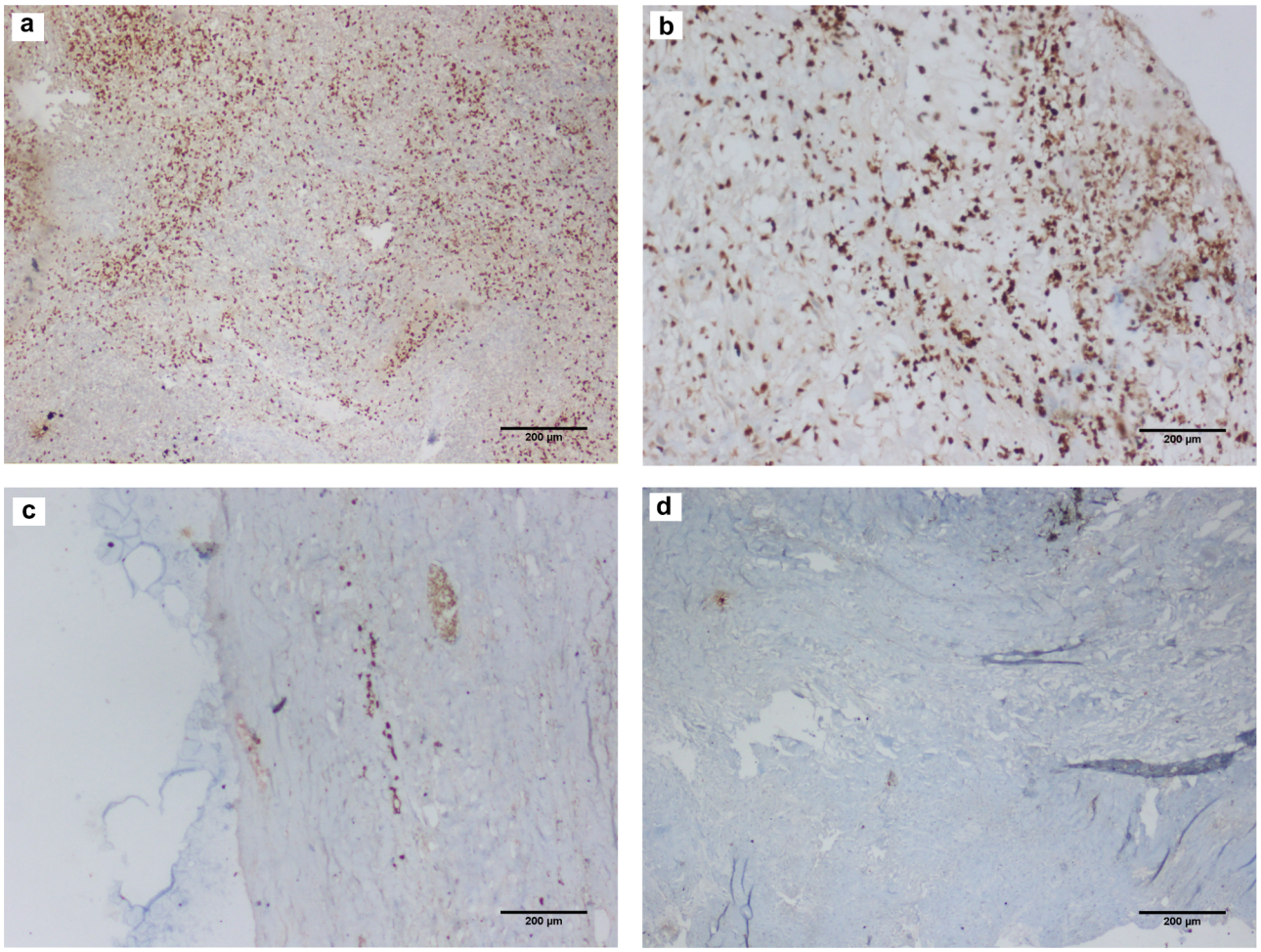

Fig. 5. Expression of S100A8/A9 in aortic tissues of patients with Takayasu Areritis. Representative photographs showing expression of S100A8/A9 in the media (Fig. 5a) and adventitia of patients with TA (Fig. 5b) at 100× and 200× respectively. Images showing negative staining for S100A8/A9 expression in patients with atherosclerosis at 100× and 200× respectively (Fig. 5c & 5d).

inflammatory rheumatic diseases too [10,11]. Higher protein expression of S100A8/A9 has been noted in temporal arteries of Giant Cell arteritis (GCA) patients [12]. Calgranulin, S100A8, S100A9 & S100A12 have been shown to be highly expressed in leukocytes of patients with Kawasaki diseases (KD), a medium vessel vasculitic disease of children, during acute phase [13]. Intravenous Immunoglobulin (IVIG) treatment suppresses these gene expressions in KD [14]. In fact, we have recently published our data on calgranulin proteins as a biomarker of disease activity in our patients with TA [15]. These reports provide evidence to emphasize that calgranulins are expressed in inflammatory regions and leukocytes in vasculitis patients.

S100A12 is a common ligand for both TLR4 and RAGE [16]. Increased expression of S100A12 and TLR4 along with decreased expression of RAGE in our study probably points towards preferential engagement of TLR-4 over RAGE by S100A12. However, we did not perform protein interaction experiments to prove this explanation.

One earlier study had shown that *in-vitro* stimulation of monocytes by LPS for 4 h results in increased secretion of S100A12 [16]. *In vivo* administration of LPS also increased their plasma levels in healthy volunteers as per the same study. In the current study, LPS induced S100A12 secretion was significantly lower in TA as compared to healthy controls. Even though, the mRNA expression of calgranulins S100A8/9 and S100A12 was increased in TA patients, we did not observe a significant increase in secretion of both these proteins in supernatant of cultured unstimulated PBMCs. In-fact, the fold induction of these calgranulins after LPS stimulation was lesser in TA patients as compared to healthy controls. S100A12 secretion by neutrophil in response to stimulation by TLR-4 ligands such as monosodium urate crystals or H202 were observed by others to be dependent on generation of Reactive oxygen species (ROS) and K+ flux in these cells [17]. It is beyond the scope of this study to examine, whether these results of our study are due to an internal defect in secretion of S100A12 and S100A8/9 in TA patients caused by defective generation of ROS; this may be explored in future studies.

PBMCs from patients with TA have failed to respond to TLR4 inhibitor and showed no difference in IL-6 secretion following LPS and LPS + TAK-242 stimulation. Reason for these differences in IL-6 and TNF-α cytokines levels in our experiment might be due to the variable time required for induction and secretion of these two cytokines; IL-6 is the primary cytokine secreted much earlier by TLR4 ligand stimulated cells, whereas TNF-α could be secreted after a longer latency. We didn't perform *in-vitro* experiment to demonstrate TLR4 expression and induction of pro-inflammatory cytokines by PBMCs of TA patients in response to treatment with calgranulin, to substantiate our hypothesis. This is a major limitation of our study.

Our study has, however, generated another hypothesis that TLR-4 activation is important in pathogenesis of TA and S100A12 as well as S1008/9 are possible endogenous drivers of this activation. Studies examining the surface expression of TLR4 and RAGE in PBMCs as well as other immune cells may be required to confirm our findings. Intracellular measurement of S100 calgranulins would also be required to understand mechanisms underlying decreased secretion of these calgranulins in our study.

5. Conclusion

Basal expression TLR4 mRNA and its ligands S100A8, S100A9 and S100A12 tended to be increased in patients with TA as compared to controls. Stimulation of PBMC with LPS lead to further increase in secretion of S100A12 protein, but not that of S100A8/A9. S100A8/A9 was expressed in inflamed aortic tissues of patients with TA and it seemed to be associated with persistently raised inflammatory markers, in-spite of immunosuppressive therapy during follow up in these patients.

Funding

This work was funded by grants-in-aid from Science and Engineering Research Board, Department of Science and Technology (DST), Government of India under young investigator scheme to JK (SB/YS/LS-14/2014) and fluid research grant from Christian Medical College, Vellore (IRB no. 10970, dated 22-11-2017).

Conflicts of interest

Nil.

References

[1] A. Hata, M. Noda, R. Moriwaki, F. Numano, Angiographic findings of Takayasu arteritis: new classification, Int. J. Cardiol. 54 (Suppl) (1996) S155–S163.
[2] L. Arnaud, J. Haroche, A. Mathian, G. Gorochov, Z. Amoura, Pathogenesis of Takayasu's arteritis: a 2011 update, Autoimmun. Rev. 11 (2011) (2011) 61–67, https://doi.org/10.1016/j.autrev.2011.08.001.
[3] S. Kumar Chauhan, N. Kumar Tripathy, N. Sinha, M. Singh, S. Nityanand, Cellular and humoral immune responses to mycobacterial heat shock protein-65 and its human homologue in Takayasu's arteritis, Clin. Exp. Immunol. 138 (2004) 547–553, https://doi.org/10.1111/j.1365-2249.2004.02644.x.
[4] O. Pryshchep, W. Ma-Krupa, B.R. Younge, J.J. Goronzy, C.M. Weyand, Vessel-specific Toll-like receptor profiles in human medium and large arteries, Circulation 118 (2008) 1276–1284, https://doi.org/10.1161/CIRCULATIONAHA.108.789172.
[5] J. Deng, W. Ma-Krupa, A.T. Gewirtz, B.R. Younge, J.J. Goronzy, C.M. Weyand, TLR4 and TLR5 induce distinct types of vasculitis, Circ. Res. 104 (2009) 488–495, https://doi.org/10.1161/CIRCRESAHA.108.185777.
[6] W.P. Arend, B.A. Michel, D.A. Bloch, G.G. Hunder, L.H. Calabrese, S.M. Edworthy, A.S. Fauci, R.Y. Leavitt, J.T. Lie, R.W. Lightfoot, The American College of Rheumatology 1990 criteria for the classification of Takayasu arteritis, Arthritis Rheum. 33 (1990) (1990) 1129–1134.
[7] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods San Diego Calif. 25 (2001) 402–408, https://doi.org/10.1006/meth.2001.1262.
[8] S. Bhattacharyya, Z. Tamaki, W. Wang, M. Hinchcliff, P. Hoover, S. Getsios, E.S. White, J. Varga, FibronectinEDA promotes chronic cutaneous fibrosis through toll-like receptor signaling, 232ra50–232ra50, Sci. Transl. Med. 6 (2014), https://doi.org/10.1126/scitranslmed.3008264.
[9] D. Okuzaki, T. Fukushima, T. Tougan, T. Ishii, S. Kobayashi, K. Yoshizaki, T. Akita, H. Nojima, Genopal™: a novel hollow fibre array for focused microarray analysis, DNA Res. Int. J. Rapid Publ. Rep. Genes Genomes 17 (2010) 369–379, https://doi.org/10.1093/dnares/dsq025.
[10] C. Perera, H.P. McNeil, C.L. Geczy, S100 Calgranulins in inflammatory arthritis, Immunol. Cell Biol. 88 (2009) 41–49, https://doi.org/10.1038/icb.2009.88.
[11] D. Holzinger, C. Kessel, A. Omenetti, M. Gattorno, From bench to bedside and back again: translational research in autoinflammation, Nat. Rev. Rheumatol. 11 (2015) 573–585, https://doi.org/10.1038/nrrheum.2015.79.
[12] D. Foell, J. Hernández-Rodríguez, M. Sánchez, T. Vogl, M.C. Cid, J. Roth, Early recruitment of phagocytes contributes to the vascular inflammation of giant cell arteritis, J. Pathol. 204 (2004) 311–316, https://doi.org/10.1002/path.1660.
[13] T. Ebihara, R. Endo, H. Kikuta, N. Ishiguro, X. Ma, M. Shimazu, T. Otoguro, K. Kobayashi, Differential gene expression of S100 protein family in leukocytes from patients with Kawasaki disease, Eur. J. Pediatr. 164 (2005) 427–431, https://doi.org/10.1007/s00431-005-1664-5.
[14] K. Hirono, D. Foell, Y. Xing, S. Miyagawa-Tomita, F. Ye, M. Ahlmann, T. Vogl, T. Futatani, C. Rui, X. Yu, K. Watanabe, S. Wanatabe, S. Tsubata, K. Uese, I. Hashimoto, F. Ichida, M. Nakazawa, J. Roth, T. Miyawaki, Expression of myeloid-related protein-8 and -14 in patients with acute kawasaki disease, J. Am. Coll. Cardiol. 48 (2006) 1257–1264, https://doi.org/10.1016/j.jacc.2006.02.077.
[15] R. Goel, A. Nair, J. Kabeerdoss, H. Mohan, V. Jeyaseelan, G. Joseph, D. Danda, Study of serial serum myeloid-related protein 8/14 as a sensitive biomarker in Takayasu arteritis: a single centre study, Rheumatol. Int. 38 (2018) 623–630, https://doi.org/10.1007/s00296-017-3881-4.
[16] D. Foell, H. Wittkowski, C. Kessel, A. Lüken, T. Weinhage, G. Varga, T. Vogl, T. Wirth, D. Viemann, P. Björk, M.A.D. van Zoelen, F. Gohar, G. Srikrishna, M. Kraft, J. Roth, Proinflammatory S100A12 can activate human monocytes via Toll-like receptor 4, Am. J. Respir. Crit. Care Med. 187 (2013) 1324–1334, https://doi.org/10.1164/rccm.201209-1602OC.
[17] M. Tardif, L. R, J.A. Chapeton-Montes, A. Posvandzic, Pag&#xe9, Nathalie, C. Gilbert, P.A. Tessier, M. Tardif, L. R, J.A. Chapeton-Montes, A. Posvandzic, Pag&#xe9, Nathalie, C. Gilbert, P.A. Tessier, Secretion of S100A8, S100A9, and S100A12 by Neutrophils Involves Reactive Oxygen Species and Potassium Efflux, Secretion of S100A8, S100A9, and S100A12 by Neutrophils Involves Reactive Oxygen Species and Potassium Efflux, J. Immunol. Res. 2015 (2015) e296149. https://www.doi.org/10.1155/2015/296149.