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Atherosclerosis

Chronic *Staphylococcus aureus* Superantigen Toxic Shock Syndrome Toxin-1 Exposure Accelerates the Progression of Atherosclerosis in Rabbits

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Background: It has been reported that infectious agents contribute to the atherosclerotic process. However, it is unclear whether *Staphylococcus aureus* superantigen (SAg) toxic shock syndrome toxin-1 (TSST-1) has an effect on atherosclerosis progression. The present study was designed to investigate the pathogenic role of TSST-1 exposure in the atherosclerotic process in rabbits.

Methods: New Zealand White rabbits were exposed to TSST-1 through Alzet miniosmotic pumps with a constant pumping rate. Aortic atherosclerosis was evaluated by histological and morphometric methods. Using a biochemical analyzer/enzyme-linked immunosorbent assay/immunostaining, we further analyzed various atherosclerosis-related factors.

Results: The gross atherosclerotic lesion area in the aortic arch increased by 15.3% in high-fat-diet rabbits exposed to TSST-1 compared to that in the control group. In the atherosclerotic lesions, TSST-1 exposure increased the content of smooth muscle cells. Moreover, TSST-1 treatment up-regulated serum tumor necrosis factor alpha (TNF-α) level, but did not affect serum lipids (except for triglycerides) and endotoxin in the rabbits.

Conclusions: Our data validated that chronic stimulation with TSST-1 facilitates the progression of atherosclerosis in rabbits independently of endotoxins, indicating that *S. aureus* and its SAgs may be targets to prevent and treat atherosclerosis.

Key Words: Atherosclerosis • Smooth muscle cell • *Staphylococcus aureus* • Toxic shock syndrome toxin-1 • Tumor necrosis factor alpha

INTRODUCTION

Atherosclerosis is one of the leading causes of morbidity and mortality worldwide. According to the World Health Organization (WHO), about 20 million deaths each year are caused by atherosclerosis.[1] As a chronic inflammatory disorder, atherosclerosis causes tissue damage and fibrosis.[2,3] However, the ongoing stimuli promoting such chronic inflammation is still unclear.

Studies have shown that alterations in gut microbial composition in obese patients are associated with the progression of atherosclerosis.[4,5] The most obvious change is the reduction in the proportion of *Bacteroidetes* and the increase in *Firmicutes*.[4,5] Of note, the latter mainly consists of Gram-positive bacteria such as Staphylococcus *spp*.[6] Such changes in the microbiota may induce chronic low-grade inflammation in host

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gut,[7,8] and hypothetically, further accelerate the development of cardiovascular diseases such as atherosclerosis. In addition, many microorganisms, including chlamydia, mycoplasma, bacteria and viruses, have been detected in human atherosclerotic plaques.[2,9,10] Moreover, increasing evidence suggests that these pathogens may facilitate atherosclerotic processes by directly infecting vascular cells and/or via indirectly inducing cytokines and acute phase proteins by infecting non-vascular sites.[2,11] Therefore, infectious agents are now increasingly considered to be risk factors for atherosclerosis.

Staphylococcus aureus (*S. aureus*) widely exists in various environments, and the highly virulent strains cause substantial morbidity and mortality. Roughly 30 to 40% of individuals are asymptomatically colonized with *S. aureus* on their mucosal surfaces.[12,13] For obese people, the rates of *S. aureus* nasal colonization and skin infection are higher than those in lean individuals.[14] Considering the robust positive correlation between obesity and atherosclerosis[7] and the proposed role of microbes in obese subjects, it is likely that the colonization and infection of *S. aureus* in obese people are conducive to the formation of atherosclerosis.

S. aureus pathogenicity is mediated by numerous virulence factors,[15] among which the staphylococcal superantigens (SAgs) play a key role.[16] toxic shock syndrome toxin-1 (TSST-1), an SAg with the capacity to boost systemic inflammation, possesses the quintessential characteristics of pyrogenicity, including the initiation of non-cognate antigen T-cells and promotion of the toxic effects of endogenous endotoxins.[13,16] Although these findings suggest a positive association between *S. aureus* SAg TSST-1 exposure and atherosclerosis, there is no direct evidence for a causal relationship between TSST-1 and atherosclerosis.

The objective of this study was to investigate the pathogenic role of TSST-1 exposure in atherosclerosis progression using a rabbit model. We hypothesized that TSST-1 may contribute to the progression of atherosclerosis through its inflammation-causing effects. To test our hypothesis, we administered TSST-1 with miniosmotic pumps to New Zealand White rabbits, and evaluated aortic atherosclerosis using histological and morphometric methods. Possible mechanisms involved in TSST-1-mediated effects were also explored.

MATERIALS AND METHODS

Animal model

All animal experiments were carried out under the approval of the Animal Ethics Committee of Shanghai Jiaotong University School of Medicine and conducted in accordance with the principles of laboratory animal care. Twenty male New Zealand White rabbits (8 months old at the beginning of the experiment) were obtained from the Experimental Animal Center, Shanghai General Hospital. The animals were maintained in a facility with constant temperature (25 ± 2 °C), a 12-hour light and dark cycle and free access to water and laboratory diet. After 1 week of adapting to the new environment, the animals were randomly divided into four groups of five animals each, designated as the Normal Diet (ND) group, Normal Diet+TSST-1 (ND+TSST-1) group, High-Fat Diet (HFD) group, and High-Fat Diet+TSST-1(HFD+TSST-1) group. The ND and ND+TSST-1 groups were fed standard rabbit chow, and the HFD and HFD+TSST-1 groups received the same diet but containing 2% (w/w) cholesterol and 6% (w/w) lard. The body weight of the rabbits was monitored every 3 weeks. After 6 weeks of feeding as described above, Alzet miniosmotic pumps (DURECT Corp., CA, USA), containing either TSST-1 (1.3 mg/mL, 200 μL) or PBS (200 μL) with a constant pumping rate of 0.15 μL/h for 42 days, were implanted subcutaneously in the right lumbar regions of sodium pentobarbital-anesthetized rabbits. Alzet miniosmotic pumps were used in order to obtain a chronic and constant release of TSST-1 over the entire study period.

Preparation and purification of recombinant TSST-1

To obtain recombinant TSST-1 (rTSST-1), the expression vector of the *tst* gene was constructed. This experiment was carried out according to the relevant guidelines issued by the Chinese government. The nucleotide coordinates of the primers were derived from the sequence of the *tst* gene encoding the mature TSST-1 in *S. aureus* N315 genome (GenBank accession number BA000018.3). Both primers, *tst*-NcoI-F (5′-CATGCCATGG CATCTACAAACGATAATATAAAGGA-3′) and *tst*-XhoI-R (5′-CCGCTCGAGATTAATTTCTGCTTCTATAGTTTTT-3′) were added with NcoI and XhoI restriction sites (underlined sequences), respectively. The 603-bp polymerase chain reaction (PCR) product was cut with NcoI-XhoI (Thermo

Fisher Scientific, MA, USA) and cloned into pET28a cut with the same endonucleases. The recombinant plasmid was then transferred into *E. coli* DH5α cells (Tiangen Biotech Co., Ltd., Beijing, China), and screened on plates containing kanamycin. The kanamycin-resistant clones were tested by PCR using universal primers T7-pro (TAATACGACTCACTATAGGG) and T7-ter (TGCTAGTTATT GCTCAGCGG). The PCR product was sequenced to confirm the success of the ligation and transformation of pET28a-TSST-1. The newly generated plasmid pET28a-TSST-1 was then transformed into the *E. coli* expression strain BL21 (DE3) for the expression of rTSST-1. The bacteria containing the recombinant plasmid were grown at 37 °C in 400 mL of LB medium with 50 μg/mL of kanamycin. Once the OD_{600} reached approximately 0.5, the expression of rTSST-1 was induced for 3 hours at 37 °C by adding IPTG (isopropyl β-D-1-thiogalactopyranoside) to a final concentration of 0.5 mM. Cells were harvested by centrifugation and re-suspended in 10 mL of Buffer A (20 mM sodium phosphate, 500 mM NaCl, 10 mM imidazole, pH 7.4), and were then broken using an UP50H sonicator (Hielsher, Germany). His-Tag protein was purified using nickel magnetic beads (Bimake, TX, USA) according to the manufacturer's instructions. The purified toxin was quantified with a Bradford protein assay (Takara, Daliang, China) and analyzed by SDS-PAGE.

Analysis of blood biochemical parameters

Blood samples at each timepoint were collected from marginal ear veins of the rabbits after 16 hours of fasting, and were allowed to clot for 90 minutes before centrifugation at 2500 g to obtain serum. All serum samples were stored at -80 °C until assay. The levels of triglycerides (TGs), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and lipoprotein (a) [Lp (a)] were measured using a DxC-800 biochemical analyzer (Beckman Coulter Inc., CA, USA). All tests were performed by the same analyzer in the same laboratory.

Measurement of serum cytokine and endotoxin levels

Serum tumor necrosis factor alpha (TNF-α) and endotoxin levels were measured using commercially available ELISA kits (Novateinbio, MA, USA) and the *Limulus* amebocyte lysate (LAL) test (Sigma-Aldrich, MO, USA), respectively, according to the manufacturer's instructions.

Morphometric and immunohistochemical analysis

All rabbits were euthanized by injection of an overdose of sodium pentobarbital solution at the end of the experiment. The aortic trees and livers were isolated and fixed in 4% buffered formaldehyde (pH 7.4). The obtained aortas were dissected and stained with Oil Red O, and the atheroma plaque area was evaluated using the image analysis software Image J. For microscopic lesion analysis, paraffin-embedded aortic arches were cross-sectioned serially into three sections (3 μm thick) and dyed with hematoxylin and eosin (H&E). The intimal and medial thicknesses were quantitatively measured using Leica Qwin Pro image analysis software. To visualize the cellular components in the lesion area, primary antibodies were used for immunohistochemical staining including muscle actin monoclonal antibody (mAb, HHF35, Enzo Biochem., NY, USA) and mAbs against macrophages (RAM11, Agilent Technologies, CA, USA). Positively immunostained areas of cells were quantified using Image J and were expressed as percentages.

Estimation of TSST-1 production *in vivo*

Based on a previous study[6] that a person colonized with TSST-1-producing *S. aureus* would be continually exposed to at least 1 μg of TSST-1, and that the hide permeability of SAgs is approximately 1/10 to 1/100 over a 24-h period, the estimate of continuous subcutaneous exposure is 10 to 100 ng for an individual. It has been shown that rabbits and humans share the same extent of susceptibility to Sags.[17] In addition, a previous study reported that a rabbit model of systemic inflammation was successfully established when the animals were exposed to 0.25 μg of TSST-1 per hour for 6 weeks.[6] However, our preliminary experiments found that exposure to this stimulus of the SAg affected the appetite of the rabbits and caused weight gaining, indicating that the dose of TSST-1 should be reduced. Therefore, in this study, a chronic and constant release of 0.2 μg of TSST-1 per hour over a 6-week period was established using the Alzet miniosmotic pumps.

Statistical analysis

All data were expressed as mean ± SD. To determine

statistically significant differences, ANOVA with a LSD post-hoc test were used to compare the measurement data among the four groups. The Student's t-test was used to compare the data of other assays. In each case, statistical significance was indicated by p values less than 0.05. SAS 9.3 for Windows software (SAS Institute Inc., NC, USA) was used for all analyses.

RESULTS

TSST-1 accelerated atherosclerotic plaque formation

Analysis of *en face* quantification of Oil Red O-stained atherosclerotic lesions revealed that TSST-1 treatment markedly promoted the lesion area, with the greatest extent at the aortic arch and far less in the abdominal aorta. The HFD+TSST-1 exposure group exhibited a more noticeable increase in total plaque formation compared with the HFD group (51.0 ± 6.67% vs. 39.4 ± 4.51%, p = 0.0122) (Figure 1A and B). The gross lesion area in the HFD+TSST-1 exposure group was enlarged by 15.3% (p = 0.0058) in the aortic arch, 3.2% (p = 0.1903) in the thoracic aorta, and 6.3% (p = 0.4257) in the abdominal aorta compared to the HFD group (Figure 1B). No visible aortic lesion area was detected in the animals in the ND+TSST-1 and ND groups (Figure 1A).

We then analyzed the morphological characteristics of atherosclerotic plaques in each group. The results showed that the intima was significantly thickened at the endpoint of the experiment in the HFD and HFD+TSST-1 groups; the mean value for the intimal/medial thickness ratio was increased by 8% in the HFD+TSST-1 group compared to the HFD group, however, the difference was not statistically significant. Although the ratio in the ND+TSST-1 exposure group was only increased by 5.3% compared with the ND group, the difference was statistically significant (p = 0.0028) (Figure 1C).

H&E staining revealed that the aortic arch structure in the ND group was characterized by neat vascular intima, media and adventitia, and full integrity. No foam cell formation was observed (Figure 2A). However, in the TSST-1-treated groups (ND+TSST-1 and HFD+TSST-1 groups), the structure of the vascular media was changed with derangement of vascular smooth muscle cells (SMCs) (Figure 2A). In contrast, the vascular media in the TSST-1-free exposure groups (ND and HFD groups) showed fewer changes (Figure 2A). Additionally, the immunohistochemical staining showed that the distribution of macrophages in the lesion areas did not differ significantly between the HFD+TSST-1 and HFD groups (Figure 2B), whereas SMC infiltration (23.0% ± 2.0% vs. 9.3% ± 2.5%, p = 0.0018) was markedly increased in the HFD+TSST-1 group compared with the HFD group (Figure 2C).

TSST-1 had a small impact on rabbit body weight and serum lipid profiles

The body weight of the rabbits and serum lipid profiles were monitored every 3 weeks during the experiment. All of the animals on a high-fat diet had a significantly increased body weight (Figure 3A) and serum levels of TGs, TC, LDL-C, HDL-C and Lp (a) (Figure 3B, C, D and F). TSST-1 exposure did not significantly affect the

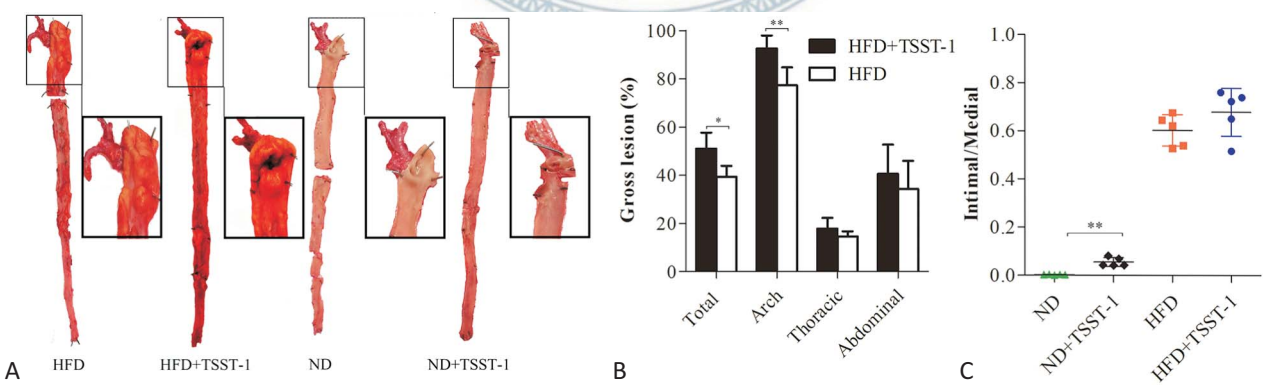

Figure 1. *TSST-1 increased atherosclerotic plaque formation in high fat diet rabbits. (A) The entire aortas were stained with Oil Red O and photographed at the end of week 12. (B) The ratio of the atherosclerotic plaque area of total aorta, aortic arch, thoracic aorta and abdominal aorta compared with the respective aortic surface area. (C) The intimal/medial thickness ratio in rabbit aorta was quantified using a digital microscope. Statistically significant differences were determined by Student's t test. * p < 0.05, ** p < 0.01. HFD, high-fat diet; ND, normal diet; TSST-1, toxic shock syndrome toxin-1.*

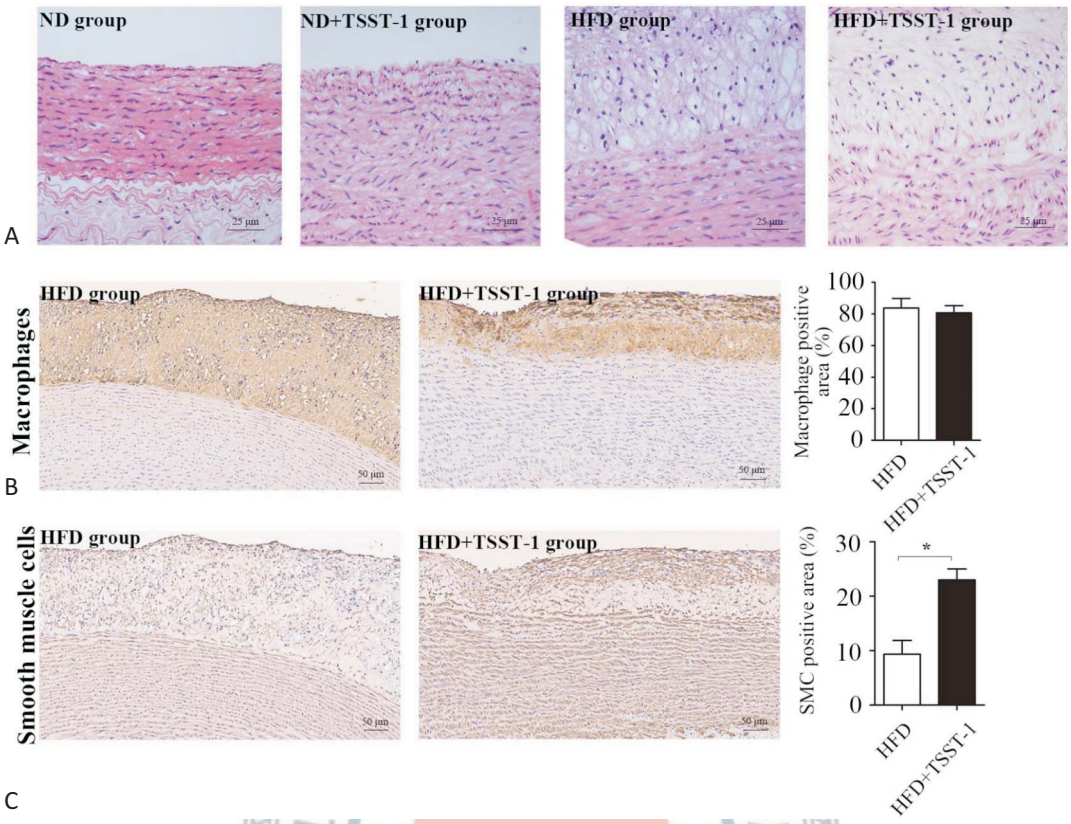

Figure 2. *Obvious migration and proliferation of vascular smooth muscle cells (SMCs) were revealed in the aortic arch of rabbits. (A) Representative micrographs of hematoxylin and eosin stained cross-sections of the aortic arch in rabbits. (B) Immunohistochemical staining with RAM11 for macrophages and quantified positive-stained areas of immunostained macrophages. (C) Immunohistochemical staining with HHF35 for SMCs and quantified positive-stained areas of immunostained SMCs. Statistically significant differences were determined by Student's t test. The values are expressed as mean ± SD. * p < 0.05, HFD+TSST-1 group versus HFD group. HFD, high-fat diet; SD, standard deviation; TSST-1, toxic shock syndrome toxin-1.*

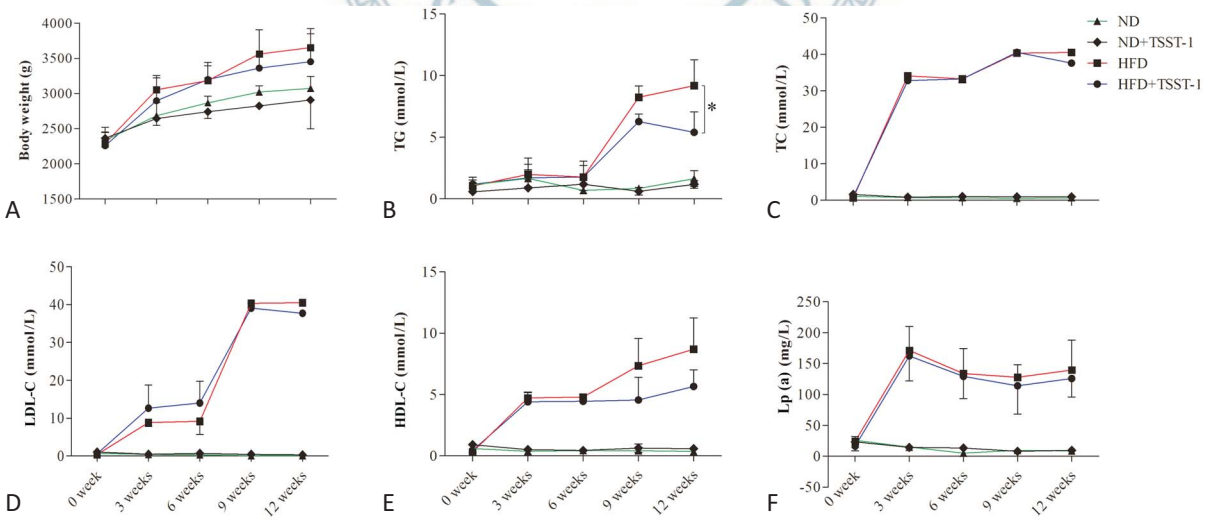

Figure 3. *Rabbit's body weight and serum lipid profile. The body weight and serum lipid level in each rabbit were measured at week 0, 3, 6, 9, 12. n = 5 for each group. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp (a), lipoprotein (a); TC, total cholesterol; TG, triglyceride. Statistically significant differences were determined by Student's t test. The results are expressed as mean ± SD. * p < 0.05, HFD+TSST-1 group versus HFD group at week 12. HFD, high-fat diet; ND, normal diet; TSST-1, toxic shock syndrome toxin-1.*

levels of the above indexes except for TGs at any time point (ND group vs ND+TSST-1 group, and HFD group vs HFD+TSST-1 group, each $p > 0.05$) (Figure 3A, C, D, E and F). Figure 3B shows that the level of TGs remarkably decreased in the HFD+TSST-1 group at week 12 compared to that in the HFD group (5.4 ± 1.7 mmol/L vs. 9.2 ± 2.1 mmol/L, $p = 0.0294$).

TSST-1 induced systemic inflammation

The results of serological assays revealed that the TSST-1-treated rabbits had markedly higher levels of TNF-α in the circulation compared to the corresponding control groups (ND and HFD groups, respectively) at week 12, and the differences were statistically significant ($p = 0.0011$ and 0.008, respectively) (Figure 4A). Although the level of TNF-α in the HFD+TSST-1 group increased more obviously in comparison with that in the ND+TSST-1 group, no statistical significance was found. These data indicated that TSST-1 treatment could promote chronic systemic inflammation in vivo.

TSST-1 did not promote the atherosclerotic process through bloodstream endotoxin levels

Our findings showed that the circulating endotoxin levels increased in the HFD-treated rabbits (HFD and HFD+TSST-1 groups) compared to the ND-treated rabbits (ND and ND+TSST-1 groups) at week 12, however, no significant differences were found between the TSST-1-treated rabbits and the corresponding controls (ND and HFD groups) (Figure 4B). Furthermore, morphometric analysis of liver tissue revealed that there were more histopathologic changes (hepatocellular steatosis formation and inflammatory cell infiltration) in the ND+TSST-1, HFD and HFD+TSST-1 groups compared to the ND group (Figure 5A, B, C and D). Although there was no obvious difference in steatosis formation between the HFD and HFD+TSST-1 groups, inflammatory infiltration was more common in the livers of the HFD+TSST-1 group than that in the HFD group (Figure 5C and D). Taken together, these findings demonstrated that TSST-1 played a role in promoting liver damage, but this was not correlated with endotoxins.

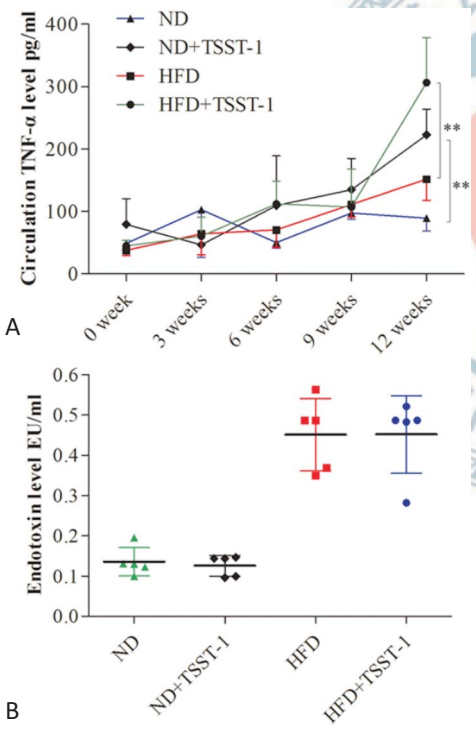

Figure 4. *TNF-α and endotoxin levels in serum collected from four groups of animals. (A) ELISA analysis of serum TNF-α levels in rabbits throughout the experiment. (B) Average endotoxin levels in the serum of four groups of animals after 6 weeks of TSST-1 or PBS treatment. Data are expressed as the means ± SD, n = 5 for each group. Statistically significant differences were determined by Student's t test. ** $p < 0.01$, ND group versus ND+TSST-1 group and HFD+TSST-1 group versus HFD group at week 12. HFD,high-fat diet; ND, normal diet; TSST-1, toxic shock syndrome toxin-1.*

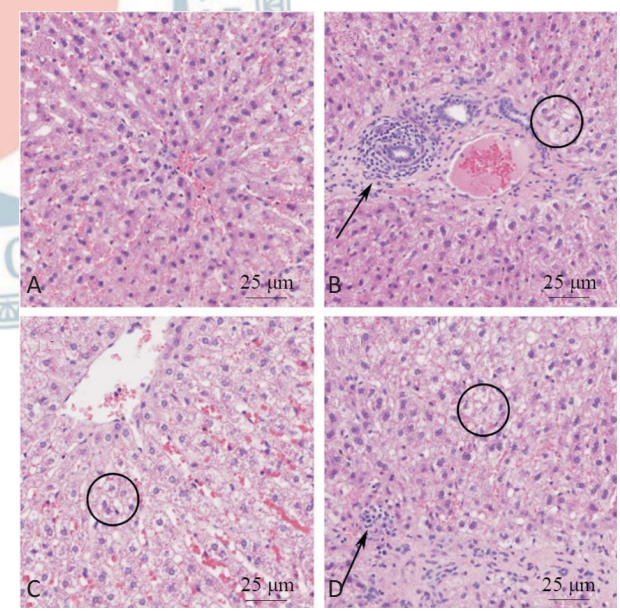

Figure 5. *Pathological changes in the liver after TSST-1 or PBS exposure for 6 weeks. Representative micrographs of rabbit liver from (A) ND group, (B) ND+TSST-1 group, (C) HFD group, and (D) HFD+TSST-1 group, stained with hematoxylineosin (H&E) after 6 weeks of TSST-1 or PBS treatment. Circles indicate areas of steatosis. Arrows highlight inflammatory cells infiltration. HFD, high-fat diet; ND, normal diet; TSST-1, toxic shock syndrome toxin-1.*

DISCUSSION

To the best of our knowledge, this is the first study to report the causal relationship between TSST-1 and atherosclerosis in a rabbit model. Our findings revealed that chronic infusion of TSST-1 significantly advanced atherosclerosis independently of endotoxins and rather through increasing systemic inflammation, as reflected by circulating TNF-α. Moreover, we demonstrated that TSST-1 exposure increased the content of smooth muscle cells within atherosclerotic lesions while it did not affect serum lipids (except for triglycerides) in the rabbits.

Inflammation is involved in many factors and is known to play an important role in the initiation and progression of atherosclerosis.[18] Putative anti-inflammatory therapeutic strategies specifically targeting factors causing chronic inflammation have attracted increasing attention.[19-22] SAgs can form a cross-bridge between various regions of the β-chain of T cell receptors (Vβ-TCRs) and α- and/or β-chains of major histocompatibility complex class II (MHC-II) molecules on antigen-presenting cells,[23] which leads to the uncontrolled release of pro-inflammatory cytokines by T cells and macrophages. It has been reported that the magnitude of immune stimulation by SAgs is usually 10-500,000-fold higher than that by conventional antigens.[23] Our findings revealed that *S. aureus* TSST-1 could promote chronic systemic inflammation in vivo and advanced atherosclerosis, indicating that TSST-1-producing *S. aureus* colonization is a potential risk factor for atherosclerosis, and that anti-TSST-1 is a promising therapeutic strategy. This is consistent with a recent study reporting that TSST-1 mini-permeated in a rabbit model of type 2diabetes mellitus (DM II) resulted in enhanced systemic inflammation.[6] However, Frodermann et al.[24] reported that heat-killed *S. aureus* could protect against the development of atherosclerosis by inducing an anti-inflammatory effect in an LDL receptor-deficient mouse model, and the authors concluded that people suffering from atherosclerosis may benefit from commensalism with *S. aureus.* This effect was reported to be mainly attributed to Staphylococcal cell wall components.[24] TSST-1 is a secretory protein that acts as a SAg to produce an uncontrolled "inflammatory cascade", of which the effect is different from *S. aureus* cell wall components. Therefore, our findings and those of Frodermann et al. are not contradictory.

SMC proliferation and migration are an essential part of remodeling[25] due to the potential expression of various receptors including LDL and VLDL receptors.[26] Inflammatory stimuli such as IL-1 and TNF-α give rise to the expression of these receptors, and this has been shown to stimulate the migration and proliferation of local SMCs.[27] In addition, TNF-α can promote foam cell formation by enhancing the binding of LDL to SMCs.[26] In this study, we found that TSST-1 exposure increased the content of SMCs in atherosclerotic lesions, indicating that TSST-1-mediated TNF-α expression may affect the phenotype of SMCs and thus the development of atherosclerosis. However, further studies are needed to investigate which signaling pathway is actually involved in the process. Another possible mechanism of TSST-1-mediated SMC migration is via endothelial cell function. TSST-1 can regulate the expression of factors involved in endothelial cell activation and function, resulting in dysregulated immune protective and pathological responses.[28] We hypothesize that endothelial cells may secrete SMC chemoattractants such as platelet-derived growth factor (PDGF) to induce SMC migration through responses to shear stress[29] and TSST-1.

A number of studies have reported that endotoxins are closely connected with atherosclerosis due to their ability to induce pro-inflammatory cytokine production and vascular dysfunction.[30] Under normal conditions, low levels of endogenous endotoxins are considered to be cleared by Kupffer cells in the liver. Previous studies have suggested that acute and chronic TSST-1treatment can induce rabbit hepatic Kupffer cell damage, and that the reduced eliminating ability of the liver then leads to the elevation of endogenous endotoxin levels in the blood of rabbits.[6,31] In this study, we found morphological distinctions of the livers between the TSST-1-treated groups and the respective controls. However, treatment with TSST-1 or PBS yielded a similar endotoxin level. One potential explanations for this difference is that we used an exposed dose of 0.2 μg of recombinant TSST-1 per hour, whereas the previous studies used a dose of 0.25 μg of the toxin per hour.[6] This may indicate that minor damage does not affect the clearing capacity of the liver. Therefore, our results suggest that TSST-1, without acting synergistically with endotoxin, could have a sig-

nificant impact on the development of atherosclerosis. Not all atherosclerotic patients suffer from liver disease, although correlations between endotoxin clearance dysfunction and atherosclerosis have been well established.[31] In addition, we also found HFD-induced endotoxemia in this study, which was not as severe as in the HFD+TSST-1 group. Endotoxins can diffuse from the gut to the systemic circulation at low concentrations via paracellular permeability or absorption by enterocytes under HFD conditions.[32] Cani et al.[33] found an obvious increase in plasma endotoxin concentration in mice fed a 4-week HFD, which is consistent with our findings.

It is interesting to note that the serum level of triglycerides, a risk factor for atherosclerosis,[34] strikingly decreased after TSST-1 treatment for 6 weeks in the HFD+TSST-1 group, although the reason for this finding is unknown (Figure 3B). Bonventre et al.[35] speculated that it was not the TSST-1 but infection itself that was responsible for the abnormality in triglycerides, since they found that both rabbits infected with TSST-producing isolates and those treated with TSST-1 antibodies displayed severe elevations of triglycerides. We believe that the possibility that TSST-1 could be linked to the reduced level of triglycerides cannot be ruled out, because other bacterial components may influence or even hide the effect of TSST-1. Further work is required to confirm the connection between TSST-1 and triglycerides. In addition, TSST-1 exposure did not affect the concentrations of rabbit serum TC, LDL-C, HDL-C and Lp (a), indicating that TSST-1 may work through inducing systemic inflammation but not changing serum lipid profile levels.

Other investigators have suggested that the recombinant TSST-1-producing strain can cause similar clinical symptoms and histopathological manifestation in rabbits to those described for humans with toxic shock syndrome (TSS).[36] Moreover, Asano and coworkers found that purified rTSST-1 was able to suppress autophagy in autophagic-induced cells in the same manner as TSST-1-secreting *S. aureus*.[37] Therefore, we assume that recombinant TSST-1 has similar function to its natural form. To avoid the requirement for technically demanding thin-layer isoelectric focusing,[38] which is a common method of toxin purification, we chose recombinant TSST-1 in this study to explore the relationship between this toxin and atherosclerosis.

There are some limitations to the present study. First, no biomarkers were detected for SMC proliferation, and in vitro investigations were lacking. Further evidence is needed to demonstrate the possible mechanism of TSST-1-induced atherosclerosis. Second, the sample size is relatively small, which may explain the discrepancy of our results that the values of intimal/medial thickness of the HFD+TSST-1 group were higher than the controls as expected, while the difference was not statistically significant ($p > 0.05$; Figure 1C).

CONCLUSIONS

The discovery of a positive link between *S. aureus* SAgs and the risk of atherosclerosis has broad health-related implications. The present study demonstrated that chronic exposure to TSST-1 may lead to enhanced aortic atherosclerosis in rabbits. Based on our data, it is possible that treatments targeting *S. aureus* colonization and SAgs may be valuable for therapeutic interventions for atherosclerosis.

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CONFLICT OF INTEREST

All authors declare no conflicts of interest.

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