Original Research *Highlight article*

CD248+CD8+ T lymphocytes suppress pathological vascular remodeling in human thoracic aortic aneurysms

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Impact statement

In spite of recent evidence indicating that CD248 is linked to microvasculature remodeling, immune response, and MMP activity, the functions of CD248 in human TAA remain unexplored. In this work, by analyzing cellular components of *in situ* as well as of blood circulation of human TAA, we have identified a novel T cell subset, the CD248+CD8+ T cells, which exhibits anti-inflammatory properties, and that we have also provided the first evidence that these cells not only suppress endothelial expression of ICAM1/VCAM1 and MMP2/3, but also inhibit endothelial migration, thus uncovering a CD248-mediated cellular mechanism against pathological vascular remodeling in human aortic aneurysms.

Abstract

Aortic aneurysms are characterized by vascular inflammation, neovascularization, and extracellular matrix destruction of the aortic wall. Although experimental studies indicate a potential role of CD248 in microvessel remodeling, the functions of CD248 in human vascular pathologies remain unexplored. Here we aimed to study how CD248 interferes with pathological vascular remodeling of human aortic aneurysms. Immunofluorescent staining showed that CD248 expression was mainly localized in the CD8+ T cells infiltrating in the adventitia and media of aortic walls of patients with ascending thoracic aortic aneurysms. qPCR and immunofluorescent staining analyses revealed increased aortic CD248 expression and infiltrating CD248+CD8+ T cells in aortic aneurysms than in nonaneurysmal aortas. Flow cytometry analysis of human peripheral blood further identified a fraction of circulating CD248+ cells which was confined in the CD8+ T-cell compartment. The increased infiltrating of CD248+CD8+ T cells was coincident with reduced circulating CD248+CD8+ T cells in patients with ascending TAA when compared with patients with coronary artery diseases and healthy donors. The CD248+CD8+ T cells were characterized by upregulated IL-10 and downregulated IL-1β/INF-γ expression when compared with CD248-CD8+ T cells. Moreover, when co-cultured with human aortic endothelial cells, the CD248+CD8+ T cells not only downregulated endothelial expression of ICAM1/VCAM1 and MMP2/3 but also suppressed endothelial migration. This study shows that CD248 reduces pathological vascular remodeling via anti-inflammatory CD248+CD8+ T cells, revealing a CD248-mediated cellular mechanism against human aortic aneurysms.

Keywords: CD248+CD8+ T cell, vascular remodeling, thoracic aortic aneurysms

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Introduction

Aortic aneurysm is defined as a localized dilatation of the aortic wall.[1] Anatomically, aortic aneurysms may occur in both thoracic and abdominal regions. Ascending TAA is the most common TAA and typically asymptomatic unless a rupture occurs, rendering a high mortality in patients suffering from this disease. Apart from some congenital TAA, which are linked to specific heritable disorders, such as Marfan syndrome, the underlying mechanisms responsible for the initiation and development of the vast majority of TAA remain elusive.[2,3] The major pathological features of human TAA include vascular inflammation, neovascularization. and extracellular matrix destruction of the aneurysmal aortic wall. It is generally assumed that immune cells infiltrating into the aortic wall trigger an early cellular response, followed by abnormal productions of proinflammatory cytokines and matrix metalloproteinases (MMPs) by inflammatory infiltrates,[4,5] resulting in destructive dilatation of the affected aortic wall.[6] Previous clinical studies further indicate that neovascularization with increased

MMPs released by invasive endothelial cells may play a key role in exacerbating vascular matrix remodeling and destroying structural integrity of the aortic wall.[7]

CD248, also known as endosialin or tumor endothelial marker-1, is a 165-kDa cell surface glycoprotein with a N-terminal extracellular portion followed by a Sushi domain and three EGF-like domains.[8] The N-terminal extracellular portion shares the typical C-type lectin domain of immunoglobulin superfamily, implying that CD248 may be involved in diverse immunoregulatory functions, including cell adhesion, regulation of natural killer function, tissue remodeling, and adaptive immunity.[9] CD248 was initially identified on blood vessels of various human tumors and was subsequently defined as an abnormally expressed marker of tumor endothelial cells,[9] involving in the reorganization of tumor blood vessels.[10] Recently, emerging observations indicate that CD248 is also expressed in non-endothelial perivascular cells, such as pericytes,[11] fibroblasts,[12] mesenchymal cells,[13] and CD8+ T cells,[14] arguing that CD248-mediated actions are not confined to vascular endothelial cells but rather may extend to vascular remodeling/inflammation process. So far, the functions of CD248 in human vascular pathologies remain unexplored. Give the previous investigations showing that CD248 expression is involved in the regulation of neovascularization and MMP activity,[15,16] we hypothesized that CD248 may interfere with pathological vascular remodeling/inflammation process of human aortic aneurysms.

In this study, we show that the aortic CD248 is increased in patients with TAA, and that CD248+CD8+ T cells contribute to suppress pathological endothelial responses, therefore unveiling a CD248-mediated cellular mechanism against human aortic aneurysms.

Materials and methods

Aortic specimens and peripheral blood samples

Aortic specimens and peripheral blood samples were collected from patients with ascending TAA and patients with CAD. Aneurysmal and nonaneurysmal biopsies were obtained during aneurysmal repair and coronary artery bypass surgery, respectively.

Clinical inclusion criteria for aneurysmal group: patients diagnosed as aortic aneurysm criteria including: 1. Symptoms: feeling fatigue, chest tightness, precordial pain, dyspnea, edema, unable to supine, and other symptoms; 2. Signs: the third and fourth intercostal diastolic murmur of left sternum can be heard; 3. Auxiliary examination: ECG, chest X-ray, echocardiography, CT or MRI, which showed that the diameter of aortic root was larger than 5 cm. Clinical exclusion criteria for aneurysmal group: patients with Marfan's syndrome, autoimmune diseases, benign or malignant tumor were excluded. Clinical inclusion criteria for control group: healthy donors who are completely normal.

Control blood samples were taken from age- and gender-matched anonymous healthy donors. Informed consent was obtained in accordance with a protocol approved by the Local Research Ethics Committee at Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University. The study was performed conforming to the Declaration of Helsinki.

Immunofluorescence staining

Immunofluorescence staining was performed as described. Briefly, human aortic wall cryosections (5 μm) were fixed and incubated with 5% donkey serum (Jackson ImmunoResearch), followed by staining with monoclonal anti-human CD3, CD8, CD68 (each 1:100; invitrogen), MMP2 (1:100; Millipore), or MMP3 (1:100; Thermo Scientific), and rabbit polyclonal anti-human CD31 (1:100; Santa Cruz), or CD248 (1:100; Abcam) antibodies. After washing in 1×PBS, sections were incubated with Alexa Fluor® 488 donkey anti-mouse IgG (H + L) (1:200; invitrogen) and Alexa Fluor® 555 donkey anti-rabbit IgG (H + L) (1:200; invitrogen), and subsequently washed in 1×PBS and counterstained with DAPI (1:1000). Stained sections were examined under Nikon Eclipse 80i microscope (Nikon).

Semi-qPCR and qPCR

Total RNA was isolated from samples using TRIzol Reagent (Takara) and reverse transcribed to cDNA using reverse transcription system (Promega). Primers used for semi-qPCR and qPCR amplifications were listed in Supplemental Table 1. The relative mRNA level was calculated using β-actin as housekeeping gene.

Isolation and chracterization of ex vivo circulating CD248+CD8+ T cell population

Peripheral blood monouclear cells (PBMCs) were isolated by density gradient sedimentation. PBMCs were then stained with rabbit anti-human CD248 (1:100; Abcam), FITC/PE-conjugated mouse anti-human CD8 (BD Biosciences), and anti-rabbit secondary antibody. Flow cytometry was performed for the analysis of cell phenotype and cell sorting using a BD Accuri C6 flow cytometer or a BD Accuri FACSAria cell sorter. Data were analyzed using BD Accuri C6 flow cytometer.

Effects of T cells on VCAM1/ICAM1 and MMP2/3 expression of endothelial cells

Primary human aortic endothelial cells (PromoCell) were co-cultured with sorted human CD248+CD8+ or CD248-CD8+ T cells, which were seeded onto a transwell membrane (8 μm, Nunc), or cultured alone in 24-well plates for 18 h. Endothelial cells were collected for PCR analysis of VCAM1/ICAM1 expression. The MMP2/MMP3 expression was detected by immunofluoresence staining. For quantitatification, both total and MMP2+ or MMP3+ endothelial cells per well (1.9 cm^2) were counted, and the percentage of MMP2+ or MMP3+ endothelial cells calculated.

Effects of T cells on endothelial cell migration by wound-healing assay

Primary human aortic endothelial cells were co-cultured with sorted CD248+CD8+ or CD248-CD8+ T cells or cultured alone for 24 h. A single scratch wound was created by removing attached cells with a pipette tip. The scratch width was 0.1 cm and the length was 3.4 cm. Images of cells migrating into the scratch field of view (×100) were recorded after 15 h or when closure of the scratch wound occurred, which was considered to represent 100% migration. This experiment was repeated three times. The migration rate was determined using ImageJ program.

Statistics

Results were depicted as mean±SEM. Comparisons were analyzed by two-tailed Student *t* test or one-way ANOVA followed by Dunnett's *post hoc* test. Differences were considered significant at $P < 0.05$.

Results

Pathological features of aortic walls of patients with TAA

The clinical characteristics of patients with TAA and CAD (nonaneurysmal control subjects) are summarized in Supplemental Table 2. We first evaluated the inflammatory infiltrates in the aortic walls of patients with TAA by immunofluorescent staining. We found more T cells in aortic wall of patients with aneurysms. Significant increase of infiltrating CD3+ T cells (39.4 vs. 6.7/mm^2; Figure 1(a), (b), and (g)) and CD8+ T cells (21.8 vs. 3.5/mm^2; Figure 1(c), (d), and (h)), as well as CD68+ macrophages (22.3 vs. 3.4/mm^2; Figure 1(e), (f), and (I)) were observed in the media of aortic aneurysms compared with nonaneurysmal aortas. Theoretically speaking, the number of CD3 cells in human peripheral blood should be about twice that of CD8 and CD68 cells. However, in the actual observation and counting, we found that the number of CD3 cells in aortic aneurysm tissue is not twice that of CD8 and CD68 cells. Perhaps in aortic aneurysm samples, the proportion of CD3 cells, CD8 cells, and CD68 cells in the tissue is different from that in the peripheral blood. Notably, the density of CD31+ microvessels was higher in both adventitia and media of aortic aneurysms compared with nonaneurysmal aortas (Figure 2(a) to (d)). Double immunofluorescent staining further demonstrated that an increased MMP2/3 expression ^was mainly co-localized in those CD31+ endothelial cells. The total area of each section was about 1 cm^2. We counted all CD31+MMP2/3+ cells and divided them by the total observed area of this section to obtain the number of CD31+MMP2/3+ cells/mm^2. In addition, the number of MMP2+CD31+ (18.2 vs. 0.7/mm^2; Figure 2(b)) or MMP3+CD31+ (20.4 vs. 0.9/mm^2; Figure 2 (d)) endothelial cells was significantly higher in the media of aortic aneurysms than nonaneurysmal aortas. It is particularly worth mentioning that 70.1% of CD31+ cells expressed MMP2, and that 75.7% of CD31+ cells expressed MMP3 in the media of aortic aneurysms. In contrast, MMP2-expressing and MMP3-expressing CD31+ cells were only 4.8% and 14.3%, respectively, in nonaneurysmal aortas. Taken together, these results indicate the potential interactions between immune cells such as T cells and endothelial cells and, moreover, in line with previous findings,[8,9] the abnormal MMP-expressing CD31+ endothelial cells

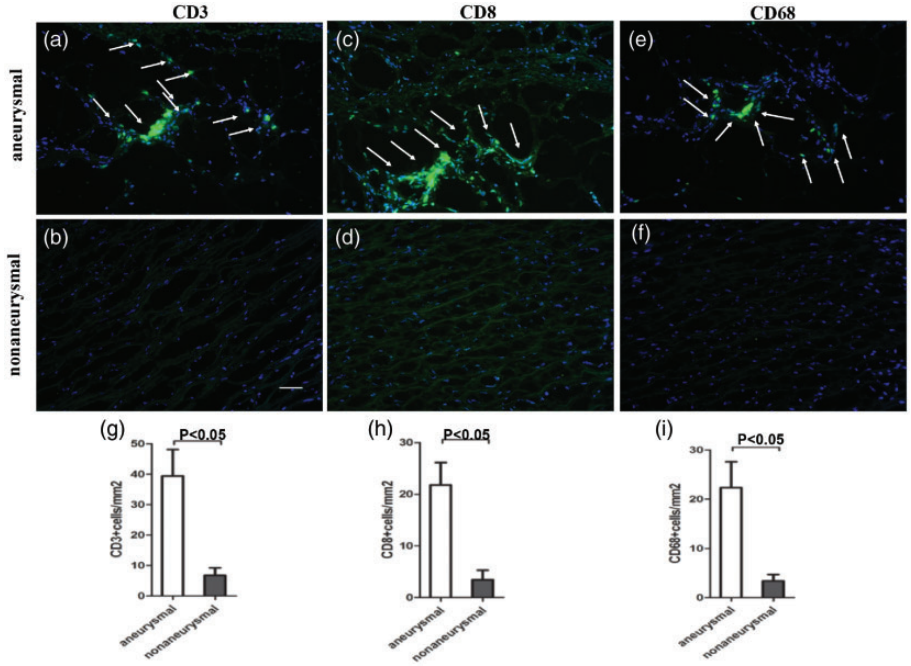

Figure 1. Increased inflammatory infiltrates in human aortic aneurysms. Representative images of stained cryosections showing CD3+ and CD8+ T cells, and CD68+ macrophages in the media of aortic aneurysms (a, c, e) and nonaneurysmal aortas (b, d, f). (g–i) Increased infiltrating CD3+ and CD8+ T cells, and CD68+ macrophages in the media of aortic aneurysms by quantitative analysis. $n = 5$ patients. Bar in Figure 1(b) stands for 50 μm and the magnification of figure (a–f) are the same.

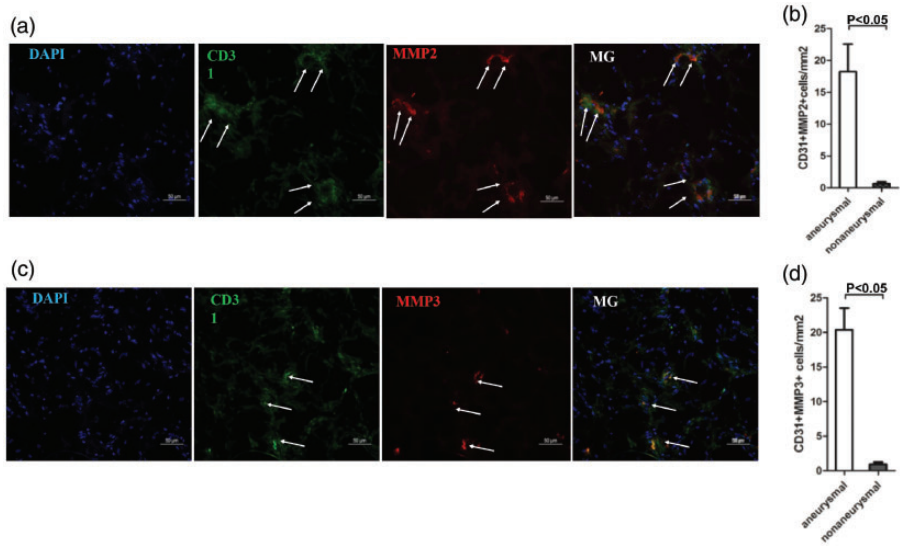

Figure 2. Increased MMP2+CD31+ and MMP3+CD31+ cells in human aortic aneurysms. (a and c) Representative images of stained cryosections showing abundant CD31+ microvessels in the media of human aortic aneurysms but not in nonaneurysmal aortas. Arrows indicate MMP2/3 expressing cells (red), CD31+ microvessels (green), or co-localization of MMP2/3 with CD31 expression (merge). DAPI (blue); (b and d)increased MMP2+CD31+ and MMP3+CD31+ cells in the media of aortic aneurysms by quantitative analysis. $n = 5$ patients. Bars in figure (a) and (c) stands for 50 μm.

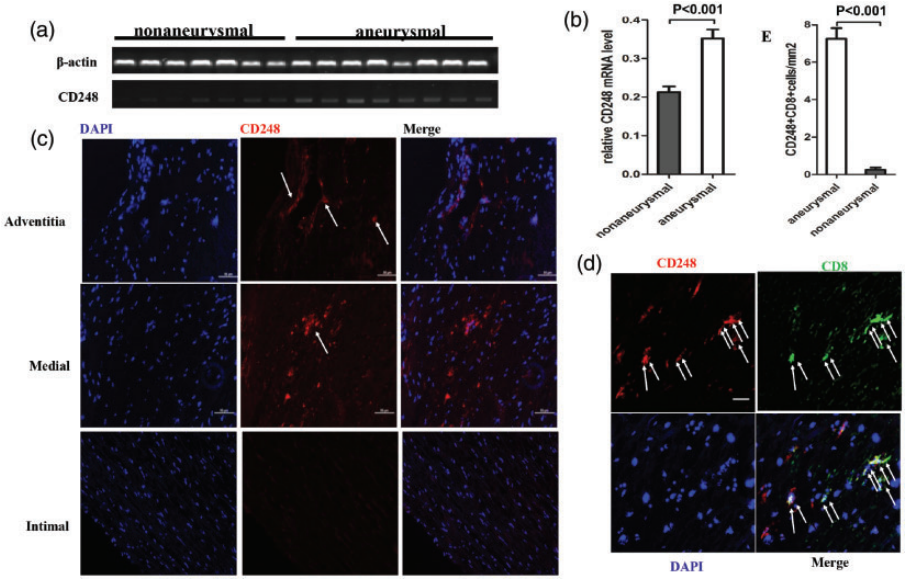

Figure 3. Increased CD248+CD8+T cells infiltrating aortic walls of patients with TAA. (a and b) Increased aortic CD248 mRNA levels in aortic aneurysms ($n = 8$) compared with nonaneurysmal aortas ($n = 7$) by semi-qPCR analysis. (c) Representative images of stained cryosections showing CD248 expression distributing in adventitia and media, but not in intima, of aortic aneurysms. Arrows, CD248+ cells (red); DAPI (blue); scale bars, 50 μm. (d) Representative images of stained aortic cryosections showing CD248 expression (red) in CD8+ T cells (green) infiltrating the media of aortic aneurysms. Arrows, CD248+CD8+ T cells; DAPI (blue); scale bars, 50 μm. (e) Increased accumulation of CD248+CD8+ T cells in the media of aortic aneurysms by quantitative analysis. $n = 5$.

could be the driving forces for pathological destruction of aortic walls of patients with TAA.

Increased CD248+CD8+T cells infiltrating aortic walls of patients with TAA

Given the previous studies showing that CD248 is closely linked to microvasculature maturation,[17] we reasoned that CD248 is also involved in pathological accumulation of microvessels in the adventitia and media of aortic aneurysms. We next examined the changes of CD248 expression in aortic walls of patients with TAA. Semi-qPCR analysis revealed that aortic CD248 expression was more abundant in aortic aneurysms than in nonaneurysmal aortas (Figure 3 (a) and (b)). Immunofluorescent staining verified that CD248 expression was mainly localized in CD8+ T cells infiltrating adventitia and media but not intima of aneurysmal aortic walls (Figure 3(c) and (d)). The infiltrating CD248+CD8+ T cells were significantly increased in aortic aneurysms than in nonaneurysmal aortas (7.3 vs. 0.3/mm^2; Figure 3(d) and (e)). The discovery of increased CD248+ CD8+ T cells infiltrating aneurysmal aortic walls

points towards a potential role of CD248 in interacting with aortic remodeling processes, probably by mediating T cell-involved cellular responses in patients with TAA.

Decreased circulating CD248+CD8+ T cells in patients with TAA

Because tissue infiltrating T cells are recruited from the circulating blood, we decided to analyze the CD248+CD8+ T cells from peripheral blood of patients with TAA and healthy donors. Both semi-qPCR (Figure 4(a)) and qPCR (Figure 4(b)) analyses revealed that CD248 mRNA level was significantly decreased in PBMCs of patients with TAA when compared with healthy donors.

In contrast, there were no differences in total CD248+ cells or CD248+CD8+ T cells between patients with CAD and healthy donors (Figure 4(c)). Interestingly, CD248+ cells were enriched only in the CD3+CD8+, but not CD3+CD4+, T cell subpopulation (Figure 4(d)). Around 4.5×10^4 CD248+CD8+ T cells, corresponding to 12.3% of circulating CD8+ T cells, were obtained from 1 mL of peripheral blood of healthy donors, while in contrast, only 1.4×10^3 CD248+CD8+ T cells, accounting for 0.3% of circulating CD8+ T cells, were isolated from that of patients with TAA (Figure 4(e)). Semi-qPCR analysis confirmed that sorted CD248+CD8+ T cells indeed expressed CD248 but CD248-CD8+ T cells did not (Figure 4(f)). These results show that human peripheral blood consists of CD8+CD248+ T cells which appear to undergo adaptive emigration in response to aortic aneurysmal injury.

Cytokine expression of CD248+CD8+ T cells

Vascular inflammation is a hallmark of aortic aneurysms. Because CD8+ T cells may interact with local inflammatory responses by the release of cytokines, we next determined the expression of immune-regulatory cytokines, including anti-inflammatory IL-10 and proinflammatory IL-1β and INF-γ, in *ex vivo* isolated circulating CD248+CD8+ and CD248-CD8+ T cells. Semi-qPCR analysis (Figure 5(a) and (b)) showed that IL-10 mRNA level was significantly upregulated by 1.49-fold, whereas IL-1β and INF-γ mRNA level was significantly downregulated by 1.68-fold and 1.57-fold, respectively, in CD248+CD8+ (vs. CD248-CD8+) T cells. These data suggest that the CD248+CD8+ T cells may be involved in modulating local inflammatory milieu of aortic aneurysms, through producing anti-inflammatory IL-10 but suppressing pro-inflammatory cytokines.

Effects of CD248+CD8+ T cells on ICAM1/VCAM1 expression in aortic endothelial cells

One of the important responses of the endothelium to vascular inflammation is the expression of key adhesion molecules, such as ICAM1/VCAM1, which mediate the emigration of blood leukocytes into vascular wall, initiating inflammatory infiltrates.[18] We next investigated the influence of CD248+CD8+ T cells on ICAM1/VCAM1 expression in co-cultured human aortic endothelial cells. Interestingly, ICAM-1 and VCAM1 mRNA level was significantly upregulated by 1.16-fold and 1.64-fold, respectively, in human aortic endothelial cells co-cultured with

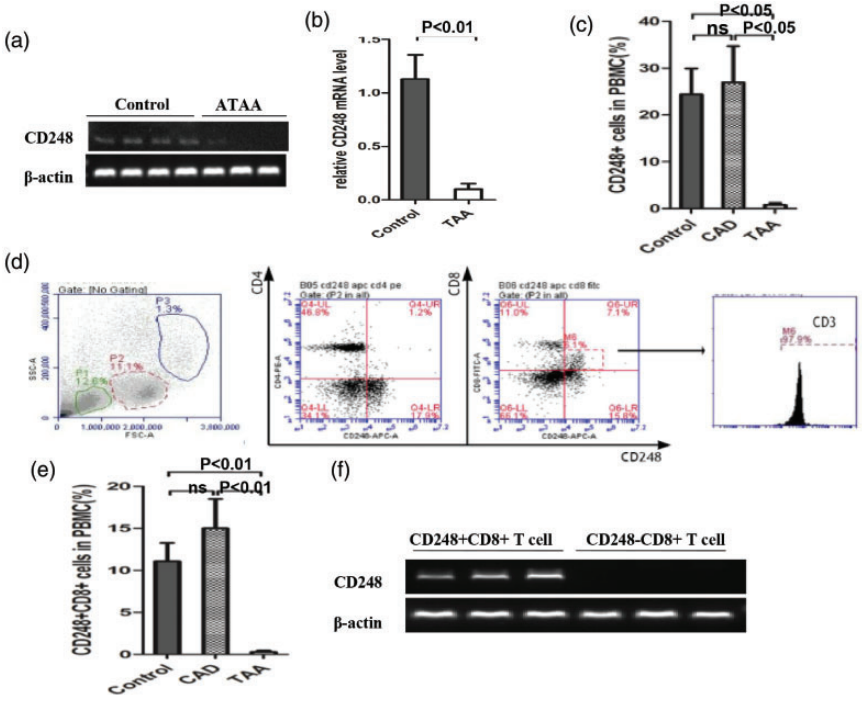

Figure 4. Reduced circulating CD248+CD8+ T cells in patients with TAA. (a and b) Downregulated CD248 mRNA in PBMC of patients with TAA ($n = 3$) compared with that of healthy donors (control) ($n = 4$) by semi-qPCR (a) and qPCR (b) analysis. (c) Comparison of frequencies of total circulating CD248+ cells from PBMCs of patients with TAA ($n = 12$), of healthy donors (control) ($n = 10$), or of patients with CAD ($n = 7$). (d) Flow cytometric analysis showing that circulating CD248+ cells are largely enriched in CD3+CD8+ T-cell but not in CD3+CD4+ T-cell compartment. (e) Reduced circulating CD248+CD8+ T cells in patients with TAA ($n = 12$), vs. control ($n = 10$) or CAD ($n = 7$); ns: not significant. (f) semi-qPCR analysis ($n = 3$) confirming the CD248 expression by CD248+CD8+ but not CD248-CD8+ T cells.

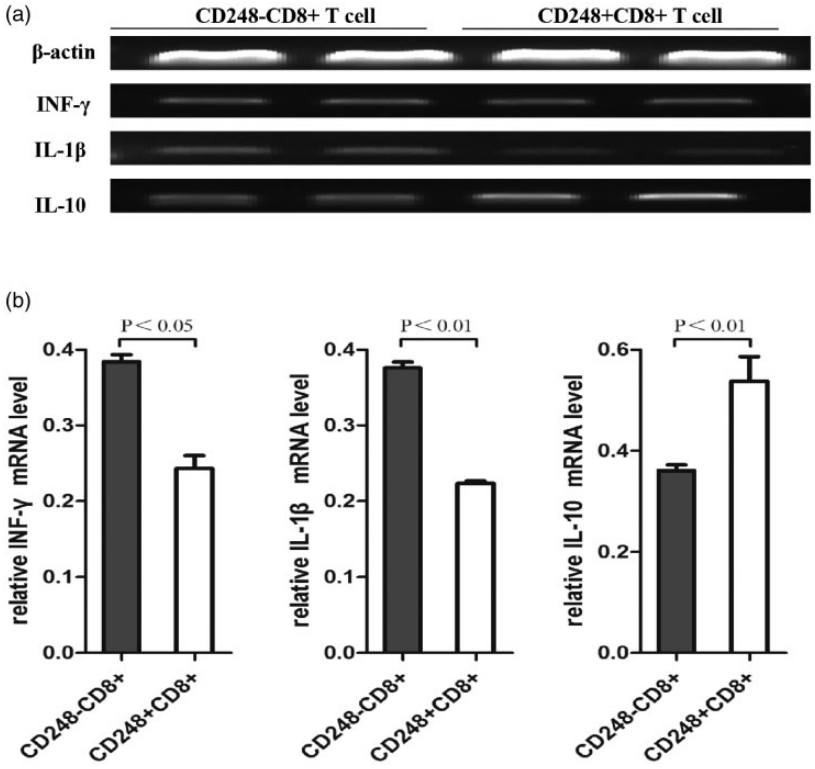

Figure 5. Cytokine expression profile of *ex vivo* isolated CD248+CD8+ T cells. (a and b) Semi-qPCR analysis showing upregulated IL-10 but downregulated IL-1β/IFN-γ mRNA in sorted CD248+CD8+ T cells compared with CD248-CD8+ T cells. $n = 3$.

CD248-CD8+ T cells, compared with endothelial cells cultured alone (Figure 6(a) and (b)). In contrast, ICAM1 and VCAM1 mRNA level was significantly downregulated by 1.29-fold and 1.49-fold in human aortic endothelial cells co-cultured with CD248+CD8+ T cells, compared with endothelial cells cultured alone (Figure 6(a) and (b)). These data indicate that CD248+CD8+ T cells may help dampen vascular inflammatory response of aortic aneurysms, through reduced expression of key adhesion molecules by aortic endothelial cells.

Effects of CD248+CD8+T cells on MMP2/3 expression in aortic endothelial cells

One of the featured pathologies of aortic aneurysm is MMPs-induced destruction of aortic media.[8] In light of the observations that aneurysmal aortic walls were characterized by accumulating MMP2/3-expressing CD31+ endothelial cells in the media (Figure 2(a) to (d)), we decided to address the effects of CD248+CD8+ T cells on MMP2/3 expression in co-cultured human aortic endothelial cells. The expression of MMP2 and MMP3 was significantly induced in aortic endothelial cells co-cultured with CD248-CD8+ T cells, compared with aortic endothelial cells cultured alone (Figure 6(c)). However, the MMP2 and MMP3 expression was significantly reduced in aortic endothelial cells co-cultured with CD248+CD8+ T cells (Figure 6 (c)). These data demonstrate that CD248+CD8+ T cells may be involved in protecting against destructive vessel remodeling by suppressing MMPs production in human aortic endothelial cells.

Effects of CD248+ CD8+ T cells on migration of aortic endothelial cells

Pathological neovascularization, a major feature of aortic aneurysms, is closely associated with MMPs-induced degradation of vascular matrix and increased migration capacities of vascular endothelial cells.[2,8,9] Therefore, we further assessed the potential impact of CD248+CD8+ T cells on migration capacity of human aortic endothelial cells. In a cellular wound assay, the CD248-CD8+ T cells led to an increased migration capacity of co-cultured aortic endothelial cells by 1.21-fold (Figure 6(d) and (e)), in comparison to aortic endothelial cells cultured alone. Notably, CD248+CD8+ T cells significantly suppressed migration capacity of co-cultured aortic endothelial cells by 1.23-fold (Figure 6(d) and (e)), when compared with aortic endothelial cells cultured alone. These data indicate that CD248+CD8+T cells help reduce pathological vascular remodeling through inhibiting abnormal formation of neovascularization during human aortic aneurysms.

Discussion

The pathogenesis of TAA is characterized by vascular inflammation, neovascularization, and extracellular matrix destruction of aneurysmal aortic wall.[19] In spite of recent evidence indicating that CD248 is linked to microvasculature remodeling, immune response, and MMP activity,[20] the functions of CD248 in human TAA remain unexplored. In this work, by analyzing cellular components of *in situ* as well as of blood circulation of human TAA, we have identified a novel T cell subset, the CD248+CD8+ T

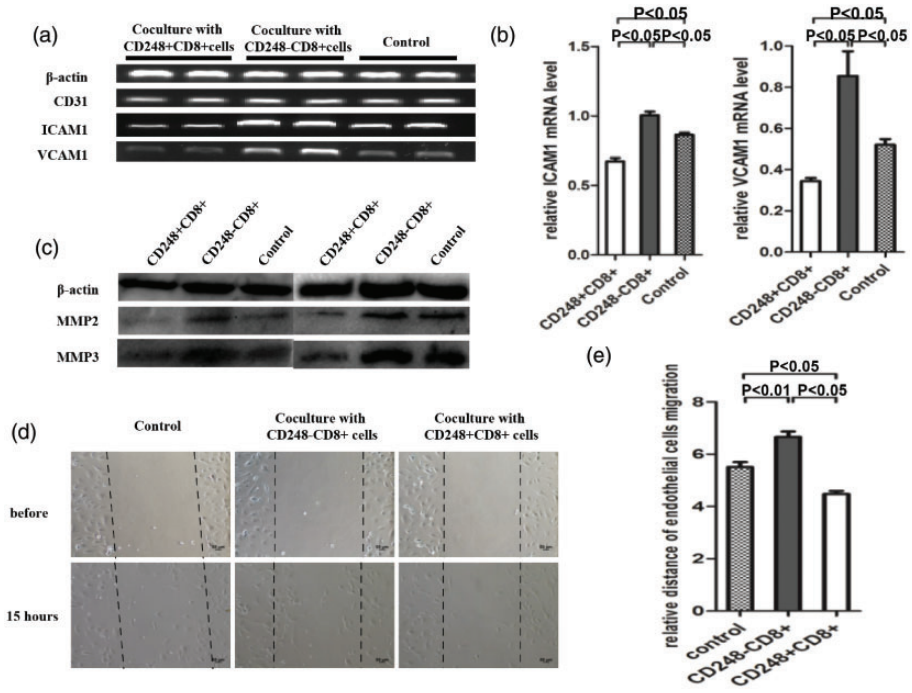

Figure 6. Effects of CD248+CD8+ T cells on pathological endothelial responses. (a and b) semi-qPCR analysis showing that VCAM1/ICAM-1 mRNA expression levels in human aortic endothelial cells are upregulated by co-cultured CD248-CD8+ T cells but downregulated by co-cultured CD248+CD8+ T cells, when compared with endothelial cells cultured alone (control). $n = 3$ different samples. (c) Western blot of MMP2/MMP3 expression in human aortic endothelial cells co-cultured with CD248-CD8+ or CD248+CD8+ T cells or cultured alone. (d) *In vitro* migration of human aortic endothelial cells co-cultured with CD248-CD8+ or CD248+CD8+ T cells or cultured alone, by cellular wound-healing assay. Scale bar, 50 μm. (e) Quantitative analysis showing the migration capacity of human aortic endothelial cells is increased by co-cultured CD248-CD8+ T cells but suppressed by co-cultured CD248+CD8+ T cells, when compared with endothelial cells cultured alone (control). $n = 10$.

cells, which exhibits anti-inflammatory properties, and that we have also provided the first evidence that these cells not only suppress endothelial expression of ICAM1/VCAM1 and MMP2/3, but also inhibit endothelial migration, thus uncovering a CD248-mediated cellular mechanism against pathological vascular remodeling in human aortic aneurysms.

CD248 shares the typical C-type lectin domain of the immunoglobulin superfamily, potentiating a role for CD248 in regulating innate and adaptive immunity.[21] Although initially identified on tumor blood vessels and defined as an abnormally expressed marker of tumor endothelial cells,[22] CD248 has been recently shown to be expressed in non-endothelial[23] immunocompetent cells, including CD8+ T cells,[24,25] supporting its potential link to T cell-involved immune reaction. Vascular inflammatory reactions seem to play a pivotal role in the development and progression of aortic aneurysms.[17] In the aortic tissues of patients with TAA, here we found that aortic CD248 was significantly increased mainly by the CD8+ T cells (CD248+CD8+) infiltrating the adventitia and media of aortic aneurysms. Interestingly, the recruitment of CD248+CD8+ T cells *in situ* was coincident with the reduced circulating CD248+CD8+ T cell subpopulation in patients with TAA when compared with patients with CAD and healthy subjects, strongly indicating that CD248 may participate in pathological vascular remodeling via infiltrating CD8+ T cell-involved vascular inflammatory response, particularly in patients with TAA. In support of this notion, similar phenomenon has been previously observed in studies showing increased Th1-cell activity in the inflamed joint but reduced Th1 response in the periphery.[18]

Naive CD8+ T cells can be differentiated into different subsets with diverse immune functions.[26] It has been previously reported that CD8+ regulatory T cells, which produce predominantly anti-inflammatory cytokines like IL-10, can be generated from circulating naive CD8+ T cells of healthy donors[27] or identified in the heart, spleen, and circulating blood of rats after myocardial infarction,[28,29] as well as in livers of patients with chronic hepatitis C virus infection.[30] In this study, the human CD248+CD8+ T cells also exhibited immune regulatory potential, because they express IL-10 but not IL-1β/INF-γ. Localized adhesion and transmigration of inflammatory infiltrates across the endothelial barrier are attributable to locally expressed adhesion molecules such as ICAM1/VCAM1.[31] The CD248+CD8+ T cells appear to act in eliminating adhesion and transmigration of inflammatory infiltrates into the aneurismal aortic walls as they were able to reduce the expression of ICAM1/VCAM1 of human aortic endothelial cells. Together, the present data indicate that the CD248+CD8+ T cells contribute to dampen pathological vascular remodeling by suppressing vascular inflammation. It would be interesting to further elucidate whether this CD248+CD8+ T cell population, as a previously unrevealed CD8+ T cell subset, could possess additional immunoregulatory properties in controlling vascular inflammation.

Pathological vascular remodeling involves structural and functional modifications that destabilize the ordered multilayered structure of the vascular wall.[32,33] Neovascularization, which is characterized by abnormal endothelial cell proliferation/migration and formation of new microvessels, has been previously implicated in the vascular remodeling process, occurring during the aneurysmal pathogenesis.[34] Indeed, in this study, we have shown that the density of CD31+ microvessels was higher in adventitia and media of human aortic aneurysms when compared with nonaneurysmal aortas. The process of neovascularization requires active participation of MMPs, which degrade the basement membrane and structural matrix of vascular wall, to allow for the proliferation/migration of endothelial cells.[35] Besides inflammatory infiltrates, it has been shown that the proliferating and immature neovessels are also the relevant sources of MMPs in human aortic aneurysms.[36] Our finding that the number of MMP2/3 expressing CD31+ endothelial cells was significantly increased in media of human aortic aneurysms, strengthens the important contributions of pathological endothelial response in promoting human aneurysmal pathologies. In this regard, the present study provides the first evidence that the CD248+CD8+ T cells exerted protective actions against pathological vascular remodeling by suppressing endothelial expression of MMP2/3 and inhibiting endothelial migration, revealing a CD248-mediated cellular mechanism against human aortic aneurysms. Our data seemed to contradict two previous reports that CD248 regulates tumor growth through facilitating activation of MMP-9 and cell proliferation/migration.[15,37] However, taking into account that CD248 is a CD8+ T cell-specific transcript[24] and CD248-mediated anti-proliferation effect has been exclusively shown in human CD8+ T cells,[25] it is most likely that CD248 act differentially depending on cellular context to interfere with pathological vascular remodeling via infiltrating CD8+ T cell-involved adaptive inflammatory response.

In conclusion, this study provides a new biological insight into the functional relevance of CD248 in human vascular pathologies. The present findings clearly indicate that CD248 contributes to reduce pathological endothelial responses via anti-inflammatory CD8+CD248+ T cell subpopulation, thereby unraveling a CD248-mediated cellular mechanism against human aortic aneurysms.

Authors' contributions: All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript; Jun Li and Xiaojuan Hu conducted the experiments, Jun Li supplied critical reagents, Xiaojuan Hu and Tingwu made the data analysis and wrote the manuscript, and Chenxi Wang supplied the clinical samples.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICAL APPROVAL

Informed consent was obtained in accordance with a protocol approved by the Local Research Ethics Committee at Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University. The study was performed conforming to the Declaration of Helsinki.

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SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online.

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