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Mast cells drive pathologic vascular lesions in Takayasu arteritis

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Background: Takayasu arteritis (TAK) is a large vessel vasculitis resulting in artery wall remodeling with segmental stenosis and/or aneurysm formation. Mast cells (MCs) are instrumental in bridging cell injury and inflammatory response. **Objectives:** This study sought to investigate the contribution of MCs on vessel permeability, angiogenesis, and fibrosis in patients with TAK.

Methods: MC activation and their tissue expression were assessed in sera and in aorta from patients with TAK and from healthy donors (HDs). *In vivo* permeability was assessed using a modified Miles assay. Subconfluent cultured human umbilic vein endothelial cells and fibroblasts were used *in vitro* to investigate the effects of MC mediators on angiogenesis and fibrogenesis.

Results: This study found increased levels of MC activation markers (histamine and indoleamine 2,3-dioxygenase) in sera of patients with TAK compared with in sera of HDs. Marked

expression of MCs was shown in aortic lesions of patients with TAK compared with in those of noninflammatory aorta controls. Using Miles assay, this study showed that sera of patients with TAK significantly increased vascular permeability *in vivo* as compared with that of HDs. Vessel permeability was abrogated in MC-deficient mice. MCs stimulated by sera of patients with TAK supported neoangiogenesis (increased human umbilic vein endothelial cell proliferation and branches) and fibrosis by inducing increased production of fibronectin, type 1 collagen, and α -smooth muscle actin by fibroblasts as compared to MCs stimulated by sera of HD.

Conclusions: MCs are a key regulator of vascular lesions in patients with TAK and may represent a new therapeutic target in large vessel vasculitis. (J Allergy Clin Immunol 2021;■■■:■■■-■■■.)

Key words: Large vessel vasculitis, mast cell, vascular remodeling, Takayasu

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Large vessel vasculitis (LVV) includes mainly Takayasu arteritis (TAK) and giant cell arteritis. LVV may lead to segmental stenosis, occlusion, dilatation, and/or aneurysm formation in the aorta and/or its main branches and is found in 50% to 70% of patients with TAK.^{1,2} In LVV, histological lesions are characterized by an inflammatory infiltrate located mainly in the inner part of the aortic wall (media) and the adventitia. Previous studies have provided evidence that LVV inflammation is driven by T_H1 and T_H17 cells in the peripheral blood and inflamed tissues.³⁻⁵ This inflammatory reaction is associated with hyperplasia of *vasa vasorum* and subsequently with a medio-adventitia sclerosis and intimal proliferation.⁶⁻⁸ The inflow of inflammatory cells in arterial inflamed walls is mainly due to their interaction with specific adhesion molecules expressed on the inflamed endothelium. Neovessels and adventitial *vasa vasorum* are the main sites where adhesion molecules for leukocytes are expressed, and therefore the main sites through which circulating leucocytes are recruited into the vascular wall. The mechanisms resulting in the vascular remodeling in LVV are still unclear. Innate immune response is instrumental in bringing immune cells to the artery and in the tissue-damaging inflammation. Mast cells (MCs) are unique, multifunctional innate immune cells of our defense system, with characteristics such as widespread tissue distribution, expression of receptors capable of recognizing both endogenous and exogenous agents, and the capability to respond rapidly to triggering factors by selective mediator release.⁹

Abbreviations used

α -SMA:	α -Smooth muscle actin
HD:	Healthy donor
HUVEC:	Human umbilic vein endothelial cell
IDO:	Indoleamine 2,3-dioxygenase
LVV:	Large vessel vasculitis
MC:	Mast cell
PDGF:	Platelet-derived growth factor
qRT-PCR:	Quantitative reverse transcriptase PCR
TAK:	Takayasu arteritis

Of note, MCs are instrumental in remodeling vascular structures as highlighted in abdominal aortic aneurysm,¹⁰ myocardial infarction,¹¹ and atherosclerosis.¹² MCs and their mediators promote leucocyte infiltration by increasing vascular permeability, inducing expression of specialized adhesion molecules on endothelial cells and by secretion of proinflammatory molecules. MCs can also affect the behavior of structural cells within inflammatory sites such as fibroblasts.¹³ Moreover, MCs are capable of producing proangiogenic factors.¹⁴

In LVV, Mäyränpää et al^{15,16} have shown a spatial association of activated MCs, T cells, and neovessels, suggesting that MCs may contribute to the pathogenesis of such diseases.

Because MCs represent an important link between cell injury and an inflammatory response that can lead to vicious circle driving vascular remodeling, we aimed to investigate the implication of MCs in vascular lesions of patients with TAK.

METHODS**Patients**

The study population consisted of 30 patients with TAK (median age at diagnosis: 32.4 [interquartile range, 27.2; 53.2] years), fulfilling the international criteria for TAK.¹⁷ Clinical characteristics of patients with LVV are presented in Table I. Patients treated with biologics and/or steroids >10 mg/day were excluded. Blood samples from 20 healthy donors (HDs) >18 years of age and sex-matched, obtained from the Etablissement Français du Sang (Hôpital Pitié-Salpêtrière), were used as controls (15 women (75%), median age, 35.2 [interquartile range, 29.0; 35.1] years). Aorta biopsies were obtained from patients with TAK undergoing surgical repair for aortic aneurysm or dissection in our hospital center. Six noninflammatory aorta biopsies were provided by Saint Louis Hospital's human tissue bank and obtained from vessels taken for transplant in multiorgan and tissue donors. The study was approved by the Paris VI ethics review board and was performed according to the Helsinki Declaration. All patients gave informed consent.

Patient involvement

We provided instructional support to foster knowledge of research concepts and terminology. If the patients asked, we provided them information about the course of the study.

Culture of human MCs

Human ROSA^{KIT^{WT}} MCs^{18,19} were cultured exactly as previously described: they were cultured in Iscove's Modified Dulbecco's Medium (ref 21980-032, Gibco, Thermo Fisher Scientific, Waltham, Mass) including the following supplements (penicillin 1%, streptomycin 1%, sodium pyruvate 1%; Minimum Essential Medium Vitamin solution 1%; Minimum Essential Medium Non Essential Amino Acids Solution 2%; and L-Glutamine 1%, Insulin-Transferrin-Selenium 1%, Gibco; Bovine Serum Albumin 0.3% CHO-KL 10%, Sigma-Aldrich, St Louis, Mo), in the presence or the absence

TABLE I. Main demographical and clinical characteristics of the cohort of patients with TAK studied

	TAK N = 30
Demographic features	
Age, median (IQR)	32.4 (27.2; 53.2)
Female	24 (79.6)
Geographic origin	
Caucasian	11 (33)
African	8 (27)
North African	8 (27)
Other	3 (10)
Clinical features	
Numano classification	
I	4 (13)
II	5 (17)
III	3 (10)
IV	1 (3)
V	17 (57)
Ischemic cardiac disease	3 (10)
Stroke	6 (20)
Aortic aneurysms	11 (37)
Treatment	
Steroids <10 mg/d	20 (66)
DMARDs	15 (50)
Biologics	0 (0)

DMARDs, Disease modifying antirheumatic drugs; IQR, interquartile range. Values are n (%) unless otherwise indicated.

of recombinant IL-33 (10 ng/mL) or of neutralizing anti-IL33 (10 μ g/mL), IgE (2 μ g/mL; ref 401152, Merck Millipore, Burlington, Mass). MCs were also cultured with quercetin (Q4951, Sigma-Aldrich), cromolyn (C0399, Sigma-Aldrich), and neutralizing IL-6 antibody (MAB2061, R&D Systems, Minneapolis, Minn). Levels of histamine (ELISA Kit, IBL International GmbH, Tecan Group, Männedorf, Switzerland) and total tryptase (α and β) (ImmunoCAP Tryptase, Thermo Fisher Scientific) were measured in culture supernatants 1 hour after stimulation. Levels of platelet-derived growth factor (PDGF) and of TGF- β (Milliplex, Merck Millipore) were measured 5 days after stimulation.

Immunohistochemistry

Detection of IL-33⁺, ST2⁺, and tryptase⁺ cells was performed on fixed, paraffin-embedded samples (aorta) from patients with TAK and noninflammatory aorta controls. After dewaxing in baths of xylene and ethanol, slides were submitted to antigen retrieval by heating (110°C) in citrate buffer at pH 6.0. Before incubation with primary antibodies, Fc receptors were blocked with normal goat serum (3.3%). Slides were then incubated overnight with mouse monoclonal anti-IL-33 (dilution 1:1000; Enzo Life Sciences, Farmingdale, NY), rabbit polyclonal anti-ST2 (dilution 1:100; Sigma-Aldrich), monoclonal mouse anti-tryptase (1/200; Dako, Agilent, Santa Clara, Calif), or with isotype control: polyclonal rabbit IgG or monoclonal mouse IgG (Abcam, Cambridge, UK). Slides were then incubated for 2 hours at room temperature with Cy3-conjugated goat anti-mouse immunoglobulin (working dilution 1:1000; Jackson ImmunoResearch, West Grove, Pa) and Alexa 488 donkey anti-rabbit Ig (working dilution 1:1000; Life Technologies, Thermo Fisher Scientific), mounted in Mowiol and evaluated under fluorescence microscopy (Leica Microsystems, Wetzlar, Germany). MCs were also quantified by counting the number of tryptase⁺ cells per field in 100 fields per sample.

Gene expression quantification at the mRNA level in aortic tissues and fibroblasts

Quantification of mRNA expression was performed on paraffin-embedded samples from patients and controls with noninflammatory aorta aneurysms.

Quantification of mRNA expression was also performed on fibroblast cultured with serum or with MC supernatant. TGF- β and PDGF gene expression was analyzed. The theoretical and practical aspects of real-time quantitative reverse transcriptase-PCR (qRT-PCR) using the ABI Prism 7900 Sequence Detection System (PerkinElmer Applied Biosystems, Foster City, Calif) have been described in detail elsewhere. Briefly, total RNA was extracted using the High Pure FFPE RNA Isolation Kit (Roche Diagnostics, Rotkreuz, Switzerland) and was reverse-transcribed before real-time PCR amplification. Quantitative values were obtained from the threshold cycle number at which the increase in the signal associated with exponential growth of PCR products began to be detected using analysis software, according to the recommendations of the manufacturer (PerkinElmer Applied Biosystems).

In vivo permeability assays

All animal experiments were performed in compliance with the guidelines for the care and use of laboratory animals and were approved by the Local Ethics Committee (approval 13-14122011). *In vivo* permeability was assessed using a modified Miles assay.²⁰ Briefly, Evans blue dye (Sigma-Aldrich) was retro-orbitally injected into C57BL/6 mice (used as wild-type controls) and into MC-deficient C57BL/6 Kit^{W-sh/W-sh} mice (carrying a natural mutation in the MC growth factor cKIT)²¹ at 8 weeks of age (100 μ L of 1% Evans blue in 0.9% NaCl). After 10 minutes, 40 μ L of serum was injected intradermally. Twenty minutes later, the shaved dorsal skin at the injection area was excised using a calibrator ring, dye was extracted with formamide overnight at 55°C, and the dye absorbance was further measured at 630 nm. For IL-33 inhibition experiments, 5 μ g/mL neutralizing anti-human IL-33 antibody (AF3625, R&D Systems) or of its corresponding isotype antibody control (AB-108C, R&D Systems) were incubated with serum for 1 hour before the injection.

In vitro angiogenesis assay

Proliferation. Subconfluent human umbilic vein endothelial cells (HUVECs) were starved for 12 hours and were then incubated for 24 hours with serum (1:10) or MC culture supernatant (1:10). Proliferation was assayed by 5-bromo-2-deoxyuridine incorporation using the Cell Proliferation Assay Kit (Roche Diagnostic).

Tube formation. Subconfluent HUVECs were starved for 12 hours. Ninety-six-well plates were coated with 50 μ L of growth factor-reduced Matrigel (BD Biosciences, San Jose, Calif) for 30 minutes at 37°C. HUVECs (20,000 cells/well) were seeded on Matrigel for 12 hours in presence of serum (1:10) or MC supernatant (1:10). Capillary-like structures and branch points were counted using ImageJ version 1.44 software (National Center for Biotechnology Information, Bethesda, Md). Each experiment was run in triplicate.

In vitro culture of fibroblasts

Arterial fibroblasts were isolated from the adventitia of noninflammatory arteries. Briefly, the adventitial layer was separated from the media layer and then digested with collagenase solution for 3 hours at 37°C. Filtered cells were washed and then cultured. Fibroblasts (20,000 per well) were thereafter incubated for 5 days with TAK or HD serum (10%). Experiments were performed with P2 or P3 fibroblasts. Arterial fibroblasts were cultured in an endothelial cell growth medium with supplements (C-22211, Endothelial Cell Growth Medium 2 Kit, PromoCell, Heidelberg, Germany).

To assess the impact of MCs on the differentiation of fibroblasts, arterial fibroblasts were also incubated for 5 days with supernatants (10%) of MCs previously cultured with TAK or HD serum for 24 hours. Production of collagen (COL1A), fibronectin, α -smooth muscle actin (α -SMA), TGF- β , and PDGF- α was assessed by qRT-PCR. Briefly, total RNA was extracted by TRIzol reagent (Invitrogen, Thermo Fisher Scientific) and reverse-transcribed using SuperScript VILO cDNA Synthesis Kit (Invitrogen) both according to the manufacturer's instruction. Gene expression was determined by real-time PCR. Each cDNA sample was amplified in triplicate using SYBR Green (Applied Biosystems, Thermo Fisher Scientific) on 7500 FAST Real-time PCR System (Applied Biosystems). The thermal cycling conditions

comprised an initial denaturation step at 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 65°C for 1 minute. Quantitative values were obtained from the threshold cycle number at which the increase in the signal associated with exponential growth of PCR products began to be detected. *GADPH* gene was used as an endogenous control.

Statistics

Continuous variables were presented with the median and extreme values or with the mean \pm SEM. Categorical variables were presented with counts and proportions. Statistical comparisons were performed by using the Mann-Whitney test for quantitative unpaired data, and the Wilcoxon matched pairs test for quantitative paired data. All statistical tests were 2-tailed with a significance level of .05. Statistical significance was evaluated using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, Calif).

RESULTS

MCs are activated and abundant in inflammatory lesions of TAK.

In vivo, we observed an increased level of MC activation markers in sera of patients with TAK compared with in sera of HDs. Histamine level (11.23 \pm 1.3 vs 7.15 \pm 0.5 nmol/L; $P = .008$), indoleamine 2,3-dioxygenase (IDO) activity (6.4% \pm 2.7% vs 3.6% \pm 0.6%; $P = .009$), and TNF- α (795 \pm 633 vs 239 \pm 135 μ mL; $P = .001$) were increased in patients with TAK compared with in HDs, respectively (Fig 1, A). Level of tryptase was not statistically different in patients with TAK and HDs ($P = .4$). We then confirmed these results *in vitro*. Human MC (ROSA^{KIT} WT cell line) stimulated with 10% sera from patients with TAK produced an increased level of histamine and IDO activity as compared to that of HDs (11.19 \pm 7.5 vs 3.2 \pm 0.2 μ mol/L; $P < .001$; and 5.9% \pm 2.2% vs 2.9% \pm 0.3%; $P = .003$, respectively) (Fig 1, B). Level of TNF- α was not different. As observed in sera, tryptase level was not statistically different in patients with TAK and in HDs ($P = .08$). In the aorta, we confirmed that MCs were markedly increased in patients with TAK as compared to noninflammatory aorta controls. Indeed, number of tryptase+ cells per field in 100 fields was 51.6 \pm 53.5 versus 3.3 \pm 3.5 ($P = .005$) in TAK versus control samples, respectively (Fig 1, C and D). Chromogenic immunohistochemistry of TAK aorta sections showed that MCs localize mostly in the adventitia layer and to a lesser extent in the intima (see Fig E1 in this article's Online Repository at www.jacionline.org). Quantification of tryptase mRNA from aorta by qRT-PCR indicated that tryptase mRNA expression aorta was increased in aorta from patients with LVV as compared to those from noninflammatory aorta controls, respectively (8.5 \pm 10.2 vs 1.3 \pm 0.2; $P = .01$) (Fig 1, E).

Increased vessel permeability in TAK is dependent on MCs

We previously showed that MCs are activated and abundant in arterial inflammatory lesions of patients with TAK. Because MCs can be an important player in vascular permeability,²² we next examined the effects of TAK sera and MCs on vascular permeability by performing a Miles assay to study Evans blue extravasation.²⁰ We showed that injection of sera from patients with TAK induce increased vessel permeability as compared to sera of HDs (0.42 \pm 0.03 vs 0.2 \pm 0.1 OD; $P = .0001$) (Fig 2, A and B). To demonstrate that vessel permeability was dependent on MCs,

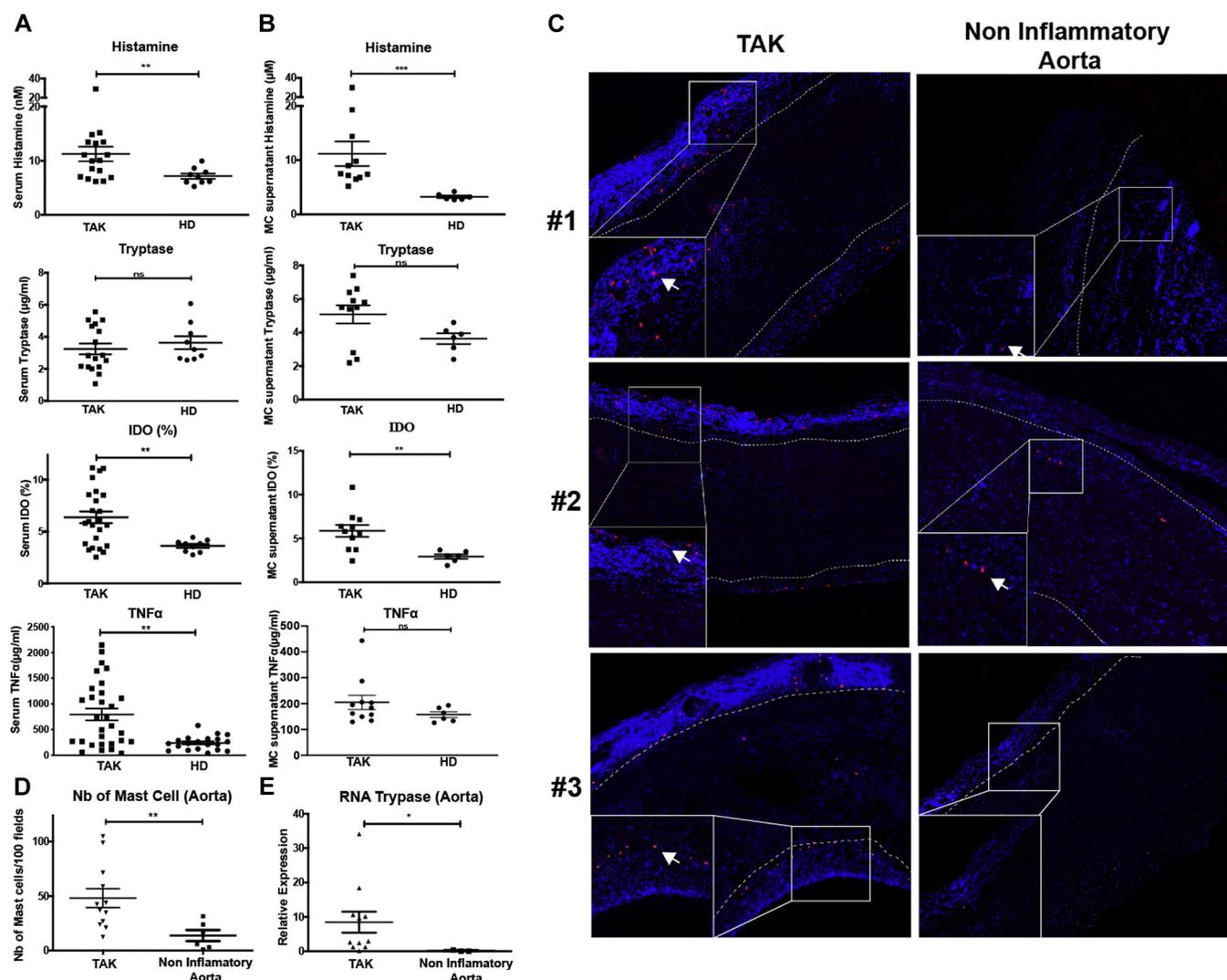


FIG 1. MCs are activated and abundant in inflammatory lesions of TAK. Levels of histamine, tryptase, IDO, and TNF- α were quantified **(A)** in the serum of HDs ($n = 9$) and of patients with TAK ($n = 17$) and **(B)** in the supernatant of human MCs cultured for 5 days with 10% of serum from patients with LVV ($n = 11$) or of HDs ($n = 5$). **(C)** Representative immunofluorescence staining of aorta obtained from 3 patients with LVV and 3 HDs. Tryptase⁺ MCs are stained in red (white arrows) and 4'-6-diamidino-2-phenylindole, dihydrochloride (DNA) in blue. Original magnification 640 \times . **(D)** Number of MCs (tryptase⁺ cells) counted per 100 fields of aorta section of 12 patients with LVV and of 6 noninflammatory controls. **(E)** Quantification of MCs (tryptase RNA) by qPCR from aorta obtained from patients with LVV ($n = 11$) and noninflammatory controls ($n = 4$). Data are shown as mean \pm SEM. For statistical analyses, Mann-Whitney test was used. * $P < .05$; ** $P < .01$; *** $P < .001$. NS, Not significant.

we then performed the same Miles assay on MC-deficient mice. Evans blue extravasation after injection of TAK sera was dramatically decreased in MC-deficient mice as compared to that observed in wild-type mice (0.2 ± 0.02 vs 0.42 ± 0.05 OD; $P < .0001$) (Fig 2, C).

We further explored the vascular permeability mechanisms induced by TAK sera and MC dependence. Because IL-33 is a well-known cytokine involved in vessel permeability and MC activation,²³ we assessed IL-33 levels in TAK sera. MC activation markers (ie, histamine and tryptase levels) were also investigated in supernatant from MC previously stimulated with and without recombinant IL-33. IL-33 sera level was increased in patients with TAK compared with in HDs (155 ± 70 vs 21 ± 19.6 pg/mL; $P = .001$) (Fig 3, A). Recombinant IL-33 significantly

increased the secretion of histamine (10% vs 1.6%; $P = .001$) and of tryptase (8.2% vs 0.5%; $P = .002$) by MCs stimulated with TAK sera with compared those stimulated with HD sera (Fig 3, B). Moreover, IL-33 blockade in sera with an IL-33 neutralizing antibody significantly reduced levels of histamine and tryptase in MC supernatant (see Fig E2, A in this article's Online Repository at www.jacionline.org). In addition, MCs and ST2 (the IL-33 receptor) colocalized in aortic lesions of patients with LVV (Fig 3, C). Lastly, Evans blue extravasation was significantly decreased by the addition of neutralizing anti-IL-33 antibody (anti-IL33) (0.42 ± 0.03 vs 0.32 ± 0.03 OD; $P = .001$) (Fig 3, D and E). Altogether, these data showed that increased vessel permeability in TAK is dependent on MCs and at least partially on mediators such as IL-33.

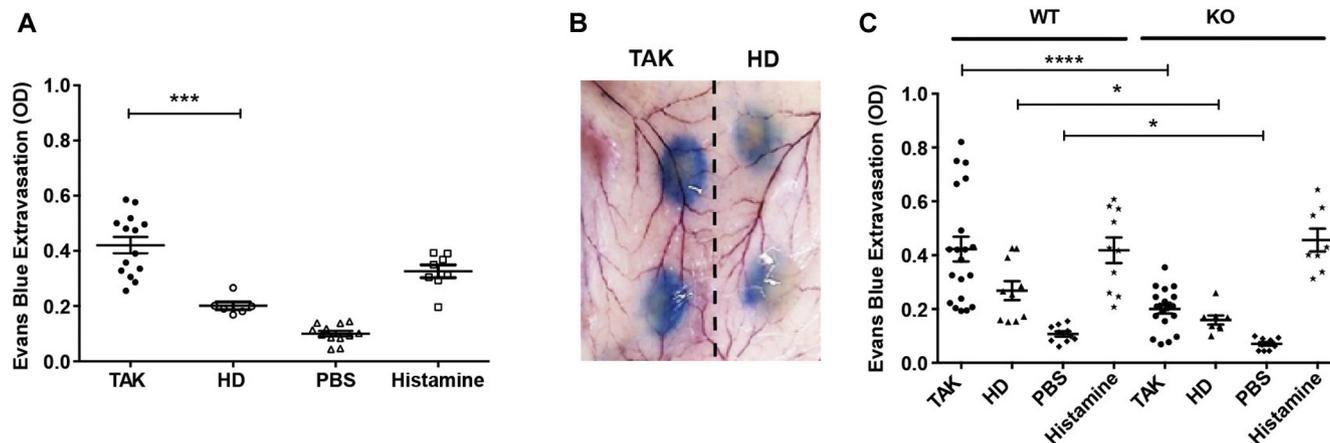


FIG 2. Increased vessel permeability in TAK is dependent on MCs. Vessel permeability induced by sera was evaluated by performing a Miles assay. Extravasation of Evans blue dye, as a measure of vascular leakiness, was analyzed using spectrophotometry. **(A)** Miles assay on wild-type (WT) mice after injection of sera obtained from patients with TAK ($n = 14$) and HDs ($n = 6$) (PBS and histamine were injected as a negative and positive controls, respectively). **(B)** Representative illustration of Miles assay performed on WT mice using injection in duplicate of sera from a patient with TAK (*left*) or from a HD (*right*). **(C)** Miles assay on WT and MC-deficient mice. Vascular permeability induced by injection of sera from patients with TAK was significantly decreased in MC-deficient mice compared with in WT mice (PBS and histamine were injected as a negative and positive control, respectively) ($n = 20$ in each group). Data are shown as mean \pm SEM. For statistical analyses, Mann-Whitney test was used; * $P < .05$; *** $P < .001$; **** $P < .0001$. KO, Knockout.

MCs support neo-angiogenesis in TAK

We next investigated whether MCs could have a functional effect on angiogenesis in TAK. We first assessed HUVEC proliferation and showed that supernatant of MCs incubated with TAK sera significantly increased HUVEC proliferation (1.77 ± 0.03 OD) as compared to supernatant of MC incubated with HD sera (1.6 ± 0.03 OD; $P = .03$) and to TAK and HD sera alone (0.70 ± 0.02 OD; $P = .002$; and 0.67 ± 0.01 OD; $P < .001$, respectively) (Fig 4, A). Incubation of the MC supernatants with an IL-33 neutralizing antibody decreased in HUVEC proliferation induced by MC supernatant preincubated with TAK sera (1.8 ± 0.03 vs 1.7 ± 0.04 OD; $P = .003$), whereas such treatment had no effect on MC supernatant incubated with HD sera (1.6 ± 0.02 vs 1.6 ± 0.02 OD; $P = .43$) (Fig 4, A). Thus, HUVEC proliferation is increased by MC supernatant stimulated by TAK sera and is partially dependent on IL-33. Similarly, in Matrigel assays, we showed that MC supernatant incubated with TAK sera significantly increased HUVEC branches (58 ± 7.8) as compared to MC supernatant incubated with HD sera (30 ± 8.4 ; $P = .04$) and to LVV sera alone (32.7 ± 5.9 ; $P = .02$) (Fig 4, B). In addition, treatment of MC supernatant preincubated with TAK sera with IL-33 neutralizing antibody decreased HUVEC branches formation as compared to MC supernatant preincubated with TAK sera (46.7 ± 8.5 vs 26.7 ± 4.7 ; $P = .01$), whereas it had no effect on MC supernatant incubated with HD sera (34.3 ± 8.0 vs 16.6 ± 3.8 ; $P = .1$) (Fig 4, B). We then showed that MC supernatant incubated with TAK sera tend to increase HUVEC pseudotube formation (65 ± 6.5) as compared to MC supernatant incubated with HD sera (46 ± 8.2 ; $P = .09$) and to TAK sera alone (44 ± 6.9 ; $P = .05$) (Fig 4, C). Finally, IL-33 neutralizing antibody decreased HUVEC pseudotube formation stimulated by MC supernatant preincubated with TAK sera (56.1 ± 8.3 vs 37.1 ± 5.3 ; $P = .03$), whereas it had no effect on MC supernatant incubated with HD sera (45.7 ± 8.2 vs 35.2 ± 7.1 ; $P = .9$) (Fig 4, C and

D). Interestingly, supernatant of IgE-stimulated MCs enhanced HUVEC branches and pseudotubes formation as compared to other conditions but had little effect on HUVEC proliferation (Fig 4, A-C). Altogether, these data show that increased neo-angiogenesis induced by TAK sera is dependent on MC activation.

MCs support fibrosis in TAK

As we have shown that MC could promote endothelial cells neo-angiogenesis, we next hypothesized that MC could also modulate the phenotype of fibroblasts. We first highlighted that fibroblasts stimulated with MC supernatant incubated with TAK sera expressed more fibronectin, collagen 1, and α -SMA than did fibroblasts simulated with TAK sera alone (fibronectin, 21 ± 2.9 vs 10.2 ± 1.3 ; $P = .005$; collagen 1, 6.1 ± 0.8 vs 1.7 ± 0.2 ; $P < .001$; and α -SMA, 0.016 ± 0.003 vs 0.006 ± 0.001 ; $P = .001$) (Fig 5, A). These profibrotic molecules (ie, fibronectin, collagen 1, and α -SMA) were also expressed markedly higher by fibroblasts stimulated with MC supernatant stimulated with TAK sera as compared to MC supernatant incubated with HD sera (fibronectin, 21 ± 2.9 vs 3.4 ± 2.5 ; $P = .002$; collagen 1, 6.1 ± 0.8 vs 1.5 ± 0.8 ; $P = .001$; and α -SMA, 0.016 ± 0.003 vs 0.005 ± 0.003 ; $P = .03$) (Fig 5, A). Interestingly, supernatant of IgE-stimulated MC enhanced fibronectin and α -SMA production by fibroblast but seems to have little effect on collagen 1 production (Fig 5, A). Unlike for permeability and angiogenesis, serum IL-33 blockade did not modified fibronectin, collagen 1, and α -SMA production by fibroblast cultured with TAK serum (Fig E2, B) nor with MC supernatant incubated with TAK serum plus anti IL-33 (see Fig E3 in this article's Online Repository at www.jacionline.org).

We then aimed to determine the mechanism that could modulate fibroblast phenotype. We found by qRT-PCR that the

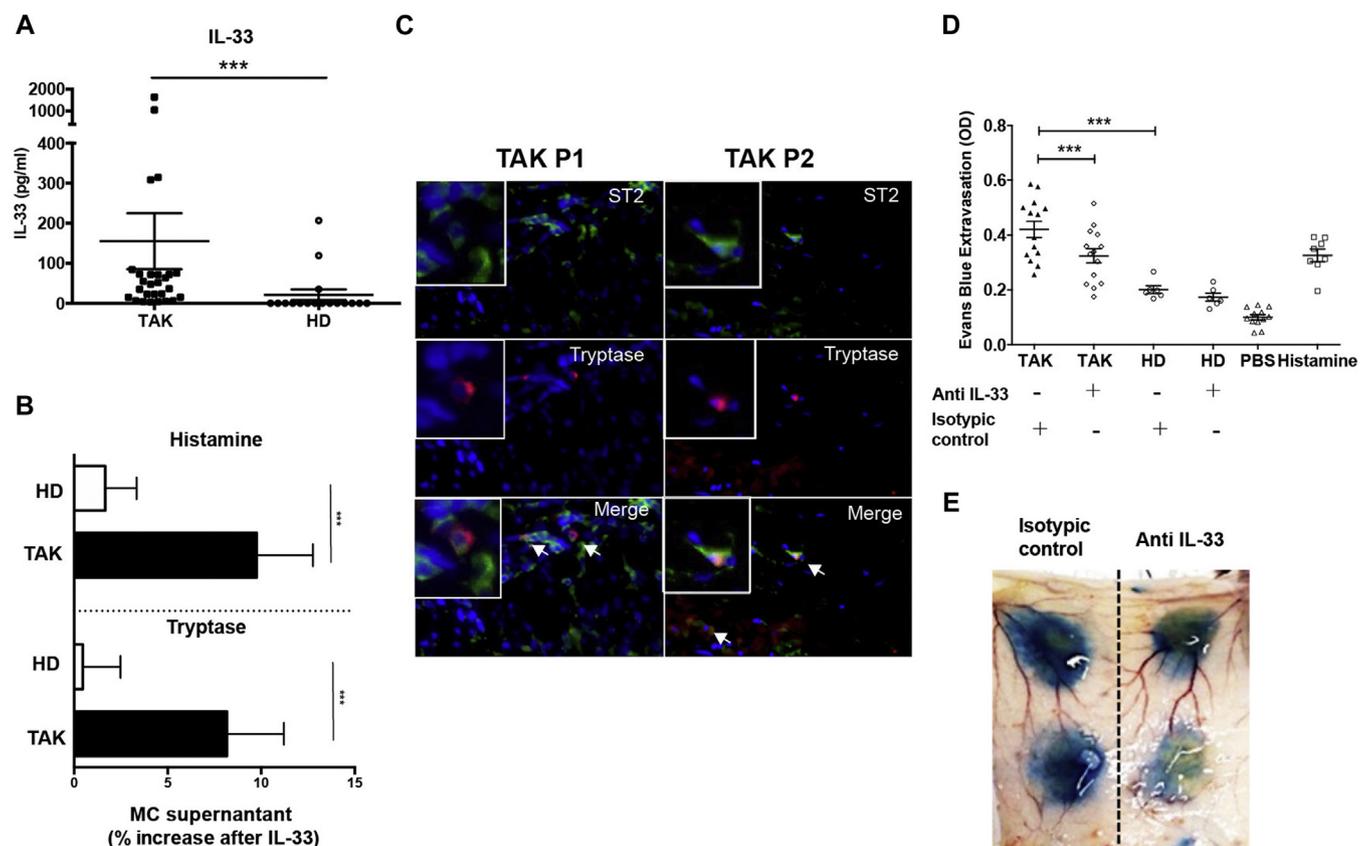


FIG 3. MC-induced vessel permeability in TAK is partially mediated by IL-33. **(A)** Level of IL-33 in sera of HDs and patients with TAK. **(B)** Levels of histamine and of tryptase in the supernatant of MCs cultured for 5 days with 10% TAK ($n = 11$) or HD ($n = 6$) serum previously incubated 1 hour with or without human recombinant IL-33. **(C)** Immunofluorescence staining of TAK inflammatory lesions showed expression of ST2 (in green) on MCs (tryptase, in red) (white arrows). Original magnification 640 \times . **(D)** Miles assay were performed on WT mice as described in Fig 2. Vascular permeability induced by injection of sera from patients with LVV was decreased when sera from patients were previously incubated 1 hour with neutralizing anti-IL-33 antibody. **(E)** Representative illustration of Miles assay (Evans blue extravasation) performed on WT mice using injection in duplicate of sera from a patient with TAK with isotypic control antibody (left) or with anti-IL-33 antibody (right). Data are shown as mean \pm SEM. For statistical analyses, Mann-Whitney test and Wilcoxon matched pairs test for paired groups were used. *** $P < .001$.

relative expressions of 2 major profibrotic cytokines, PDGF and TGF- β , were higher in aorta specimens obtained from patients with TAK compared with those from noninflammatory aorta (10.7 ± 1.9 vs 2.5 ± 0.8 relative expression; $P = .0047$; and 209 ± 23.8 vs 84 ± 20.7 ; $P = .01$, respectively) (Fig 5, B). We then determined the levels of PDGF and TGF- β in supernatant of MCs incubated with TAK sera as compared to those incubated with HD sera. Production of PDGF and TGF- β in supernatant of MCs cultured with TAK sera was higher than those of MCs cultured with HD sera (590 ± 91 vs 241.6 ± 46.6 pg/mL; $P = .003$; and 8352 ± 367 vs 6240 ± 490 pg/mL; $P = .003$, respectively) (Fig 5, C). IL-33 blockade did not modify production of PDGF and TGF- β in supernatant of MCs cultured with TAK sera (Fig E2, C).

To provide more insight into the nature of the primary MC activating factor found in the serum of patients with TAK, we performed the same experiment using inhibitors of MC degranulation (quercetin and cromolyn) and IL-6 blockade (a major inflammatory cytokine involved in TAK). We did not see a consistent effect of either blocking MC degranulation or anti-IL-6 (Fig E3).

Altogether, these data show that MC activated by TAK sera support fibrosis and suggest that these effects could be dependent of TGF- β and PDGF production.

DISCUSSION

Inflammation and angiogenesis are closely associated in a number of pathological processes. With regard to LVV, immune-mediated damage to aorta results in vascular remodeling such as segmental stenosis, occlusion, dilatation, and/or aneurysm formation.^{6,24}

In the present report, we identified MCs as a novel player in driving vascular lesions in TAK. Although MCs are not circulating cells, we first highlighted an increased level of MC activation markers (ie, histamine and IDO) in TAK sera. MCs are an important source of TNF- α and may act on fibroblast and endothelial cells.²⁵ We found high levels of TNF- α in sera of patients with TAK but these levels were similar in MC supernatant stimulated with TAK sera or HD sera. As histamine may be produced by basophils and IDO by dendritic cells, we confirmed these results in MC supernatant. We then identified a marked

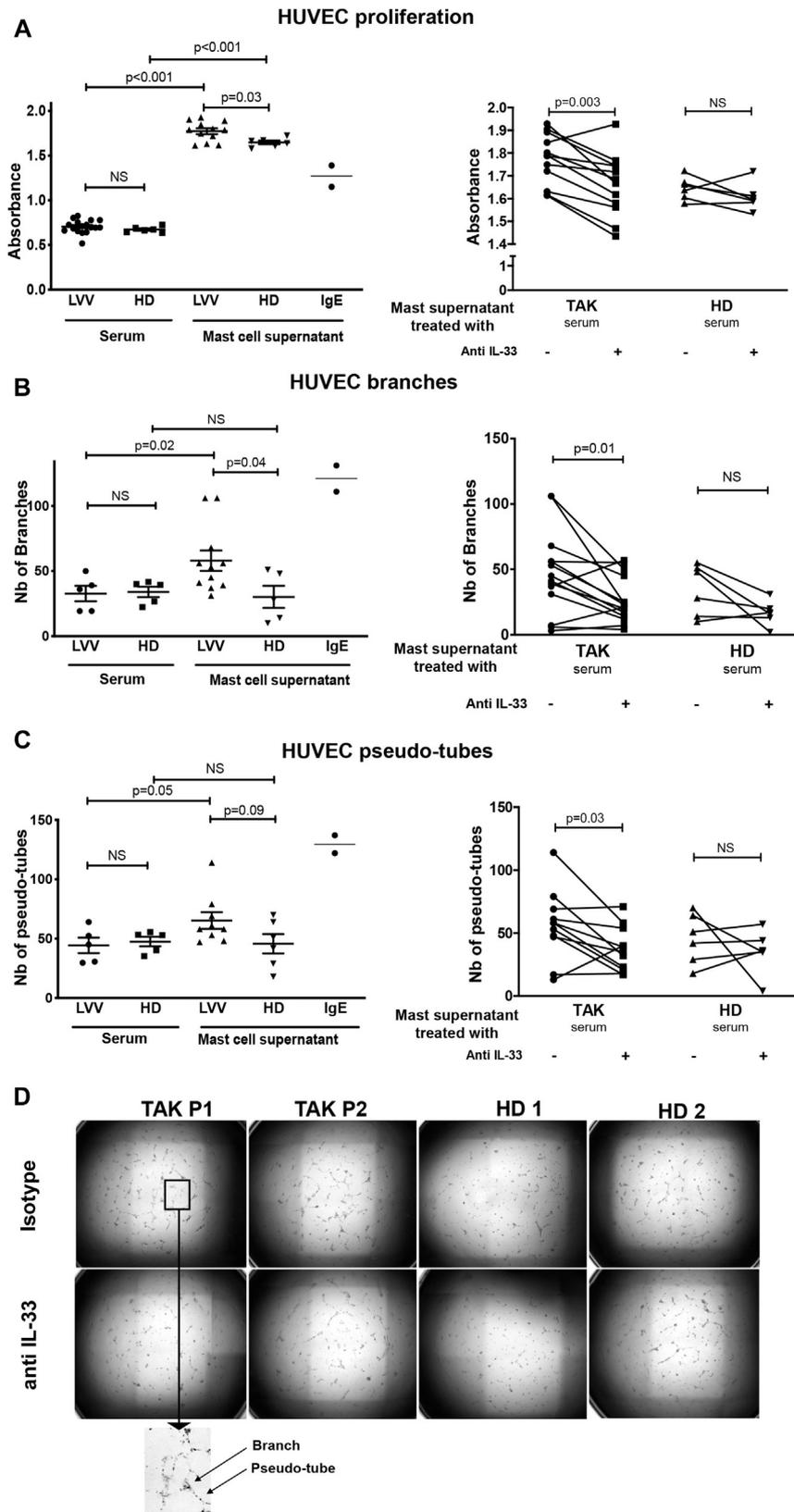


FIG 4. MCs support neoangiogenesis in TAK through IL-33. **(A)** Percentage of proliferation rate as measured by 5-bromo-2-deoxyuridine incorporation assay in HUVECs treated for 24 hours with TAK ($n = 17$) or HD ($n = 6$) sera and with supernatant of MCs cultured for 5 days with 10% TAK ($n = 12$) or HD ($n = 6$) sera with or without anti IL-33. **(B and C)** *In vitro* angiogenesis assay on growth factor reduced Matrigel. HUVECs treated for 12 hours with TAK or HD ($n = 5$) sera and MC supernatant cultured for 5 days with 10% TAK ($n = 9$) and HD ($n = 6$) sera previously incubated 1 hour with or without anti-IL-33. **(D)** Representative experiment of capillary tube formation by HUVECs treated with TAK sera preincubated with neutralizing anti-IL-33 or the same TAK sera preincubated with isotype antibodies. Data are shown as mean \pm SEM. For statistical analyses, Mann-Whitney test and Wilcoxon matched pairs test for paired groups were used.

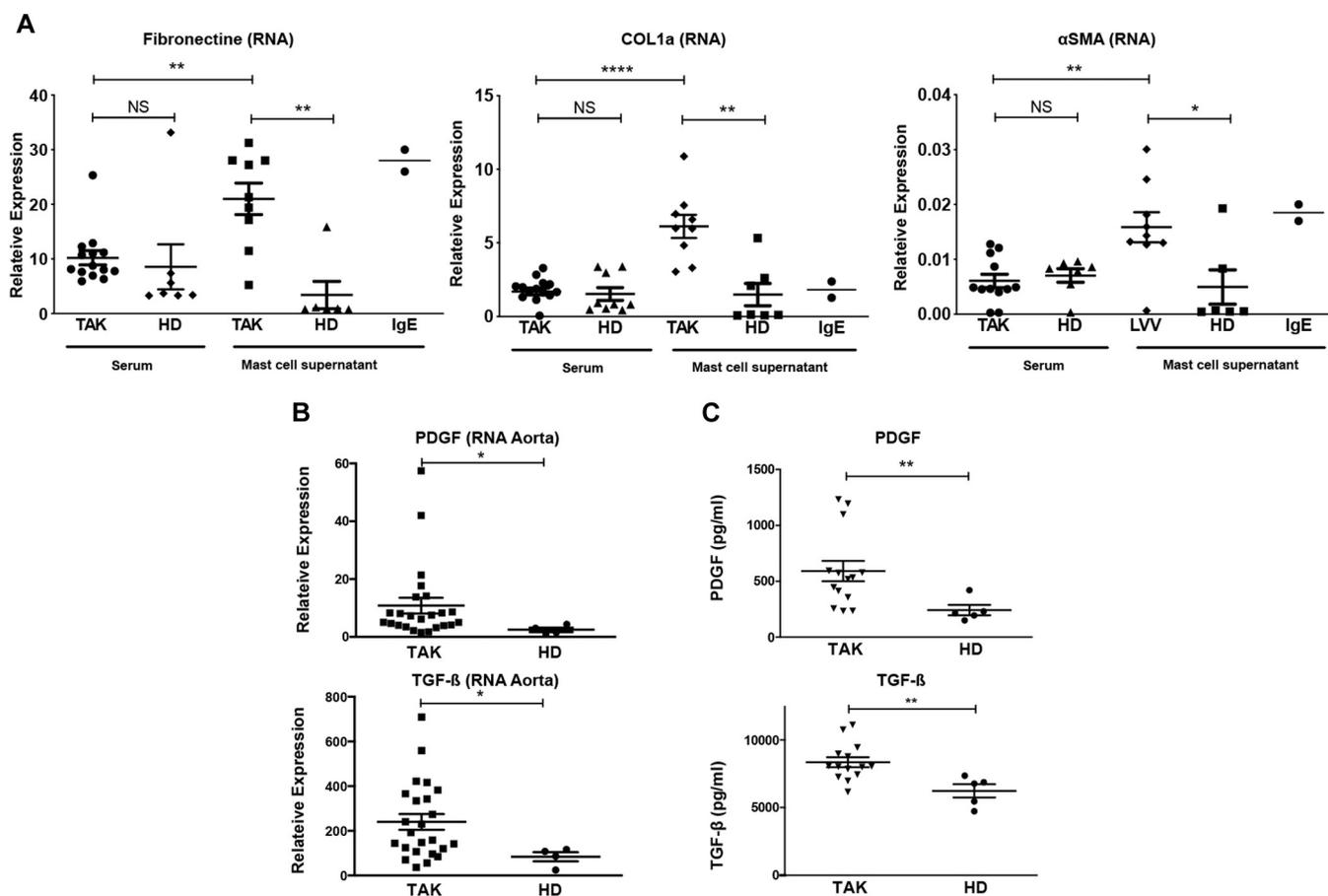


FIG 5. MCs support fibrosis in TAK. **(A)** Production of fibronectin, type 1 collagen, and α -SMA by fibroblasts cultured for 5 days with TAK ($n = 14$) or HD ($n = 7$) sera and MC supernatant cultured for 5 days with 10% TAK ($n = 9$) and HD ($n = 6$) sera. **(B)** PDGF and TGF- β mRNA quantification in aorta obtained from patients with TAK ($n = 37$) and HDs ($n = 4$) by qRT-PCR. **(C)** PDGF and TGF- β were measured in the supernatant of MCs cultured for 5 days with 10% of HD ($n = 5$) or TAK ($n = 13$) sera. Data are shown as mean \pm SEM. For statistical analyses, Mann-Whitney test and Wilcoxon matched pairs test for paired groups were used * $P < .05$; ** $P < .01$; **** $P < .0001$.

increase in MC numbers in TAK arterial lesions. MCs localized mostly in the adventitia layer, indicating that these cells are optimally positioned for sensing danger signals in the arterial wall, via the *vasa vasorum*, which are restricted to the adventitia layer. The high density of MCs in aorta is unexpected because MCs are mainly found at sites close to the external environment, such as the skin and mucosal surfaces of the lung and gut. Along this line, the presence of MCs has been pointed out in temporal arteries affected by giant cell arteritis in patients.¹⁶ MCs have also been implicated in abdominal aortic aneurysm,¹⁰ myocardial infarction,¹¹ and atherosclerosis¹² lesions, by promoting leucocyte infiltration through increasing vascular permeability.

In addition, in the present report, we provide strong evidence, using a Miles assay (Evans blue extravasation), that treatment of MCs with sera from patients with TAK is able to induce vascular leakage and that significantly more than treatment with sera from HDs can do. In addition, vascular permeability induced by injection of sera from patients with TAK was dramatically decreased in MC-deficient mice as compared to wild-type mice, underlining the critical role of MCs in such phenomenon.

We then showed that MCs stimulated by sera of patients with TAK have angiogenic properties by promoting HUVEC

proliferation, pseudotubes, and branch formation. Several factors can enhance MC-mediated angiogenesis. Indeed, MCs produce a large spectrum of proangiogenic factors such as vascular endothelial growth factor, TGF- β , fibroblast growth factor 2, vascular permeability factor.²⁶ Moreover, it has been shown that tryptase secreted by MCs greatly enhanced angiogenesis²⁷ and that histamine stimulates new vessel formation by acting through both H1 and H2 receptors.²⁸ Lastly, MCs also contain preformed matrix metalloproteinases, such as MMP-2 and MMP-9, which enable MCs to directly modulate extracellular matrix degradation. This, in turn, allows for tissue release of extracellular matrix-bound angiogenic factors.²⁶ Because IL-33 is a potent regulator of MC functions^{23,29} and is involved in both vessel permeability and angiogenesis, we examined its role in vascular homeostasis in TAK. We pointed out the ability of IL-33 to activate MCs. Because we observed that MCs and ST2 (the IL-33 receptor) colocalized in inflamed aorta of patients with TAK, we sought to investigate the effect of blocking IL-33 effects on MCs in such context. Interestingly, we observed that blocking IL-33 inhibited the MC-dependent vascular permeability and angiogenesis induced by TAK sera. Altogether, these data showed that IL-33 and MCs might constitute an important link between

cell injury and an inflammatory response that can lead to restoration of tissue function and homeostasis, but might, under other circumstances, contribute to a vicious circle driving tissue remodeling. In an inflammatory context, MCs can promote vascular permeability and thus can amplify the recruitment of inflammatory cells.³⁰

In TAK, inflammatory response is followed by hyperplasia of *vasa vasorum* and subsequently by medio-adventitia fibrosis and intimal proliferation. However, the inflammatory and stenotic phases of the disease coexist frequently. MCs can promote fibrosis through various mechanism such as fibroblast proliferation and activation, fibroblast recruitment, and induction of secretion of matrix protein by fibroblasts.³¹ Indeed, MCs have been associated with numerous fibrotic conditions, such as idiopathic lung fibrosis, cardiac fibrosis, renal fibrosis, scleroderma, chronic graft-versus-host disease, and liver cirrhosis.³²⁻³⁵ MC degranulation and proliferation have been documented in fibrotic processes *in vivo*, and several MC mediators such as PDGF and TGF- β have been found to exert fibrogenic effects by causing fibroblast proliferation or by increasing collagen synthesis.^{36,37}

Interestingly, we demonstrated here that MCs stimulated by sera of patients with TAK led to increased production of collagen, fibronectin, and α -SMA by fibroblasts. In addition, MCs stimulated with TAK sera secreted higher levels of TGF- β and PDGF than those stimulated with HD sera did, suggesting a role for MCs in the profibrotic phenotype of fibroblasts. Furthermore, the mRNA expression of these 2 mediators was markedly increased in inflamed aorta of patients with TAK. The mechanisms involved in vascular remodeling in TAK are unknown. Unlike in vessel permeability and angiogenesis, we did not find any effect of IL-33 on fibrosis. To provide more insight into the nature of the primary MC activating factor, we used inhibitors of MC degranulation (quercetin/cromolyn) to assess the effect of MC degranulation and IL-6 blockade (a major inflammatory cytokine involved in TAK) but did not find any effect on MC-mediated fibrosis in TAK. Moreover, the effects of LVV sera in endothelial cells and fibroblasts did not fully phenocopy the IgE effect. This supports the concept of a novel MC activation pathway present in LVV. Further studies are needed to find the missing activating factor(s) present in TAK sera and involved in MC-mediated fibrosis. In the late phase of TAK, scarring can be seen in the tunica media with disruption and disorganization of the remaining elastic fibers. Dense adventitial fibrosis is also often present. However, the precise mechanisms involved in vascular remodeling in TAK were largely unknown.

In conclusion, we provide here strong evidence that MCs play a critical role in regulating vessel permeability, angiogenesis, endothelial activation, and fibrogenesis in TAK. IL-33 and MCs may constitute an important link between cell injury and an inflammatory response in such diseases.

Key messages

- MCs are activated and abundant in inflammatory aortic lesions of patients with TAK.
- Increased vessel permeability in TAK is dependent on MCs.
- MCs support neo-angiogenesis and fibrosis in TAK.

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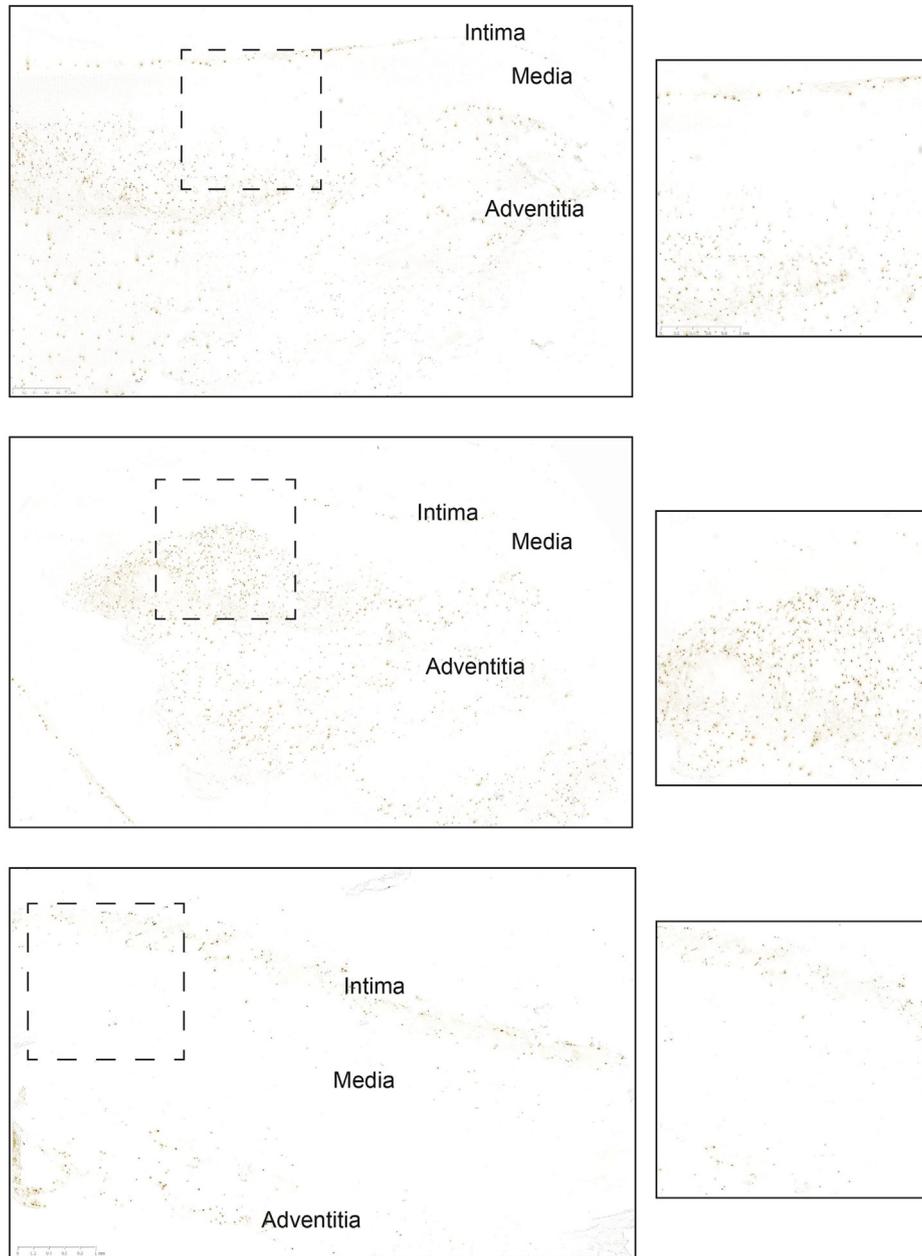


FIG E1. MCs localize mostly in the adventitia layer. Chromogenic immunohistochemistry of 3 TAK aorta sections showing that MCs localize mostly in the adventitia layer and to a lesser extent in the intima.

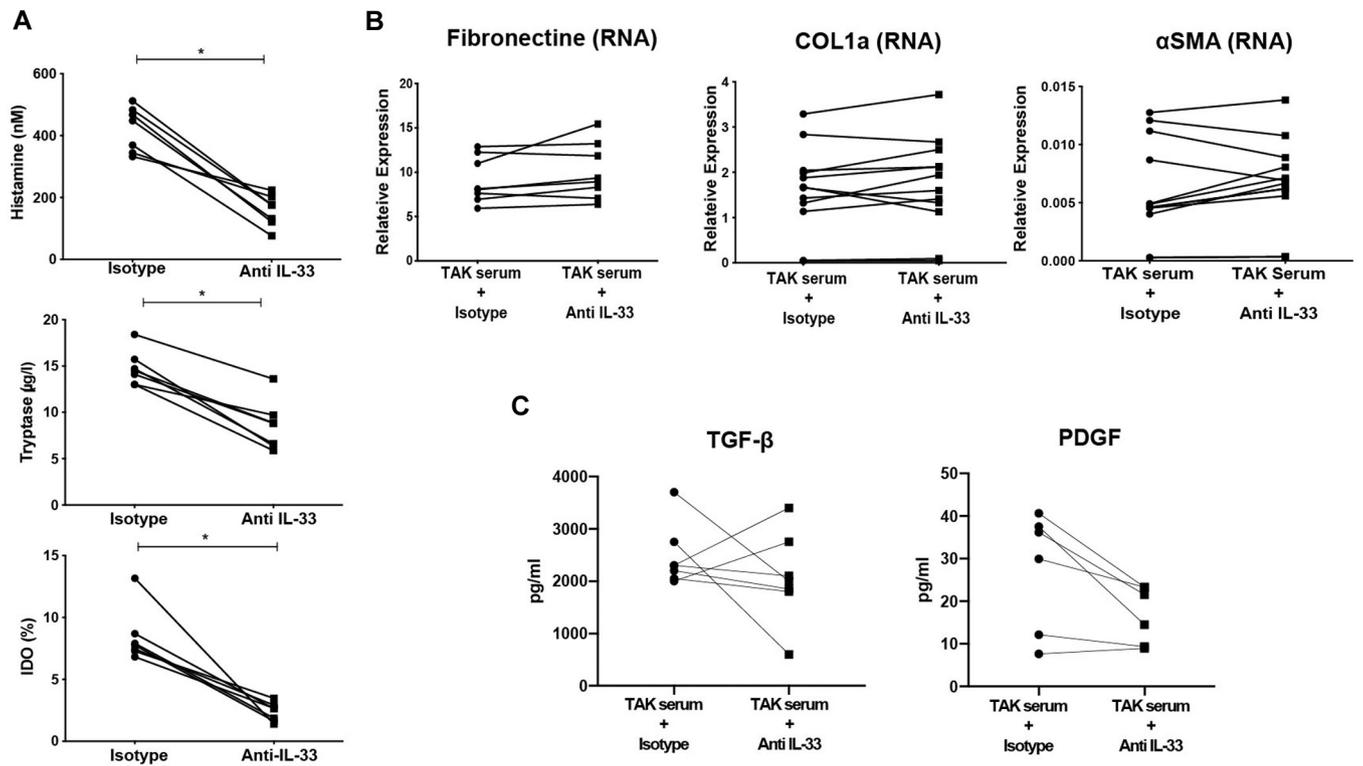


FIG E2. Effect of serum IL-33 blockade on MC function. **(A)** IL-33 blockade in TAK sera significantly reduced levels of histamine, tryptase, and IDO in MC supernatant. **(B)** IL-33 blockades in TAK sera have no effect on fibronectin, COL1A, and α -SMA production by fibroblast. **(C)** IL-33 blockades in TAK sera have no effect on PDGF and TGF- β production by MCs. * $P < .05$.

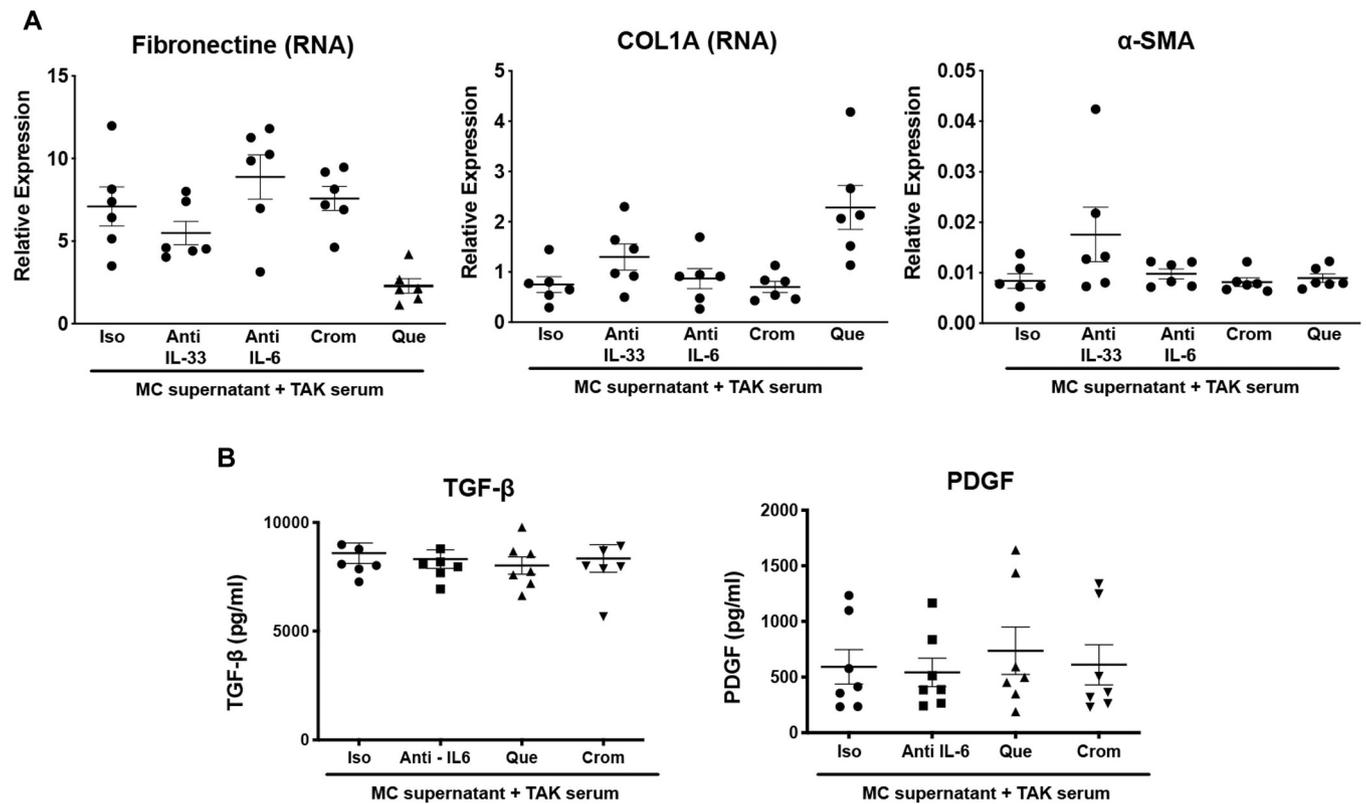


FIG E3. Assessment of putative MC activating factors on fibroblast function. **(A)** Production of fibronectin, type 1 collagen, and α -SMA by fibroblasts cultured for 5 days with TAK MC supernatant cultured for 5 days with 10% TAK sera plus one of the following: isotype (*Iso*), anti-IL-33, anti-IL-6, cromolyn (*Crom*), or quercetin (*Que*). **(B)** PDGF and TGF- β were measured in the supernatant of MCs cultured for 5 days with 10% TAK plus one of the following: isotype, anti-IL-6, cromolyn, or quercetin.