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Original article

mTOR pathway is activated in endothelial cells from patients with Takayasu arteritis and is modulated by serum immunoglobulin G

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Abstract

Objectives. Takayasu arteritis (TA) and GCA are large-vessel vasculitides characterized by vascular remodelling involving endothelial cells (ECs) and vascular smooth muscle cells. Mammalian target of rapamycin (mTOR) pathway has been involved in vascular remodelling. We hypothesized that the mTOR pathway was involved in the pathogenesis of large-vessel vasculitis.

Methods. We used IF analysis on aortic and temporal artery biopsies from patients with TA and GCA to assess the involvement of the mTOR pathway and searched for antibodies targeting ECs in serum by IIF and cellular ELISA. We evaluated *in vitro* the effect of purified IgG from patients on mTOR pathway activation and cell proliferation.

Results. IF analyses on tissues revealed that both mTORC1 and mTORC2 are activated specifically in ECs from TA patients but not in ECs from GCA patients and healthy controls (HCs). Using IIF and ELISA, we observed higher levels of antibodies binding to ECs in TA patients compared with GCA patients and HCs. Using western blot, we demonstrated that purified IgG from TA patients caused mTORC1 activation in ECs, whereas this effect was not observed with purified IgG from GCA patients or HCs. Purified IgG from TA patients induced a significant EC proliferation compared with to GCA and HC IgG, and this effect was decreased after EC exposure with sirolimus, a specific mTOR inhibitor and PI3K inhibitor.

Conclusion. Our results suggest that antibodies targeting ECs drive endothelial remodelling in TA through activation of the mTOR pathway, but not in GCA. Inhibition of the mTOR pathway could represent a therapeutic option in TA.

Key words: giant cell arteritis, large-vessel vasculitis, mTOR pathway, sirolimus, Takayasu arteritis

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CLINICAL SCIENCE

Rheumatology key messages

- Mammalian target of rapamycin pathway is activated in endothelial cells in patients with Takayasu arteritis.
- Antibodies targeting endothelial cells caused mammalian target of rapamycin complex 1 activation and endothelial cell proliferation in Takayasu arteritis.
- Inhibition of mammalian target of rapamycin pathway with sirolimus could represent an alternative therapeutic option in Takayasu arteritis.

Introduction

Takayasu arteritis (TA) and GCA are the two main idiopathic large-vessel vasculitides [1]. TA typically occurs in women in the second and third decades and causes inflammation of the aorta and its main branches, leading to segmental stenosis and occlusion [2]. In contrast, GCA preferentially affects persons >50 years of age and causes vasculitis of the extracranial branches of the aorta leading to ischaemic complications and aortitis in 45–65% of cases [3, 4]. Both of these are associated with an increase in biological inflammatory parameters, but paucisymptomatic disease progression without systemic inflammation is a common feature in TA [2, 4, 5]. Despite these epidemiological, clinical and topographic differences, studies suggest that both diseases share some pathophysiological similarities, with a prominent role of Th1 and Th17 immune responses and IL-6 [6–8], and evidence of granulomatous inflammation in vessel walls [2, 4, 9]. These findings explain why IL-6-targeting therapies are under evaluation, with promising but only suspensive efficacy in GCA [10, 11] and contrasting results in TA [12, 13]. Vascular inflammation observed in both diseases drives vascular remodelling, especially the migration and proliferation of vascular smooth muscle cells (VSMCs) and endothelial cells (ECs), leading to intimal hyperplasia responsible for vascular occlusion [14, 15]. However, molecular pathways driving this vascular inflammation and remodelling have been partially deciphered [16, 17].

The mammalian target of rapamycin (mTOR) pathway has been involved in vascular remodelling [18–22]. mTOR is a serine/threonine kinase driving various signalling pathways, among which is the phosphoinositide 3-kinase (PI3K)-Akt pathway, and playing a central role in the regulation of cellular growth, proliferation and survival [23]. mTOR forms two distinct molecular complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC 2). mTORC1 activation leads to increased ribosomal biosynthesis and translation of proteins critical for proliferation through phosphorylation of S6 ribosomal protein (S6RP) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1). In contrast, mTORC2 has been shown to phosphorylate Akt on Ser473 and to be involved in cytoskeleton rearrangements [23]. Previous findings have shown that neovascularization is regulated by mTOR activation [18, 19]. Also, the mTOR pathway has been demonstrated to be involved in vascular lesions associated with APS [20], an autoimmune disease affecting mainly young women, like TA, and sharing intimal hyperplasia and vascular remodelling with large-vessel vasculitis.

In the present study we hypothesized that the mTOR pathway could drive vascular remodelling in large-vessel vasculitis.

Methods

Patients

Patients with TA ($n = 20$), GCA ($n = 20$) and healthy controls (HCs; $n = 20$) were included in this study. All TA and GCA patients were followed in the French National Center for Systemic Vasculitis in Cochin Hospital (Paris, France). Patients with TA fulfilled the ACR 1990 criteria for a diagnosis of TA [24]. Patients with GCA fulfilled the ACR 1990 criteria for GCA [25], and all of them had a positive temporal artery biopsy. Patients with other identified vasculitides were excluded. The characteristics of TA and GCA patients are summarized in Table 1. At the time of enrolment into the study, 9 patients with TA had active disease and 11 were in remission, whereas all patients with GCA had untreated and active disease. Blood samples from 20 HCs were obtained from Etablissement Français du Sang (Hôpital Saint-Antoine, Paris, France). Aorta and temporal artery biopsies from patients with TA and GCA, respectively, were obtained from the Department of Pathology of Hôpital Européen Georges-Pompidou (P.B.) and from the University Hospital of Dijon (M.S.). The study was performed according to the principles of Declaration of Helsinki and informed consent was obtained from all subjects. The local ethical body that approved our study was CPP île de France 3.

IF analysis

Paraffin-embedded vascular sections were blocked with 10% foetal bovine serum (FBS)/3% BSA and incubated with rabbit or mouse anti-human P-AKT (Ser473) antibodies (Cell Signaling Technology, Danvers, MA, USA), rabbit anti-human P-S6RP antibodies (Cell Signaling Technology), mouse anti-human α smooth muscle actin (α-SMA) antibodies (Sigma-Aldrich, St. Louis, MO, USA) and rabbit anti-human connective tissue growth factor antibodies (Thermo Fisher Scientific, Waltham, MA, USA) after appropriate antigen retrieval, as previously described [20]. Primary antibodies were revealed with appropriate Alexa Fluor 488 or 555 conjugated secondary antibodies (goat anti-mouse and goat anti-rabbit; Molecular Probes, Eugene, OR, USA). To confirm the specificity of immunostainings, incubation with secondary antibodies alone was done (supplementary Fig. 1, available at *Rheumatology* online). Tissues from six subjects with TA, five subjects with GCA and three atheromatous aortas were analysed. IF staining was visualized using the LSM 700 confocal

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Table 1 Main characteristics of the patients

Characteristics	Patients with TA ($n = 20$)	Patients with GCA ($n = 20$)
Demographics		
Age at diagnosis, median (range), years	32 (17–50)	74 (60–90)
Female, *n* (%)	15 (75)	15 (75)
Ethnicity, *n* (%)		
White	10 (50)	20 (100)
North African	3 (16)	–
Black	6 (32)	–
Asian	1 (5)	–
Disease activity at enrolment		
NIH activity score for TA, *n* (%)		
0–1	11 (55)	–
>1	9 (45)	–
Active disease for GCA, *n* (%)	–	20 (100)
Increased CRP >0.5 mg/dl, *n* (%)	9 (45)	18 (90)
Treatments used during evolution, *n* (%)		
Glucocorticoids	16 (80)	19 (95)
Immunosuppressants	12 (60)	0
MTX	8 (40)	–
AZA	2 (10)	–
MMF	1 (5)	–
Anti-TNF-α antibody	5 (25)	–
Anti-IL-6 receptor antibody	1 (5)	–

microscope (Zeiss, Oberkochen, Germany) and the DMI 600 microscope (Leica, Wetzlar, Germany).

Immunohistochemistry

For the cell proliferation index, paraffin-embedded sections were incubated either with a mouse anti-Ki67 antibody (Dako, Glostrup, Denmark), followed by sheep horseradish peroxidase (HRP)-conjugated anti-mouse antibody (Amersham, Little Chalfont, UK). Staining was revealed by 3,3′-diaminobenzidine. The vascular proliferation index was calculated as the number of vessels with at least one Ki67-positive nucleus out of the total number of vessels.

Cell culture

EC lines were used in the study and were human microvascular ECs (HMEC-1) immortalized by SV40/telomerase transfection (CRL-3243, American Type Culture Collection, Manassas, VA, USA). ECs were cultured in endothelial cell growth medium 2 (Promocell, Heidelberg, Germany) supplemented with 2% FBS, 0.4% endothelial cell growth supplement, 1 ng/ml basic fibroblast growth factor, 90 μg/ml heparin and 1 μg/ml hydrocortisone.

Detection of antibodies against ECs

Antibodies binding to ECs were detected by IIF and cellular ELISA. For IIF, HMEC-1 was cultured until confluence and incubated with sera from patients with TA, GCA or HCs at 1:100 dilution. Secondary goat anti-human IgG antibody conjugated with FITC (Invitrogen, Waltham, MA, USA) was then added. IF staining was visualized using the Leica DMI 600 microscope. Stainings were compared with similar settings. Fluorescence intensity was calculated using the following formula: corrected total cell fluorescence = integrated density − (area of selected cell × mean fluorescence of background readings). Anti-endothelial cell antibodies were also detected by cellular ELISA, as previously described [26]. Optical density (OD) was measured at 450 nm in an automated reader. OD values greater than the mean value + 2 S.D. of HCs were considered positive. Overall, serum from 20 patients with TA, 20 patients with GCA and 20 HCs was tested individually in duplicate with these two methods.

IgG purification

Human serum IgG from six patients with TA (TA IgG), six patients with GCA (GCA IgG) and six healthy controls (HC IgG) was purified using a protein G sepharose column and quantified by spectrophotometry at 260 and 280 nm. The purity of IgG preparations was assessed by SDS gel on a 10% acrylamide gel stained with Coomassie (supplementary Fig. S2, available at *Rheumatology* online).

Cell experiments

HMEC-1 was grown until 80% confluence and then starved in EC medium with 0.2% FBS. Cells were next incubated with different concentrations of purified TA IgG, GCA IgG or HC IgG, during various time points. Positive control was incubation with 20% FBS in EC medium. The reaction was stopped by washing with ice-cold PBS and solubilizing in radioimmunoprecipitation assay buffer (Thermo Fisher Scientific). For mTORC pathway inhibition experiments, HMEC-1 was incubated for 1 h with sirolimus (10 nM; Cell Signaling Technology) and

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LY294002 (50 μM; Cell Signaling Technology). Cells were next incubated with TA IgG, GCA IgG or HC IgG.

Western blot analysis

Briefly, protein extracts from HMEC-1 were subjected to electrophoresis (SDS-PAGE) before transfer onto polyvinylidene difluoride membrane and incubated with anti-P-AKT (Ser473) antibodies (Cell Signaling Technology), anti-P-AKT (Thr308) antibodies (Cell Signaling Technology), anti-P-S6RP antibodies (Cell Signaling Technology), anti-ERK1/2 antibodies (Cell Signaling Technology) and anti-β-actin antibodies (Sigma-Aldrich). Immunoreactive bands were visualized using appropriate HRP-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA, USA). Images were acquired using the Fusion Fx system (Vilber Lourmat, Marne-la-Vallée, France) and analysed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Cell viability assay

Proliferation of HMEC-1 incubated with purified TA IgG, GCA IgG or HC IgG was determined in real-time using the xCELLigence system (ACEA Biosciences Inc.) [27]. After cells were seeded onto E-plate, TA IgG, GCA IgG and HC IgG were added at a concentration of 100 μg/ml in the presence or absence of sirolimus and LY294002 (Cell Signaling Technology). The impedance value of each well was automatically monitored by the xCELLigence system for 120 h and expressed as a cell index value.

aPL measurement

aCL and anti-β2-GPI antibodies were measured with ELISA as previously described [28]. For the anticardiolipin antibodies, values were expressed as GPL units (1 GPL unit = 1 μg of affinity-purified IgG anticardiolipin from an original index serum sample) and were considered positive when >22 GPL units were reported. For anti-β2-GPI antibodies, values were expressed as GPL units and were considered positive when >20 GPL units were reported.

Characterization of target antigens of antibodies binding ECs

Target antigens of antibodies binding to ECs were analysed by western blotting. Briefly, a monolayer of HMEC-1 was subjected to electrophoresis (SDS-PAGE), electrotransferred from gel onto a polyvinylidene difluoride membrane and incubated with serum samples from patients. IgG reactivity was detected using secondary anti-human IgG peroxidase-conjugated antibody (Jackson Immuno Research Laboratories).

Statistical analysis

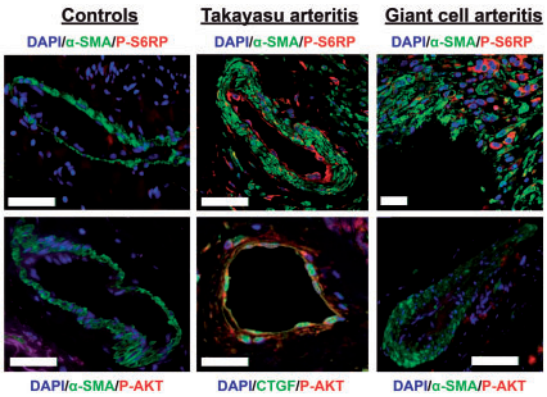
Data are expressed as means (S.E.M.) for continuous variables and as percentages for qualitative variables. Fisher's exact test was used to compare qualitative variables and the non-parametric Mann–Whitney test was used to compare continuous variables, as appropriate. *P*-values <0.05 were considered significant. Statistical analyses were performed using GraphPad Prism software (GraphPad Software, La Jolla, CA, USA).

Results

Activation of the mTOR pathway in ECs from TA patients

To study activation of the mTOR pathway in injured vessels from patients with TA and GCA, we evaluated phosphorylation of S6RP (p-S6RP) and AKT [p-AKT (Ser473)], reflecting activation of mTORC1 and mTORC2, respectively. Adventitial vessels from vasa vasorum of the aorta from TA patients were strongly positive for both p-S6RP and p-AKT (Ser473) (Fig. 1), and co-staining with α-SMA or connective tissue growth revealed that p-S6RP and p-AKT (Ser473) were expressed in ECs and not in vascular smooth muscle cells. In contrast, IF analysis did not reveal any positive staining for p-S6RP and p-AKT (Ser473) on vascular sections from control aortas and from the temporal artery of GCA patients, but the latter showed some positive staining within the media of GCA patients (Fig. 1). These results demonstrated that both mTORC1 and mTORC2 were activated specifically in ECs from patients with TA and not in ECs from patients with GCA.

Fig. 1 Activation of mTOR pathway in endothelial cells from TA patients but not GCA patients



Upper panels show the results of double immunostaining of phosphorylated S6 ribosomal protein (S6RP) (red) and α-SMA (green) in atheromatous aorta from controls, aorta from patients with Takayasu arteritis and temporal artery from patients with GCA. Lower panels show results of double immunostaining of phosphorylated AKT (Ser473) (red) and endothelial cell marker CTGF or α-SMA (green) in atheromatous aorta from controls, aorta from patients with Takayasu arteritis and temporal artery from patients with GCA. DAPI staining (blue) was used to detect nuclei of tissue specimens. Results are representative of six subjects with TA, five subjects with GCA and three atheromatous aortas (magnification ×40). DAPI: 4′,6-diamidino-2-phenylindole.

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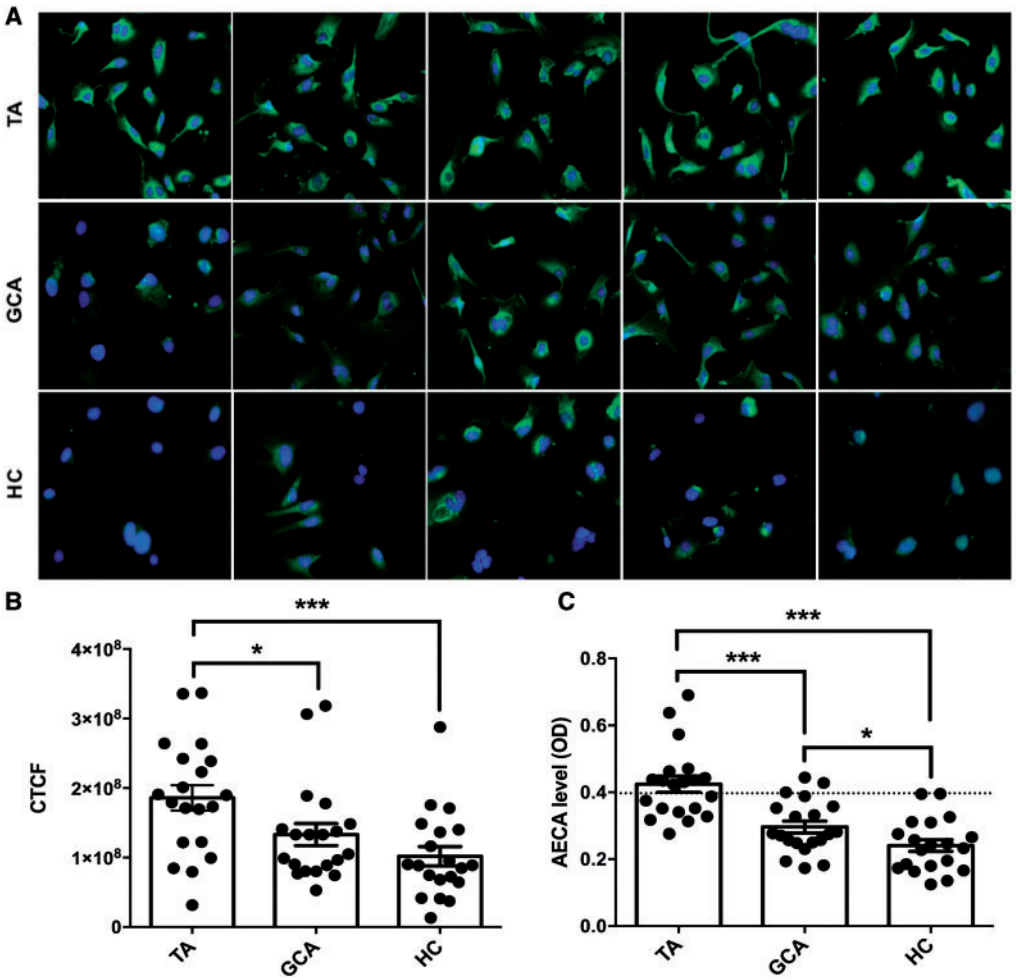
Presence of antibodies binding ECs in TA

To investigate the potential role of antibodies binding to ECs in the activation of the mTOR pathway, sera from TA and GCA patients and HCs were screened using IIF and ELISA. First, fluorescence against the HMEC-1 cell line was significantly more intense with serum IgG from TA patients compared with serum IgG from GCA patients [1.9×10^8 (S.D. 0.82×10^8) *vs* 1.3×10^8 (S.D. 0.71×10^8), $P = 0.02$] and HCs [1.9×10^8 (S.D. 0.82×10^8) *vs* 1×10^8 (S.D. 0.63×10^8), $P = 0.001$] (Figs 2A and B). Second, using cellular ELISA, antibodies targeting ECs were present in the sera of 11/20 (55%) TA patients compared with 2/20 (10%) GCA patients and 0/20 HCs ($P = 0.006$ and $P = 0.0001$, respectively) (Fig. 2C). Serum levels of these antibodies assessed by OD were also significantly higher in TA compared with GCA patients [0.42 (S.D. 0.11) *vs* 0.30 (S.D. 0.08), $P = 0.0003$] and HCs [0.42 (S.D. 0.11) *vs* 0.24 (S.D. 0.08), $P < 0.0001$]. These findings showed higher reactivity of serum IgG from TA patients against ECs than in GCA patients and HCs.

Activation of the mTOR pathway induced by serum IgG in TA

To determine whether serum IgG (including IgG binding ECs) could activate the mTOR pathway in ECs, we incubated the HMEC-1 cell line with purified IgG

Fig. 2 Reactivity of serum IgG against endothelial cells in patients with TA



(**A**) Results of IIF on human microvascular endothelial cell (HMEC-1) with sera from patients with TA, patients with GCA and HCs. Sera were tested at 1:100 dilution. Results are representative of 20 subjects in each group (magnification ×40). (**B**) Quantification of reactivity by IIF using corrected total cell fluorescence [CTCF = integrated density − (area of selected cell × mean fluorescence of background readings)] for each group. (**C**) Levels of AECAs in patients with TA and GCA and HCs. The dashed line represents the positivity threshold [mean + 2 (S.D.) in HCs]. Each dot represents data from an individual subject. Data are presented in mean (S.E.M.). The Mann–Whitney test was used for statistical analyses. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. TA: Takayasu arteritis; HCs: healthy controls; HMEC: human microvascular endothelial cell; CTCF: corrected total cell fluorescence; AECA: anti-endothelial cell antibody.

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from TA and GCA patients and HCs at different concentrations and for various durations. TA IgG induced S6RP phosphorylation but not AKT (Ser473) phosphorylation with 100 μg/ml IgG at 30 min, whereas GCA IgG and HC IgG did not (Fig. 3A and supplementary Fig. S3A, available at *Rheumatology* online). These findings suggest that serum IgG mainly activates mTORC1 rather than mTORC2 in TA.

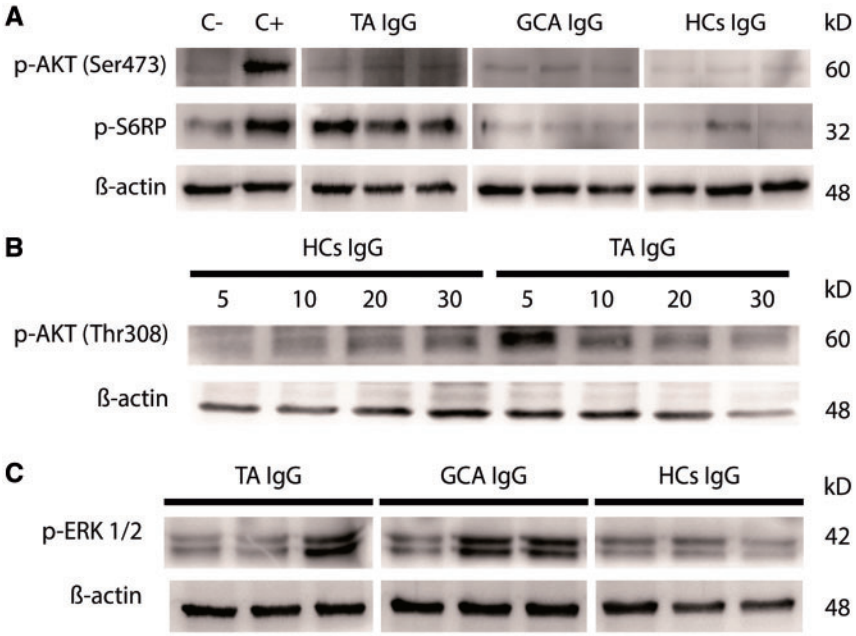
Because mTORC1 can be directly activated by different pathways [i.e. PI3K-AKT or mitogen-activated protein kinase (MAPK) pathways], we evaluated phosphorylation of AKT (Thr308), which is the target residue of the PI3K pathway, and phosphorylation of ERK1/2 reflecting activation of MAPK. Purified TA IgG induced phosphorylation of AKT on Thr308 after 5 min of incubation with TA IgG but not increased phosphorylation of ERK1/2, suggesting that mTORC1 is activated by serum IgG through the PI3K-AKT pathway (Figs 3B and C, supplementary Fig. S3B, available at *Rheumatology* online). These findings also suggest that recruitment of AKT to the cell membrane is necessary for activation of the mTOR pathway induced by serum IgG in TA.

Because aPLs were previously described in large-vessels vasculitis [29–31] and demonstrated to activate mTOR pathway in ECs [20], we screened patients' sera for aCL and anti-β2-GPI antibody by ELISA (supplementary Table S1, available at *Rheumatology* online). As recently reported [31], aPLs were detected in 2/20 (10%) TA patients and 8/20 (40%) GCA patients, indicating that these antibodies could not account for mTOR pathway activation in TA.

Activation of EC proliferation by serum IgG in TA

Activation of the mTOR pathway has been shown to trigger cell proliferation [23]. Because intimal hyperplasia is a hallmark of vasculitis lesions in TA, we analysed whether serum IgG could modulate EC viability. TA IgG induced significant EC proliferation, with a peak at 72 h, compared with GCA IgG [1.37 (S.D. 0.66) *vs* 0.36 (.D.0.57), $P = 0.03$] and HC IgG [1.37 (.D.0.66) *vs* 0.32 (.D.0.45), $P = 0.009$] (Fig. 4A). No difference was found between GCA IgG and HC IgG. Immunostaining with antibodies against Ki-67, a protein that is selectively expressed in proliferating cells, could not confirm these results in biopsies, with no difference found between TA aortas, GCA temporal arteries and control aortas (data not shown). EC viability induced by TA IgG was dramatically decreased after treatment with sirolimus, a specific mTOR inhibitor, and LY294002, a PI3K

Fig. 3 Activation of mTORC1 by antibodies binding ECs from patients with TA in endothelial cells



(**A**) Western blot of phosphorylated AKT (P-AKT Ser473) and phosphorylated S6RP (P-S6RP) were performed in HMEC-1 after exposure for 30 min to purified IgG from patients with TA (TA IgG), patients with GCA (GCA IgG) and healthy controls (HC IgG). Results are representative of six subjects with TA, six subjects with GCA and six HCs. (**B**) Western blot of phosphorylated AKT (P-AKT Thr308) was performed at different time points after exposure to TA IgG or HC IgG. (**C**) Western blot of P-ERK1-2 was performed in HMEC-1 over 30 min after exposure to TA IgG, GCA IgG and HC IgG. β-actin was used as a housekeeping protein. Positive control (C+) was exposure to medium with 20% FBS. Negative control (C^-) was exposure to medium with 0.2% FBS. All experiments were performed in duplicate. mTORC1: mammalian target of rapamycine complex 1; AKT: protein kinase B; TA: Takayasu arteritis; HCs: healthy controls; HMEC: human microvascular endothelial cells; FBS: foetal bovine serum; P-ERK: phosphorylated extracellular signal-regulated kinases.

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inhibitor (Fig. 4B), demonstrating the implication of the mTOR pathway in EC proliferation induced by serum IgG in TA. In addition, exposure with sirolimus completely inhibited phosphorylation of S6RP induced by serum IgG (data not shown). These results demonstrate that the mTOR pathway plays a key role in EC proliferation in TA.

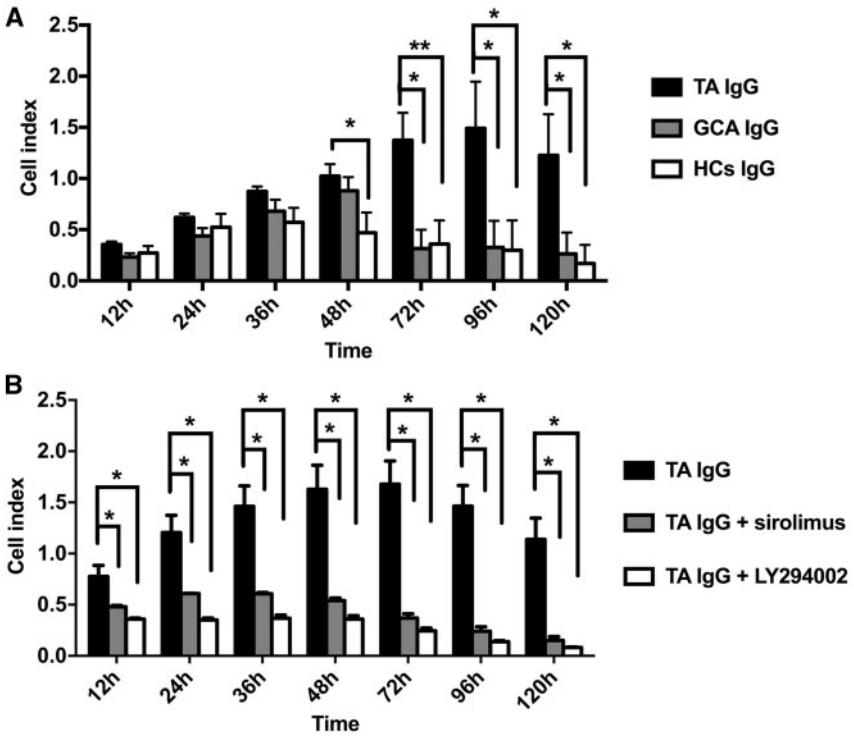
Target antigens of antibodies binding to ECs

Target antigens of antibodies binding to ECs were investigated by western blotting. Sera obtained from TA patients and with positive screening of antibodies targeting ECs bound predominantly to an EC antigen with a molecular weight between 60 and 65 kDa (Fig. 5). This protein band was also recognized by one TA patient with negative screening for these antibodies but not by HCs.

Discussion

We demonstrated in the present study that the mTOR pathway is specifically activated in ECs from TA patients and not in GCA patients, that TA patients have serum antibodies targeting ECs and that serum IgG (including IgG binding ECs) is able to drive this activation. Recent studies support the key role of the mTOR pathway in vascular remodelling, especially in APS [20] or chronic allograft rejection-related vasculopathy associated with anti-class I HLA antibodies [21]. In APS, a previous study demonstrated that mTOR was involved in the development of intimal hyperplasia and that aPL activated AKT at the plasma membrane in a PI3K-dependent manner [20]. In transplant vasculopathy, antibodies directed against class I HLA molecules were found to activate the mTOR pathway, leading to cell proliferation [21]. In our study, immunostaining of patients' biopsies showed that both mTORC1 and mTORC2 are activated in adventitial ECs from TA, whereas *in vitro* experiments showed that mTORC1 is activated through the PI3K-AKT pathway rather than mTORC2. Importantly, giant cells and activated macrophages present in the vessel wall in TA are known to release VEGF and PDGF [32], which are able to activate the mTOR pathway [23]. Hence we can hypothesize that the mTOR pathway could be locally activated in TA by inflammatory cells present in the vessel wall through the production of growth factors, but also by

Fig. 4 Viability of endothelial cells induced by IgGs from patients with TA



(**A**) Proliferation of HMEC-1 incubated with purified IgG from patients with Takayasu arteritis (TA IgG), from patients with GCA (GCA IgG) and from healthy controls (HC IgG) assessed by the xCELLigence system. The impedance value of each well showing time-dependent proliferation was monitored by the xCELLigence system for 120 h and expressed as the cell index value. Analyses were done in two independent experiments, in duplicate. (**B**) Proliferation of HMEC-1 incubated with TA IgG in the presence or absence of sirolimus (specific mTORC inhibitor) and LY294002 (PI3K inhibitor). Data are presented as mean (S.E.M.). The Mann–Whitney test was used for statistical analyses. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. TA: Takayasu arteritis; HMEC: human microvascular endothelial cells; HCs: healthy controls; mTOR: mammalian target of rapamycine.

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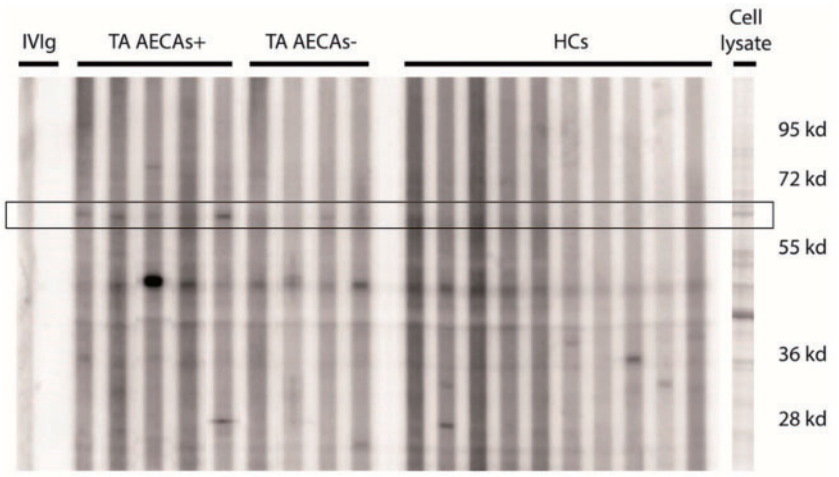
Fig. 5 Target antigens of antibodies binding ECs from patients with TA



Representative immunoblots showing molecular weights of protein bands recognized by sera from patients with Takayasu arteritis with positive anti-endothelial cell antibodies (TA AECAs^{+}), Takayasu arteritis with negative anti-endothelial cell antibodies (TA AECAs^{-}) and healthy controls (HCs). Intravenous Ig was used as a control. Sera were tested at 1:100 dilution. Cell lysate was stained with Coomassie blue.

serum IgG as demonstrated in this study. Overall, previous and our data support the key role of the mTOR pathway in vascular remodelling observed in antibody-mediated disorders. In GCA patients, IF analysis only showed activation possibly in inflammatory infiltrates within the media, but co-stainings are lacking to affirm this point.

The role of antibodies remains controversial in TA [32]. Few studies have addressed the role of B cells and antibodies against ECs in the pathophysiology of large-vessel vasculitides [26, 29, 33–41]. B cells were detected in temporal arteries [39], with tertiary lymphoid organs being recently observed in the media layer of patients with GCA [42]. Ectopic lymphoid neogenesis was also found in the aortic wall of a subset of patients with active TA, and flow cytometry analysis of these lesions showed an accumulation of germinal centre-like B cells and the presence of follicular Th cells [33]. Several autoantibodies have been reported in GCA without evidence of a pathogenic role [36, 38]. In TA, the hypothesis of an immune response against ECs that could trigger the disease was proposed [32]. Anti-aortic EC antibodies were found in up to 86% of patients with TA, compared with 9% of HCs [26], and sera from patients with antibodies against ECs induced an increased expression of adhesion molecules and the production of pro-inflammatory cytokines [26]. Some studies have confirmed the presence of antibodies binding ECs in TA and others had conflicting results [29, 34, 35, 40, 41]. It is well-known that identification and quantification of such antibodies depends of the technique used [43, 44]. Here we used two different methods with comparison with GCA patients and found significantly more frequent antibodies against ECs in TA.

We provide here evidence of a pathogenic role of serum IgG in the activation of the mTOR pathway in ECs from adventitial vessels from TA patients, leading to EC proliferation. We could not confirm this cell proliferation in biopsies from patients, but another study recently found significant EC and VSMC proliferation in vascular lesions in TA compared with controls [45]. However, data regarding controls, patients and treatments received at enrolment are lacking in this study [45] and could have influenced the observed differences in proliferation. To support the role of serum IgG, B cell depletion therapy with rituximab has been suggested to be effective in TA in a small number of patients [35, 46, 47]. We found that antibodies targeting ECs bound predominantly to an EC antigen with a molecular weight between 60 and 65 kDa, as previously suggested [26, 41]. Targeted antigens driving EC activation remain to be identified. Some authors propose Hsp60 as a potential target, an HSP that can be expressed at the plasma membrane during stress [43]. Other studies have hypothesized that annexin V, a protein exhibiting an important role in the regulation of apoptosis in vascular ECs, could be a potential target [37]. Further studies are necessary to determine which antigens are targeted by these antibodies in TA.

TA and GCA are known to share some common pathogenic features. However, in the present study we show some difference in the pathogenesis of the two diseases, with the involvement of antibodies targeting ECs and modulating the mTOR pathway in TA. This difference could be explained by TA features that are closer to an autoimmune disease (typically occurring in young women) than GCA, with data about B cells and humoral response more convincing in TA than in GCA [26, 34, 35, 40]. Second, TA is characterized by vascular stenosis and occlusion secondary to vascular remodelling and intima hyperplasia. Moreover, paucisymptomatic vascular disease progression without systemic inflammation is a

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common feature in TA but not in GCA [5]. As shown in vasculopathy associated with APS [20] or with anti-HLA class I antibodies [21], we hypothesize that mTOR pathway activation might be responsible for these TA vascular complications and evolution.

Lastly, the use of mTOR inhibitors in TA could represent an alternative therapeutic option. Glucocorticoids remain the cornerstone of TA treatment. However, although most patients initially achieve remission, relapses and corticodependence are observed in more than two-thirds of patients, with many side effects [48]. Whereas IL-6 targeting therapy has been shown to be effective in GCA [10], it does not seem to be as effective in TA [10], thus highlighting the importance of new emerging therapeutic strategies in this disease. Sirolimus, a selective inhibitor of the mTOR pathway, leads to a decrease in cellular metabolism, growth and proliferation [23]. The efficacy of mTOR pathway inhibitor has been demonstrated in diseases sharing intimal hyperplasia and vascular remodelling with TA, acting directly on ECs [20, 49]. Moreover, sirolimus-eluting stents were suggested to be effective to reduce coronary artery in-stent restenosis in TA in a few reports [50, 51]. Our data suggest the potential efficacy of mTOR inhibitors, showing that EC proliferation induced by TA IgG is strikingly decreased after pretreatment with sirolimus. However, since the mTOR pathway could also be beneficial by supporting neovascularization, further studies are necessary to demonstrate the absence of an increase in ischaemic complications in patients.

In conclusion, this study suggests that serum IgG drives endothelial remodelling in TA through activation of the mTOR pathway in ECs, leading to EC proliferation and possibly intimal hyperplasia. Importantly, this mechanism was not observed in GCA. Inhibition of the mTOR pathway could thus represent an alternative therapeutic option in TA.

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Supplementary data

Supplementary data are available at *Rheumatology* online.

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