Pharmaceutical stabilization of abdominal aortic aneurysms : changing its natural history

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Chapter 8

IL-6: A JANUS-LIKE FACTOR IN ABDOMINAL AORTIC ANEURYSM DISEASE

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ABSTRACT

Background and aims

An abdominal aortic aneurysm (AAA) is part of the atherosclerotic spectrum of diseases. The disease is hallmarked by a comprehensive localized inflammatory response with striking IL-6 hyperexpression. IL-6 is a multifaceted cytokine that, depending on the context acts as a pro- or anti-inflammatory factor. In this study we explore a putative role for IL-6 in AAA disease.

Methods

ELISA's, Western blot analysis, real time PCR and array analysis were used to explore IL-6 expression and signaling in aneurysm wall samples from patients undergoing elective AAA repair. A role for IL-6 in AAA disease was tested through IL-6 neutralization experiments (neutralizing antibody) in the elastase model of AAA disease.

Results

We confirmed an extreme disparity in aortic wall IL-6 content between AAA and atherosclerotic disease (Median [5^{th}-95^{th} percentile] aortic wall IL-6 content: 281.6 [0.0 – 1820.8] (AAA) vs. 1.9 [0.0-37.8] µg/g protein (atherosclerotic aorta), ($p<0.001$). Array analysis followed by pathway analysis showed that IL-6 hyper-expression is followed by increased IL-6 signaling ($p<0.000039$), an observation confirmed by higher aneurysm wall pSTAT3 levels, and *SOCS1* and *SOCS3* mRNA expression, ($p<0.018$).

Remarkably, preventive IL-6 neutralization i.e. treatment started one day prior to the elastase-induction resulted in 40% 7-day mortality due to aortic rupture. In contrast, delayed IL-6 neutralization (i.e. neutralization started at day 4 after elastase induction) did not result in ruptures, and quenched AAA growth ($P<0.021$).

Conclusions

AAA disease is characterized by increased IL-6 signaling. In the context of elastase model of AAA disease IL-6 appears a multi-faceted factor that appears protective upon acute injury, but that is negatively involved in the perpetuation of the disease process.

Non-standard Abbreviations and Acronyms

GAPDH: Glyceraldehyde-3-phosphate Dehydrogenase
IL-6: Interleukin 6
PBS: Phosphate Buffered Saline
pSTAT3: phosphorylated Signal Transducer and Activator of Transcription 3
SOCS1: Suppressors of Cytokine Signaling 1
SOCS3: Suppressors of Cytokine Signaling 3

INTRODUCTION

An abdominal aortic aneurysm (AAA) is common pathology that is considered part of the atherosclerotic spectrum of diseases[1]. Current clinical management fully relies on surgical repair of larger (viz. maximum diameter 55 mm or more) AAA. Yet, while traditional open repair comes with significant perioperative morbidity and mortality, endovascular therapy requires life-long follow up and the cost-effectiveness of endovascular repair is being challenged[2]. It has thus been pointed out that pharmaceutical therapy quenching or halting aneurysm growth holds many promises, both from the patient's point of view as from the socio-economical point of view[3].

It is generally assumed that AAA growth is driven by a comprehensive inflammatory response, and consequently that interference with the inflammatory response will alleviate aneurysm progression. The validity of this approach has been well shown in numerous animal studies, but thus far clinical interventions failed[4-6]; a conclusion pointing to incomplete understanding of the inflammatory aspects of AAA disease.

The inflammatory footprint of AAA is complex and includes both components of an acute inflammatory response such as neutrophils and NK cells as well as aspects of a chronic inflammation (plasma cells, tertiary follicles)[3]. On the molecular level the disease is best defined as a general pro-inflammatory response with comprehensive and intense upregulation of pro-inflammatory cytokines and chemokines, including notably high IL-6 levels[7]. In fact, it has been reported that aortic wall IL-6 protein levels in AAA exceed those in advanced atherosclerotic disease by several 100-fold, implicating IL-6 as a potential critical inflammatory factor in AAA disease[7]. This notion is supported by robust genetic evidence linking a polymorphism in the IL-6 gene promoter (IL-6-572G>C) to AAA disease (reported odds ratio: 6.00)[8].

IL-6 has long been considered a pro-inflammatory cytokine and, through its pro-inflammatory activities, a culprit in the development and complications of atherosclerotic disease[9,10]. Yet, it is now becoming apparent that IL-6 is versatile multifunctional (Janus-like) cytokine with functions that extend beyond that of a pro-inflammatory cytokine; and include coordination of immune and acute responses, regulation of hematopoeisis[9], as well as roles in tissue protection and regeneration[11, 12]. Consequently, the biology of IL-6 is complex and, depending on the context IL-6 may exert protective activities as well as detrimental actions[13].

In light of these observations we considered an evaluation of a putative role for IL-6 in AAA relevant. To that end, we evaluated the IL-6 signaling pathways in human AAA disease, and tested a role for IL-6 in the initiation and progression of AAA in the murine elastase model, an established model of AAA disease.

PATIENTS AND METHODS

Human Samples

The investigation conforms the principles outlined in the Declaration of Helsinki (2013). Sample collection and handling was performed in accordance with the guidelines of the medical ethical committee of the Leiden University Medical Center and University Medical Center Utrecht. Control infra renal aorta was selected from a tissue bank of aortic wall patches that were obtained during kidney procurement for organ donation. Characteristics of these samples have been

described elsewhere[14]. Controls were age matched and only samples displaying atherosclerotic lesions were included.

Aneurysm wall samples (anterior-lateral wall) were collected during elective surgery for asymptomatic AAA (55 mm or larger). Wall samples were divided in two parts. One half was immediately snap-frozen in CO_2-cooled iso-pentane or liquid N_2 and stored at −80°C for later analysis. The other half was fixed in 4% formalin for 24 hours followed by decalcification (Kristenssen solution). Next, these segments were paraffin embedded and 4 µm sections were processed into slices.

A further analysis of the effects of doxycycline on SOCS-1 and 3 signaling was performed on cDNA samples from an earlier doxycycline intervention trial[15].

Elastase model

All animal experiments were approved by the Leiden University Medical Center animal welfare committee, and performed in compliance with the Dutch governmental guidelines.

IL-6 activity was quenched through repeated doses (4 mg/kg) of an IL-6 neutralizing antibody (MAB406, R&D systems, Abingdon, UK). This antibody and dosing scheme was earlier shown to fully quench IL-1-induced hepatic CRP expression (a process mediated by IL-6 and STAT3 signaling) in a pilot study using human CRP transgenic mice.[16]

Eight-to-ten weeks old, male, wild-type (WT; C57BL/6) mice were obtained from Charles River (Chatillon-sur-Chalaronne, France). Animals were housed in a temperature and humidity-controlled room on a 12:12-h light–dark cycle with ad libitum access to water and normal chow diet.

Infra renal aneurysms were created via incubation of the isolated terminal aorta segment with type I porcine pancreatic elastase (4.5 U/mL; Sigma-Aldrich, Zwijndrecht, the Netherlands) as previously described[17]. Briefly, the aorta was exposed and a catheter positioned at the iliac bifurcation. The catheter was removed after elastase infusion, and the abdomen closed after hemostatic control. Mice were given 0.1 mg/kg/12hrs buprenorphine, allowed to recover with free access to food and water.

A role for IL-6 in AAA initiation and progression was assessed through repeated anti-IL-6 injections (IP) starting at the day before elastase infusion (day -1), followed by injections on day 3, day 7 and day 11 (n=9) (Figure 1). Control animals (n=9) received parallel IP injections with PBS.

There were a high number of unexpected deaths in the 24 hours following aneurysm induction in the treatment arm. To that end we set up a second experiment (n=8) in which IL-6 neutralization was delayed, i.e. therapy was initiated on day 3 after elastin treatment the infusion.

Aneurysm formation and growth in all groups was assessed by ultrasound (Vevo 770 Imaging system using RMV 704 micro-visualization scan head (Visualsonics, CA)). The maximum axial diameter of the aorta was measured at day -1 (one day prior to elastase infusion), day 7 and 14. Mice were sacrificed after the final aneurysm reading and their aorta was removed, formalin fixed and paraffin embedded.

Aneurysm wall IL-6 content

Aorta wall IL-6 protein levels were determined in 238 AAA samples from the Aneurysm-Express Biobank.[18] Twenty six atherosclerotic aortic wall samples served as control. IL-6 content was

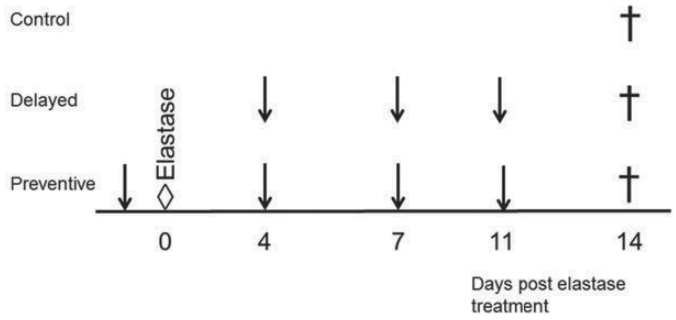

Figure 1. Study scheme of the elastase model. Arrows (↓) depict anti IL-6 injections. Mouse were sacrificed at day 14 (†) and the aorta harvested.

measured through Luminex multi-analyte profiling technology[19,20], using a bio-plex system (Bio-Rad, USA), and the content normalized on basis of total protein content (BCA protein measurement method (Pierce Biotechonology, USA)). Inter-assay coefficient of variation was <10%.

Immunohistochemistry

Human (n=10 AAA samples and n=10 control atherosclerotic aorta samples) and murine tissue sections were deparaffinized and incubated overnight at room temperature with the primary antibody diluted in PBS, 1% BSA, using the following primary antibodies for the human studies: IL-6 (Santa Cruz Biotechnology, USA), IL-6R or CD126 (Abcam, UK) and pSTAT3 (Abcam, UK). Envision mouse or Envision Rabbit (Dako, Denmark) were used as secondary antibody.[15]

Murine sections were incubated with CD45 (BD Pharmingen, USA), MAC3 (BD Pharmingen,USA), MMP9 (Santa Cruz Biotechnology, USA) and Smooth Muscle Alpha Actin (DAKO, Denmark). Further sections were stained with Sirius Red for collagen and Weigert's elastin stain to visualize elastic lamina. Eight slides per animal were used per staining for analysis and only moderate or strongly reactive cells were counted as positive. The slides were blindly evaluated. A mean value for positive staining cells in the eight sections was calculated for each animal.

Microarrays

RNA extraction was performed from full thickness aortic wall samples from 31 AAA patients (mean age 69.5 yrs. mean diameter 62.3±12.1 mm) and 9 control samples (infra renal aorta obtained during kidney procurement for donation).

RNA from aneurysm wall was labeled and hybridized to Illumina HumanHT-12 v4 BeadChips. Arrays were scanned with an Illumina iScan microarray scanner. Bead level data preprocessing was done in Illumina GenomeStudio.

Analysis of array data

Quantile normalization and background reduction were performed according to standard procedures in the Illumina GenomeStudio software.

Association of genome-wide expression data with AAA phenotype revealed 11486 transcripts with P<0.05. These differentially expressed transcripts were used as an input for pathway analysis through Ingenuity Pathway Analysis suite (http://www.ingenuity.com, accessed 2016). Levels of significance were determined using Fisher's exact tests implemented in the software.

Western blot analysis

Samples for Western blot were homogenized and lyzed in the following buffer: 150mM NaCl, 50 mM Tris (pH 8.0), 1.0% Triton X-100, 0.1% SDS, 0.5% sodium deoxycholate, 10mM orthovanadate and Protease inhibitor cocktail (Roche, The Netherlands), and Western blot was performed as earlier described,[14] using the following antibodies: pSTAT3 (GeneTex, The Netherlands), STAT3 (Santa Cruz Biotechnology, USA) and alpha actin (Abcam, Cambridge, UK) for normalization. Donkey anti-rabbit IgG-HRP (Santa Cruz Biotechnology, USA) was used as the secondary antibody. Spots were visualized and quantified using the Super Signal West Dura Extended Duration Substrate (Pierce Biotechnology, Germany), and the luminescent image workstation (Roche Diagnostics) with labworks 4.6 software.[15]

Semi quantitative RealTime PCR analysis

IL-6, IL-6R (CD126), *SOCS1* and *SOCS3* mRNA expression was quantified by semi quantitative RT, according to manufacturer's instructions in n=12 AAA samples and n=16 control atherosclerotic aorta samples. cDNA was prepared by using a Promega kit for RT-PCR. Semi quantitative RNA analysis was performed using the Taqman system for the determination of mRNA expression we used the established primer/probe sets (Life technologies, USA; *IL-6*: Hs00985641_m1; *IL6r*: Hs01075666_m1; *SOCS1*: Hs00705164_s1; *SOCS3*: Hs02330328_s1 and *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase)), the mastermix (Eurogentec, Belgium) and the ABI-7700 system (Life Technologies, USA) as previously described. GAPDH (Life Technologies, USA) was used for normalization.[15]

Statistical Analysis

An ANOVA test was performed to explore the difference between human normal aortic and the aneurysm samples. Subsequently an unpaired t-test was performed. Non-normally distributed data were log-transformed.

To evaluate the statistical significant difference between the mice treated for 15 days with anti-IL-6, mice treated for 10 days and the controls were evaluated with a Kruskal-Wallis ANOVA test. All parameters considered statistically significant ($p<0.05$) were hereafter evaluated pair-wise with the Mann-Whitney U test. Aneurysm formation in the elastase models was evaluated by the Chi square test.

All values are shown as mean (SD) or as median [5^{th} -95^{th} percentiles]. Probability values of $p<0.05$ were considered statistically significant. The analyses were performed using SPSS 23.0 (IBM, Amsterdam, the Netherlands).

RESULTS

Elevated IL-6 expression and pathway activation in aortic aneurysm walls

The majority of studies on AAA wall cytokine profiles indicate abundant IL-6 protein expression, yet the current literature is not fully consistent. We therefore first validated previous reports[7, 21] of prominent aortic wall *IL-6* mRNA expression and elevated IL-6 protein levels in samples from the Aneurysm-Express Biobank. This analysis confirmed ample *IL-6* mRNA expression ($p < 0.001$, Figure 2A), and sharply increased IL-6 protein levels in AAA wall samples (aortic wall IL-6 content in AAA: 281.6 [0.0 – 1820.8] µg/g protein vs. 1.9 [0.0 – 37.8] µg/g protein median [5th–95th percentile] in atherosclerotic controls; $p < 0.001$, Figure 2B).

IL-6 signaling is thought to occur via two distinct pathways. Firstly, through the classic signaling route involving the IL-6 receptor (CD126)/GP-130 (CD130) receptor complex, and secondly via a trans-signaling route that involves signaling through the GP-130 (CD130)/soluble-IL-6 receptor (sIL-6r)[9, 12] complex. Signaling via this later route is thought to activate the non-classical signaling route[21]. While GP-130 is ubiquitously expressed, expression of the IL-6 receptor is thought be more restricted. ELISA showed abundant sIL-6r in AAA and control wall samples (Figure 2D). Semi-quantitative RealTime

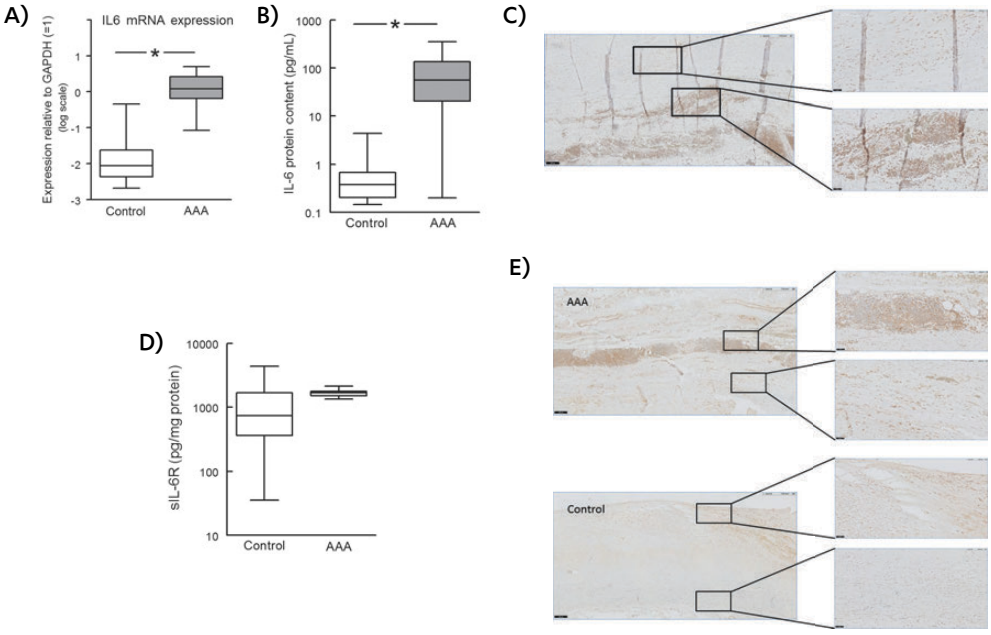

Figure 2. Aortic wall IL-6 expression. **(A.)** Aortic wall IL-6 mRNA expression relative to the house keeping gene GAPDH, in atherosclerotic controls and AAA, $^{*}p < 0.001$. **(B.)** Normalized aortic wall IL-6 protein content (ng/ml protein), $^{*}p < 0.001$. **(C.)** L-6 localization in the human aneurysm wall. IL-6 is broadly expressed in lymphocytes, macrophages as well as mesenchymal cells. Overview 40X, details 200 X. **(D.)** Similar aortic wall soluble IL-6 receptor protein content (ng/mg protein) in AAA and atherosclerotic controls. p = ns. **(E.)** IL-6 receptor (CD126) expression in AAA and atherosclerotic controls. CD126 expression in control aorta is limited to the intimal layer, whereas diffuse but selective expression (subpopulations of lymphocytes, macrophages and mesenchymal cells) was observed in AAA tissue. Overview 40X, details 200X).

PCR for *IL-6r* mRNA showed increased expression in AAA versus atherosclerotic aortic controls ($p < 0.01$). Immunohistochemical evaluation for the aneurysm wall showed diffuse expression IL-6r expression in mesenchymal cells and subpopulations of lymphocytes in the adventitial infiltrates (conclusions based on cellular morphology). IL-6r expression in the atherosclerotic controls was limited to the intimal layer (Figure 2E).

Signaling through both the classical, and non-classical route involves phosphorylation of the STAT-3 transcription factor, and induction of suppressor of cytokine signaling (SOCS) -1 and 3 expression. Ingenuity-based transcriptomics analysis shows upregulation of IL-6 signaling pathway in AAA disease (canonical IL-6 pathway: $p < 0.000039$). More detailed analysis of the signaling networks indicated selective activation of the JAK-STAT signaling cascade but not of the RAS mediated ERK1/2 route (Figure 3). Western blot analysis confirmed the array data and showed increased STAT3 phosphorylation in AAA (Figure 4A, $p < 0.018$). *SOCS1* and *3* mRNA expression was higher in AAA wall samples than in controls ($p < 0.003$, Figure 4B). Immunohistochemistry for phosphor-STAT3 indicates that IL-6 signaling in AAA and atherosclerotic controls mainly occurs in macrophages and smooth muscle cells (Figure 4C).

The tetracycline analogue doxycycline has broad anti-inflammatory properties that include quenching of aneurysm wall IL-6[15]. Paradoxically, a clinical trial concluded that doxycycline accelerates aneurysm growth[5]. We hypothesized that this effect may (partially) relate to suppression of SOCS expression. Comparison of *SOCS-1* and *3* mRNA expression in AAA wall samples from patients that did and did not receive doxycycline treatment showed that doxycycline treatment reduces AAA wall *SOCS-1* mRNA expression (Figure 4B, $p < 0.037$).

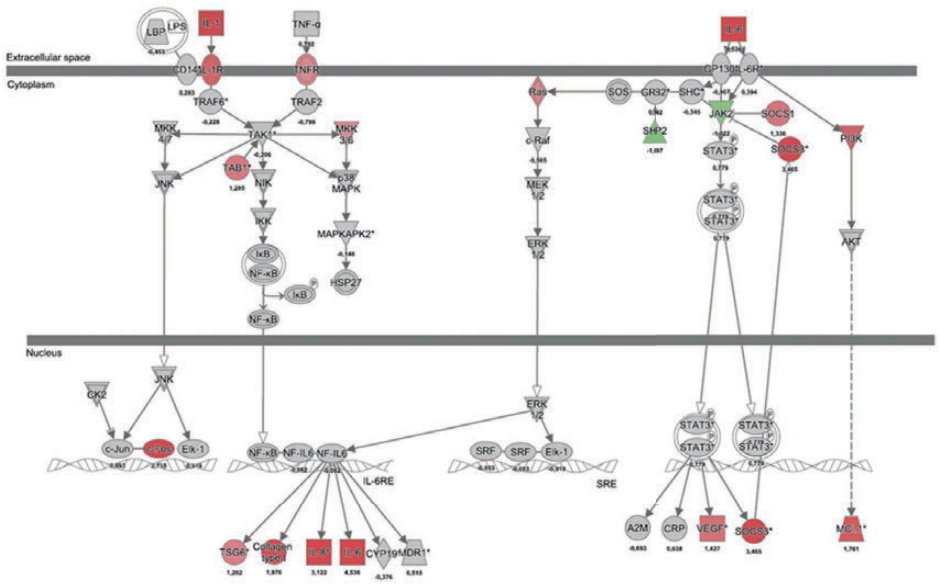

Figure 3. IL-6 signaling pathways in AAA disease (Ingenuity Pathway Analysis). Specific upregulation of STAT3 mediated IL6 signaling but not of the RAS-ERK1/2 axis in AAA disease (Ingenuity Pathway Analysis).

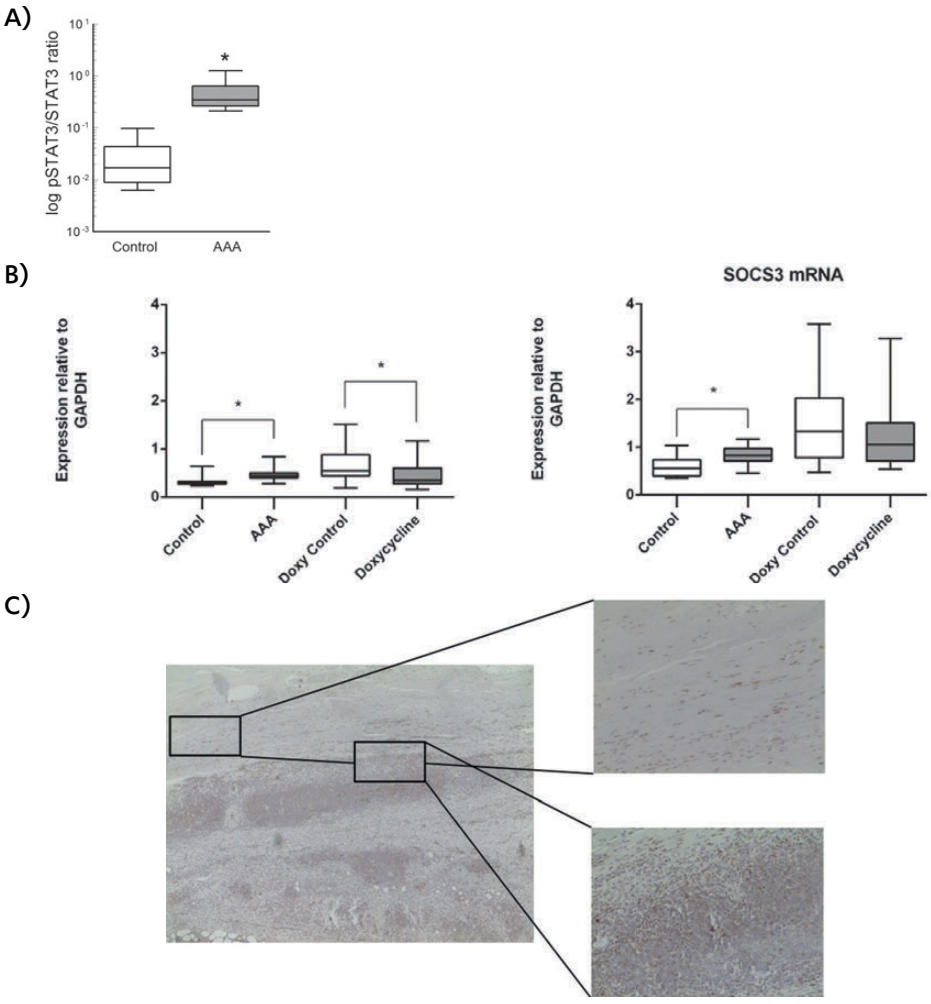

Figure 4. Enhanced IL-6 signaling in AAA disease. **(A.)** Similar aortic wall STAT3 but increased phosphorylated STAT3 (pSTAT3) content (Western blot analysis) in AAA tissue. Levels normalized on α-actin content. (STAT3 (p = ns) and pSTAT3 ($p < 0.030$)). **(B.)** Left bars: increased SOCS-1 and -3 mRNA expression in AAA tissue, ($^{*}p$ respectively <0.0008 and 0.0035). Right bars: reduced aortic wall SOCS-1 mRNA expression after doxycycline therapy. **(C.)** phosphor-STAT3 distribution in AAA tissue, showing IL-6 signaling in macrophages and mesenchymal cells.

A possible role for IL-6 in AAA disease was investigated in the murine elastase model of AAA disease through quenching IL-6 by means of a validated IL-6 neutralizing antibody (Figure 1). Unexpectedly, preventive IL-6 neutralization (i.e. therapy started at the day prior to the elastase infusion) resulted in a 40% 7-day mortality (Figure 5A). Further evaluation showed that this mortality was due to aortic rupture (Figure 5A and B). No deaths or ruptures were observed in the control group. In the surviving mice, preventive anti-IL-6 treatment did not influence the number of aneurysms formed (>50% increase in aorta baseline diameter, $p > 0.05$) (Figure 5C).

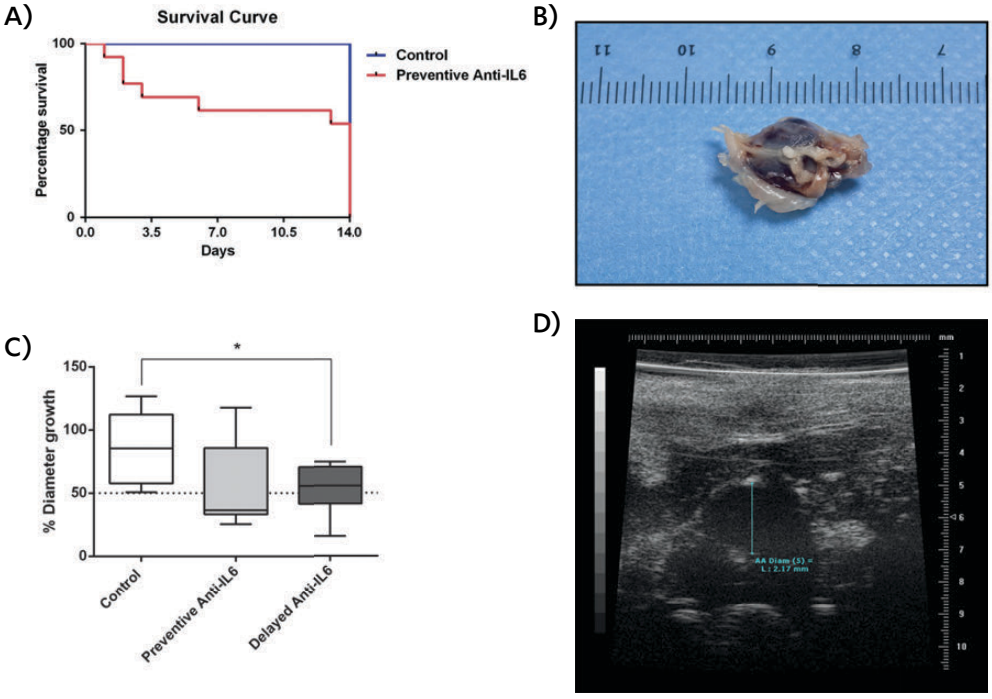

Figure 5. IL-6 neutralization in the elastase model of AAA disease. **(A.)** Survival curves of mice receiving preventive anti-IL-6 (i.e. anti-IL-6 was administered before the elastase perfusion), and vehicle treated control mice. Day 0 is the day of the elastin infusion. Mice were all sacrificed on day 14. **(B.)** Explanted aorta of one of the deceased mice that received preventive anti-IL-6 treatment showing a confined rupture (the array indicates the lumen of the aorta), and (right) an ultrasound showing a murine aneurysm. **(C.)** Delayed anti-IL6 (therapy initiated at day 4 after elastase infusion) quenches AAA formation ($p < 0.03$). No effect was seen in the mice that survived whereas the preventive treatment resulted in similar aortic growth as the controls ($p > 0.05$). **(D.)** Ultrasound image showing an aortic aneurysm in the elastase model.

The observed detrimental effects of IL-6 neutralization may relate to a possible protective role for IL-6 in the context of acute injury[13]. We therefore performed a second series of experiments in which IL-6 neutralization was delayed until day 4 (antibody treatment was started on the fourth day following elastase treatment). Delayed IL-6 neutralization did not result in rupture, moreover, the IL-6 neutralizing antibody reduced the AAA progression (Figure 5C, anti-IL-6 (delayed), $p < 0.03$). Histological evaluation on day 14 (Figure 6) showed that IL-6 neutralization did not influence aortic wall leukocyte, macrophages or MMP9 content nor the number of elastin breaks and collagen content compared to control animals (Data not shown).

DISCUSSION

The pathology of AAA is complex and poorly understood. On the molecular level, the disease is best described as an intense, localized comprehensive inflammatory response[23, 24]. At this point, it is still unclear which aspects of this inflammatory response are causatively involved in the disease

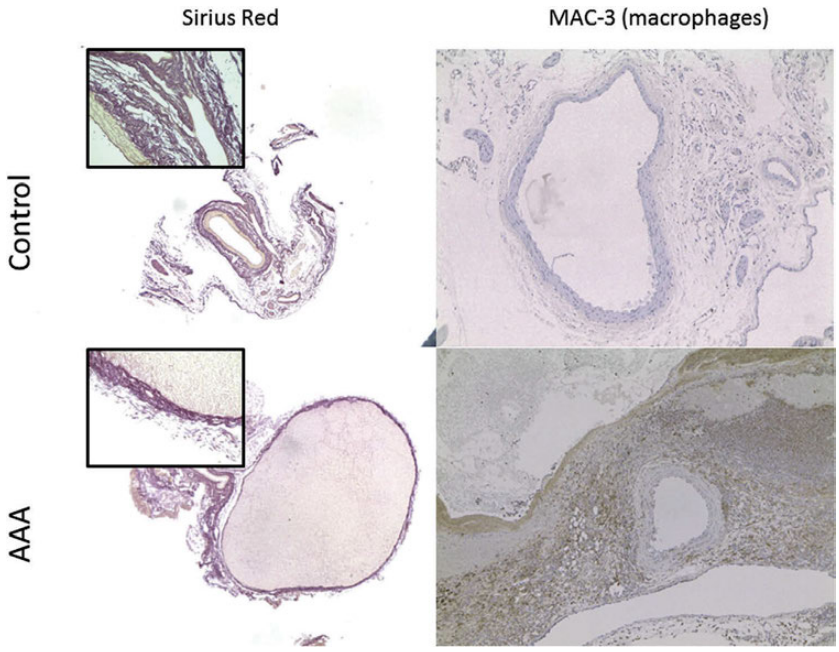

Figure 6. Histological examples of the elastase model. Histological staining for collagen (sirius red, left) and macrophages (MAC3, right) for a control and aneurysmal aorta .

process, and which factors are found by association[25]. Remarkably, while there is genetic evidence for an association between IL-6 signaling[8] and AAA disease, and while IL-6 hyperexpression has been reported as a discriminative feature of the disease[7, 21, 26], no studies to date addressed a possible role for IL-6 in the disease. Results from this study confirm ample IL-6 expression and increased IL-6 signaling in human AAA tissue. IL-6 neutralization experiments in a mouse model of AAA disease suggest that the role of IL-6 could be protective in the context of aneurysm formation, but disadvantageous in the context of AAA progression.

Genetic and observational associations imply a link between IL-6 and AAA disease. A report by Smallwood and colleagues identifies a polymorphism in the IL-6 gene promoter as a rare independent risk factor for AAA[8]. Moreover, a Mendelian randomization approach found robust evidence for a causal link between the IL-6 receptor signaling pathway and AAA disease[27]. On the (aneurysm) tissue level most, the majority, but not all studies report increased IL-6 levels[23,28, 29], and the aneurysm wall has been identified as a relevant source of plasma IL-6[30].

Given the lack of full consistency with regard to IL-6 hyperexpression[23], we first performed a validation experiment for our previous findings in a patient cohort[18] that is distinct from our earlier studies[7]. Results of this evaluation again showed an extreme disparity in aortic wall IL-6 content in AAA and atherosclerotic aorta wall samples with median AAA IL-6 content being more than 100-fold higher than in atherosclerotic controls.

Although IL-6 is generally considered a pro-inflammatory cytokine and a key-regulator of the acute phase response, it is now clear that the roles of IL-6 extend beyond its traditional function

in inflammation/infection, and also include regulation of hematopoiesis, metabolic control and roles in tissue protection/regeneration[9, 12]. These disparate roles are thought to reflect different signaling routes: the *classical* IL-6 receptor route being responsible for the anti-inflammatory/tissue regenerative activities[13], and the non-classical, *trans-signaling* route via the soluble IL-6 receptor being responsible for the pro-inflammatory activities. These observations characterize IL-6 as a Janus-like factor with the net effect depending on the activities of the two distinct signaling routes, and the cross-talk with other inflammatory mediators[9, 12, 13].

IL-6 receptor (CD126), responsible for the classical signaling route was diffusely expressed in the mesenchymal cell population within the aneurysm wall as well as in subpopulation of leucocytes within the tertiary follicles. This pattern was clear distinct from aortic atherosclerotic disease in which the receptor was essentially expressed in the atherosclerotic region in the intima. At this point both signaling routes are thought to converge on STAT3 transcription factor effector pathway, and on SOCS-1 and 3 expression[31-33]. As such, elevated STAT3 phosphorylation, and increased SOCS-1 and 3 expression in AAA show that the increased IL-6 levels in AAA disease are followed by augmented IL-6 signaling. Yet, this data doesn't allow for distinction between signaling via the tissue protective (classical) route or via the pro-inflammatory (non-classical) signaling route.

We therefore decided for an experimental approach and tested whether quenching of IL-6 via a neutralizing antibody influenced aneurysm formation and progression in the elastase model of AAA disease. Unexpectedly we experienced early ruptures in almost 50% of the anti IL-6 treated animals, a notable phenomenon since, to the best of our knowledge ruptures have not been described for the elastase model. The aggravation of acute tissue damage during IL-6 neutralization proofs the efficacy of the antibody used, but also fits in other reports showing that IL-6 is protective in the acute phases of ischemia reperfusion injury and ischemia[34-37]. Yet, it is obviously unclear how whether and how these experimental findings from an acute model translate to the process of AAA formation in man i.e. whether IL-6 has a role in protecting against AAA formation.

We therefore performed a second series of experiments with delayed anti-IL-6 therapy started at day 4 after the elastase treatment. Delayed IL-6 neutralization did not result in ruptures, suggesting that the deleterious effects of IL-6 inhibition relate to processes involved in the initiation process of AAA formation in the elastase model. Delayed therapy did result in a significant reduction of aneurysm progression.

A critical question is whether these mouse observations translate to the human context. Although the acute aortic ruptures observed in this study may not appear relevant for human AAA, diverticular perforations have been observed during treatment with Tocilizumab, a monoclonal antibody targeting the IL-6 receptor[11]. As such it cannot be excluded that IL-6 neutralization may negatively affect clinical AAA disease. Data from the experiments with delayed therapy hint at a beneficial effect of IL-6 neutralization on AAA progression. A critical question is whether quenching IL-6 has beneficial effect in the clinical context. Our earlier studies show that statins, ACE-inhibitors and doxycycline therapy all profoundly reduce aneurysm wall IL-6 levels[15,38,39], yet this is not followed by attenuation of aneurysm progression [4]. Although one could argue that halving IL-6 hyperexpression in AAA disease is not sufficient to interfere with AAA progression, it remains to be established whether a further suppression is clinically feasible. In fact, the observed accelerated aneurysm growth in patients receiving doxycycline-treatment may (partially) relate to a reduction in SOCS-1 expression in patients receiving doxycycline and/or a role of IL-6 in tissue remodeling[40].

In conclusion, this study confirms exaggerated IL-6 levels and IL-6 signaling in AAA disease, and although indications were found for a beneficial effect of AAA neutralization in the elastase model of AAA disease, we also found indications for potential detrimental effects. Moreover, indirect interference with aneurysm wall IL-6 content in clinical studies is not followed by a reduction in AAA progression. As such this study does not identify IL-6 as a key-driver of AAA progression, yet the data does not rule out a role for IL-6 in AAA formation.

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