

Increased Expression and Activation of Absent in Melanoma 2 Inflammasome Components in Lymphocytic Infiltrates of Abdominal Aortic Aneurysms

Susanne Dihlmann,¹ Philipp Erhart,¹ Arianeb Mehrabi,² Arash Nickkholgh,² Felix Lasitschka,³ Dittmar Böckler¹ and Maani Hakimi¹

¹Department of Vascular and Endovascular Surgery, University Hospital Heidelberg, Heidelberg, Germany; ²Department of General, Visceral and Transplantation Surgery, University Hospital Heidelberg, Heidelberg, Germany; ³Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany

Chronic vascular inflammation is a key hallmark in the pathogenesis of abdominal aortic aneurysm (AAA). Recent investigations have suggested that the inflammasome, a cytosolic multiprotein complex that recognizes pathogen-associated molecular patterns, plays a role in atherosclerosis. However, its role in AAA inflammation has not yet been investigated. This pilot study analyzed inflammasome activation and its intramural localization in 24 biopsy samples from 11 patients with asymptomatic AAA versus 12 aortic samples from apparently healthy controls. Using a histological inflammation scale, we identified grade 2/3 inflammatory changes with lymphoid aggregates/tertiary lymphoid organs in 21 out of 24 AAA samples, whereas only 7 of the 12 control samples exhibited local grade 1 inflammatory changes. Strong expression levels of “apoptosis-associated speck-like protein with a caspase recruitment domain” (ASC), caspase-1, caspase-5 and “absent in melanoma 2” (AIM2) were detected by immunohistochemistry in both sporadic infiltrating lymphoid cells and lymphoid aggregates located in the outer media and adventitia of AAA samples. In contrast, inflammasome-positive cells were restricted to cholesterol plaque-associated areas and to single infiltrating cells in control aortas. Analysis of gene expression using real-time polymerase chain reaction (PCR) revealed significantly increased median mRNA levels of the inflammasome core components *PYCARD* (ASC), *CASP1* (Caspase-1) and *IL1B* (*IL-1β*) in AAA tissue compared with normal aorta. Moreover, significantly increased median amounts of AIM2 protein and mature caspase-5 (p20) were found in samples associated with high rupture risk compared with paired low rupture risk samples of the same AAA patient. We conclude from our data that AAA-associated lymphoid cells are capable of inflammasome signaling, suggesting that inflammasome activation is involved in the chronic inflammatory process driving AAA progression.

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INTRODUCTION

The pathophysiology of abdominal aortic aneurysm (AAA) is considered a combination of complex mechanisms, including apoptosis of vascular smooth muscle cells, increasing fibrosis, extracellular-matrix degeneration and chronic inflammation (1). The sequential order and precise nature of these degen-

erative mechanisms, finally leading to rupture, are controversially discussed in the literature. Growing evidence from immunological and histopathological investigations of AAA supports an “outside-in” hypothesis, in which vascular inflammation is initiated in the adventitia and progresses toward the intima (2). Vascular-associated lymphoid tissue (VALT),

consisting of disseminated accumulations of immunocompetent cells such as T cells, B cells, dendritic cells and macrophages, have been identified in the outer aortic wall, mostly located in the external layer of the media and the entire adventitia (3,4). Detailed analysis of the VALT revealed that their composition resembles that of mucosa-associated lymphoid tissue (MALT) of the respiratory and gastrointestinal tracts (5), forming lymphoid follicles with a germinative center of B cells that are surrounded by T cells (3). Further phenotypic characterization of the immunocompetent cells by specific surface receptors identified different populations of T and B cells of varying activation and memory status with distinctive homing properties, indicating that these

Address correspondence to Maani Hakimi, Department of Vascular and Endovascular Surgery, University Hospital Heidelberg, Im Neuenheimer Feld 110, D-69120 Heidelberg, Germany. Phone: +49-6221-5638759; Fax: +49-6221-567654; E-mail: maani.hakimi@med.uni-heidelberg.de.

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infiltrates are relevant for development and progression of AAA (4).

In addition to the adaptive immune response, innate immunity was recently demonstrated to be crucial for vascular inflammation. In particular, inflammasome activation and subsequent reactions of macrophages in the arterial wall appear to be required for atherogenesis (6–8). Inflammasomes are cytosolic multiprotein complexes composed of intracellular receptors, adaptor molecules and effector proteins, which were originally identified in macrophages. Their major function is recognizing pathogen and danger-associated patterns (PAMPs and DAMPs) such as intracellular bacteria, viruses, crystals, cytosolic nucleic acids from damaged/senescent cells or ultraviolet B radiation, among others, and responding to these signals by activation of inflammatory caspases (-1 or -5 in humans) (8,9). Upon activation, these caspases drive host responses by releasing cytokines, such as interleukin (IL)-1 β and IL-18, into the cellular environment. In addition, inflammasomes may induce pyroptosis, a certain type of cell death that requires caspase-1 activation (9).

In the vasculature, several inflammasome components have been identified as important mediators of atherosclerosis. Cholesterol crystals and oxidized low density lipoprotein (LDL) activate the “NLR family, pyrin domain containing 3” (NLRP3) inflammasome in human macrophages, thereby driving vascular repair, foam cell formation (10) and atherogenesis (7,10–12). Moreover, the inflammasome adaptor molecule “apoptosis-associated speck-like protein with a caspase recruitment domain” (ASC) and caspase-1 were demonstrated to be critical for vascular inflammation, neointima formation and atherosclerosis in mouse models (11,12). Finally, our own recent findings, identifying induction of the cytosolic double-stranded DNA (dsDNA) receptor and inflammasome activator “absent in melanoma 2” (AIM2) in atherosclerotic carotid plaques and in the adventitia of aortic

aneurysms suggest a role of AIM2 in vascular inflammation (13).

Inflammasome activation in the aortic wall of abdominal aneurysms has not yet been examined. However, IL-1 β is considered a major cytokine that activates proteolysis in AAA (14,15), and IL-1 β release and signaling have been found to be critical for both initiation and progression of AAA (16–18). Given that inflammasome activation is an important inducer of IL-1 signaling, and this inflammatory signaling is associated with progression of AAA, we investigated inflammasome activity within the aortic wall of AAA in a pilot study.

MATERIALS AND METHODS

Tissue Samples and Vascular Biobank Heidelberg

Twenty-four tissue samples were collected during open surgery from 11 patients with asymptomatic infrarenal AAA ranging in maximum diameters from 5 to 12 cm. Patient characteristics are summarized in Supplementary Table 1. From each patient, 2–3 biopsies were taken from aneurysm areas with high or low rupture risk. For identification of respective areas with different rupture risks, finite element (FE) analysis was performed as described before (16,17). Briefly, preoperative multislice computed tomographic (CT) angiographies were generated with a 64-slice CT scanner (Somatom Definition; Siemens, Munich, Germany). The resulting data were used to reconstruct three-dimensional AAA contour models of each patient, and FE analysis was performed using a semiautomatic FE analyzing software (A4research; VASCOPS GmbH, Graz, Austria) as described earlier (16–18). In addition, 12 aortic tissue samples, free of macroscopically observable disease, were obtained during back-table procedures of organ transplantations to serve as controls. All specimens were immediately processed for analysis by quantitative reverse-transcription PCR (RT-qPCR) and immunohistochemical analysis according to standard operating proce-

dures (SOPs) of the Vascular Biobank Heidelberg (VBBH).

Tissues were collected and processed according to ethics guidelines. All patients gave their written informed consent. The study as well as tissue collection by the VBBH were approved by the Medical Ethics Committee of the University of Heidelberg, Germany (reference numbers S-301/2013 and S-412/2013).

Immunohistochemistry and Histopathological Grading

Tissues were formalin fixed and embedded in paraffin according to standard procedures for conventional histology. For immunohistochemical detection of protein expression, serial 4- μ m sections were prepared from each paraffin-embedded tissue specimen. After deparaffinizing and rehydration, samples were pretreated according to individually optimized protocols (detailed protocols are available on request; antibodies and dilutions are listed in Supplementary Table 2). Histopathological grading of aortic samples was performed according to a modified histological inflammation scale of aneurysms (HISA) that was suggested by Rijbroek *et al.* (19): grade 0, no inflammation, single infiltrating lymphocytes, no fibrosis; grade 1, mild chronic inflammation, localized lymphocyte infiltration, mild fibrosis of the media; grade 2, moderate chronic inflammation, localized and diffused lymphoplasmocytic infiltrates in the adventitia with perivascular aggregates and medial degeneration; grade 3, severe chronic inflammation and/or clinically defined inflammatory aneurysm (IAAA), with wall thickening of the adventitia, lobular arranged lymphocytes and severe dense fibrosis. Examples are shown in Supplementary Figure 1.

RNA Extraction, cDNA Synthesis and RT-qPCR

Tissue samples were immediately shock frozen in liquid nitrogen and stored at -80°C until further processing. The samples were ground with pestle and mortar in liquid nitrogen, and RNA was extracted using the RNeasy Fibrous

Tissue MiniKit (Qiagen, Hilden, Germany). For cDNA synthesis, 0.5 µg of total RNA was reverse transcribed using oligo-dT primers and SuperScript III reverse transcriptase (Invitrogen/Life Technologies [Carlsbad, CA, USA]) following the manufacturer's instructions. For real-time PCR, PowerSYBR Green master mix (Applied Biosystems/Life Technologies) was added to appropriate cDNA samples and primers (Supplementary Table 3). Samples were loaded onto 96-well PCR plates and analyzed in an ABI Prism 7300 thermocycler (Applied Biosystems/Life Technologies) as described (20). Quantitative analysis of gene expression was performed relative to expression of *GAPDH* RNA in corresponding samples by using individual standard amplification curves of each transcript.

Western Blotting

Frozen tissue samples were ground with pestle and mortar in liquid nitrogen and protein was extracted by adding 200 µL of lysis buffer (50 mmol/L TRIS-HCl [pH 7.5], 150 mmol/L NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 0.5% NP-40), which was supplemented with protease inhibitor (Mix G; Serva, Heidelberg, Germany) immediately before use. Equal amounts of protein lysates (50 µg per lane) were separated by SDS-PAGE and blotted on nitrocellulose. Antibodies and dilutions that were used for detection are listed in Supplementary Table 2. Signals were visualized by chemoluminescence as described earlier (20). For semiquantitative analysis, the blots were scanned and the signal intensity was determined using the public domain Java image processing program ImageJ (<http://rsb.info.nih.gov/ij/index.html>). The specific signal intensity of different proteins was normalized to β-actin that was detected on the same blot.

Statistical Analysis

Data analysis was performed using the GraphPad Prism 4.02 software (La Jolla, CA, USA). Statistical differences between the medians of two groups (quantitative analysis of mRNA expression) were de-

Table 1. Histological evaluation of the aortic wall using the histological inflammation scale (HISA) of aneurysms (19).

Aneurysm	No.	Inflammatory changes, n (%)	Atherosclerotic plaque, n (%)	Grade 0, n (%)	Grade 1, n (%)	Grade 2/3, n (%)
AAA	24	24 (100)	22 (91.2)	0 (0)	3 (12.5)	21 (87.5)
Aorta control	12	7 (58.3)	4 (33.3)	5 (41.7)	7 (58.3)	0 (0)

termined by the Mann-Whitney *U* test. Comparison of differences between the medians of three groups (protein levels of high-risk tissue, low-risk tissue and normal control) was performed using the Kruskal-Wallis test. A level of $p < 0.05$ was considered statistically significant.

All supplementary materials are available online at www.molmed.org.

RESULTS

Histopathological Analysis of Inflammation and the Expression Pattern of Inflammasome Components in Aortic Tissue Suggest Inflammasome Activity in Lymphoid Tissue of AAA

Inflammatory changes such as local, diffuse or clustered infiltration with lymphocytes were identified in all samples derived from AAA patients (Table 1). Out of these, 21 samples exhibited grade 2 or grade 3 lesions, with diffused lymphoplasmocytic infiltrates in the adventitia and medial degeneration or lobular arranged lymphocytes at the adventitia-media border, respectively. Three AAA samples showed only localized lymphocyte infiltration, corresponding to grade 1 lesions (for examples see Supplementary Figure 1). In contrast, five out of 12 control samples from aortic tissue were devoid of any inflammation and seven exhibited local infiltrates corresponding to grade 1. In addition, atherosclerotic lesions, characterized by cholesterol plaque and/or calcified areas, were identified in 22/24 (91.2%) of AAA samples and in 4/12 (33.3%) of the healthy control samples (Table 1).

To further characterize the inflammation, we next examined the expression

of inflammasome components in the aortic wall of AAA versus unaffected controls. As exemplarily shown in Figure 1 (upper layer), none of the inflammasome components (neither ASC, AIM2, caspase-1 nor caspase-5) was detected in the media/intima of healthy aortic wall. In addition, these tissues were free of infiltrating monocytes/macrophages (CD68-positive cells), T cells (CD3-positive cells) or B cells (CD20-positive cells). Within the adventitia, only single infiltrating cells staining positive for CD3, ASC and/or AIM2 were detected in healthy aortic wall (Figure 1, white arrows). In contrast, atherosclerotic areas neighboring cholesterol plaque were locally infiltrated with both T and B cells (exemplarily shown in Figure 1, lower layer). Expression of the inflammasome adapter molecule ASC was clearly identified in cells infiltrating both the cholesterol plaque and the adjacent medial layer. In addition, ASC was found to be expressed in the vasa vasorum of both atherosclerotic and healthy aortic adventitia (11/12 control samples, Table 2). A similar pattern was found for the double-stranded DNA (dsDNA) sensor and inflammasome receptor AIM2. AIM2 expression was restricted to the vasa vasorum in 12 out of 12 control samples and, to a minor extent, to local plaque-infiltrating cells in atherosclerotic areas (exemplarily shown in Figure 1, lower layer). In total, plaque-associated expression of ASC and AIM2 was found in each of the four atherosclerotic control samples, whereas plaque-associated expression of caspase-1 and caspase-5 was restricted to single infiltrating cells in two out of the four atherosclerotic control samples. No caspase-1 expression was detected in

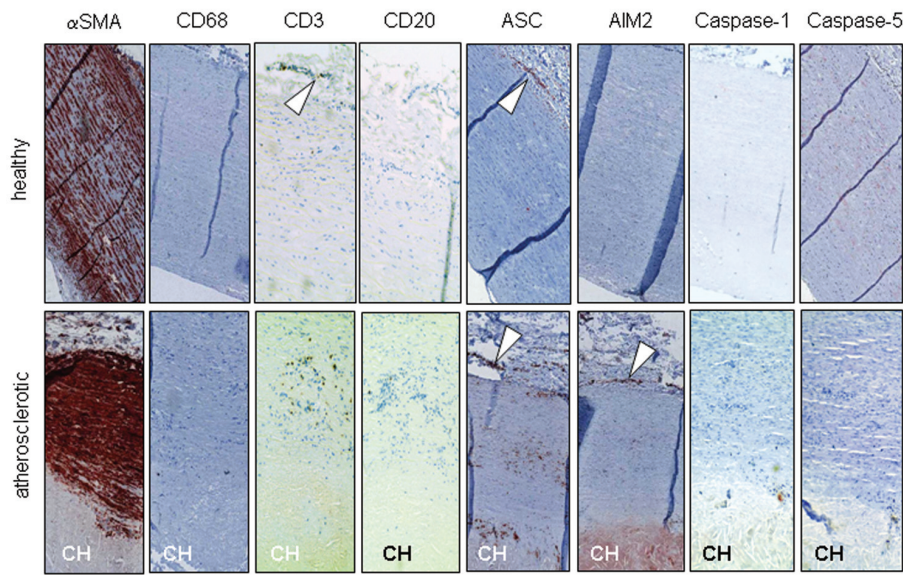


Figure 1. Immunohistochemical staining of transversal sections derived from representative healthy (upper layer) and atherosclerotic (lower layer) aortic wall. Samples were immunohistochemically stained with antibodies directed against the indicated antigens (SMA (smooth muscle actin), CD68 (macrophages), CD3 (T cells), CD20 (B cells), ASC, AIM2, caspase-1, caspase-5). Arrows point to the vasa vasorum. CH, cholesterol plaque; original magnification, 40x.

the vasa vasorum or other cells of the aortic tissue in control samples (Table 2).

In AAA samples, plaque-associated expression of AIM2, ASC, caspase-1 and caspase-5 in infiltrating cells was identified in 81.8%, 81.8%, 90.9% and 63.6% of tissues, respectively (Table 2). The strongest expression of inflammasome components was detected within the VALT and tertiary lymphoid organs (TLO) of the AAA samples (Figure 2).

A prominent ASC expression was observed in both the germinative center of active B cells, identified by CD23 and D2-40, and the surrounding T cells, identified by CD3. Similarly, AIM2 was detected in both compartments of TLOs and in the majority of lymphocytes clustering at the adventitia media border of AAA-derived aortic tissue. This lymphoid expression pattern of both ASC and AIM2 was verified in 100% of the

analyzed AAA samples. Moreover, caspase-1, predominantly expressed in the outer compartment of TLOs, where T cells and nonactivated B cells reside (Figure 2), was detected in each of the 21 grade 2/3 samples of AAA-derived aortic tissue. Caspase-5, which was predominantly expressed in the germinative center of active B cells (Figure 2), was identified in 14/19 (73.7%) informative AAA samples (Table 2).

In summary, the histological expression pattern of ASC, AIM2, caspase-1 and caspase-5 clearly indicates that inflammasomes play an important role in the inflammatory process of AAA that is mediated by lymphoid tissue and infiltrating lymphoid cells.

mRNA Levels of Inflammasome Components Are Increased in AAA Tissue Compared with Healthy Controls

Intracellular activation of the inflammasomes is regulated on different levels by a biphasic, two-signal mechanism. A priming signal is required to induce transcription of most inflammasome components before the resulting proteins can form an active multiprotein complex (21). To further support a role of the inflammasome in the pathogenesis of AAA, we therefore next investigated gene expression of *PYCARD* (ASC), *AIM2*, *CASP1* (*Pro-Caspase-1*), *CASP5* (*Pro-Caspase-5*) and *IL1B* (*Pro-IL-1β*) in aortic tissue. Eighteen out of the 24 AAA samples and 7 out of the 12 control samples were eligible for quantitative analysis upon mRNA extraction. As shown in Figure 3, the median mRNA levels of *ASC*, *Pro-Caspase-1* and *Pro-IL-1β* were significantly increased in AAA samples compared with healthy aortic tissue (*ASC*, $p = 0.0002$; *Pro-Caspase-1*, $p = 0.0003$; and *Pro-IL-1β*, $p = 0.0137$). Thus, priming signals, which induce gene transcription of these inflammasome components, appear to be active in AAA tissue. *AIM2* and *Caspase-5* mRNA, restricted to a subset of inflammatory cells, were barely detectable and could thus not be compared between the two tissue groups (data not shown).

Table 2. Expression of inflammasome proteins in AAA and controls.

Intramural location	Protein	Positive/informative AAA tissues (%)	Positive/informative control tissues (%)
Plaque associated	AIM2	18/22 (81.8)	4/4 (100)
	ASC	18/22 (81.8)	4/4 (100)
	Pro-caspase-1	20/22 (90.9)	2/4 (50)
	Pro-caspase-5	14/22 (63.6)	2/4 (50)
Vasa vasorum endothelium	AIM2	15/22 (68.2)	12/12 (100)
	ASC	16/23 (69.6)	11/12 (91.7)
	Pro-caspase-1	0/24 (0)	0/12 (0)
	Pro-caspase-5	0/23 (0)	4/12 (33.3)
Lymphoid follicles (VALT)	AIM2	19/19 (100)	Does not apply
	ASC	20/20 (100)	Does not apply
	Pro-caspase-1	21/21 (100)	Does not apply
	Pro-caspase-5	14/19 (73.7)	Does not apply

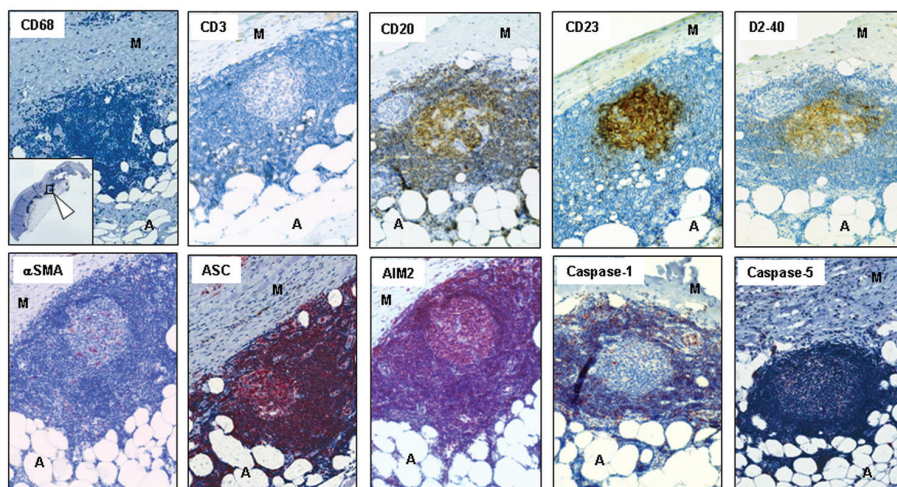


Figure 2. Representative examples of grade 3 AAA. Lobular arranged VALT at the adventitia/media border was characterized as tertiary lymphoid organs (TLA) by immunolabeling of monocytes/macrophages (CD68), T cells (CD3), B cells (CD20), activated B cells and lymphatic endothelium (D2-40, identifying the highest developmental grade of TLA). The inflammasome components ASC, AIM2, caspase-1 and caspase-5 were identified in lymphoid cells derived from different compartments within the TLAs. A, adventitia; M, media; original magnification, 200x.

Mature Caspase-5 (p17) and Induction of AIM2, ASC and Pro-Caspase-1 in AAA Tissue Indicate Activation of Specific Inflammasome Complexes That Are Involved in the Inflammatory Process of AAA

Because gene expression of its components is not sufficient for full inflammasome activation but requires further regulation on the protein level, we next compared the protein amounts of ASC, AIM2, pro-caspase-1, pro-caspase-5 and pro-IL-1 β as well as the mature cleavage products caspase-1 (p20), caspase-5 (p20) and active IL-1 β (p17) in AAA versus control samples. Semiquantitative Western blot analysis revealed significantly increased median levels of cleaved caspase-5 (p20), pro-caspase-1, AIM2 and ASC in lysates derived from AAA tissues compared with control tissues (Figure 4A). Moreover, when we compared paired biopsies of different rupture risks derived from the same aneurysm, the amount of active caspase-5 (p20) was clearly higher in eight out of 10 samples with high rupture risk compared with their counterparts with low rupture risk (Figure B). In contrast to the mature caspase-5 (p20),

the 50-kD precursor pro-caspase-5 was not detected in lysates of either tissue (data not shown). These data strongly indicate that a caspase-5-activating inflammasome is induced in the aortic wall of advanced AAA.

Similarly, the amount of AIM2 protein was higher in seven out of 10 AAA tissues with high rupture risk compared with the paired sample derived from a site with low rupture risk (Figure 4B), suggesting a prominent role of the AIM2 sensor in the inflammatory process during AAA progression. The amount of the pro-IL-1 β precursor protein (p50) was unchanged between the groups and no mature form of caspase-1 (p20) or IL-1 β (p17) was detected by Western blot analysis in lysates of either sample (data not shown).

DISCUSSION

This pilot study characterized for the first time the inflammasome components in healthy, atherosclerotic and aneurysm-derived aortic wall. ASC, caspase-1, caspase-5 and AIM2 were detected at different amounts by immunohistochemistry in sporadic infiltrating lymphoid

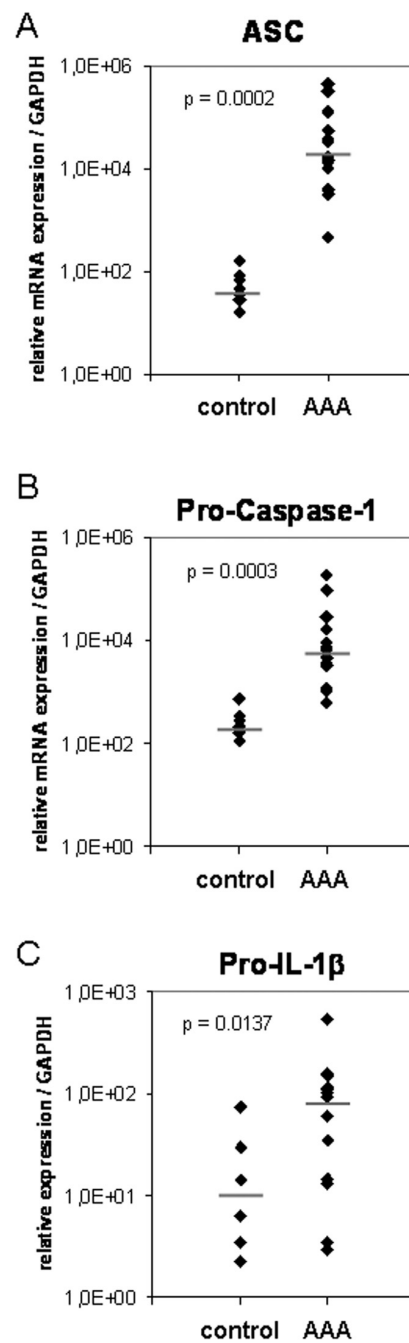
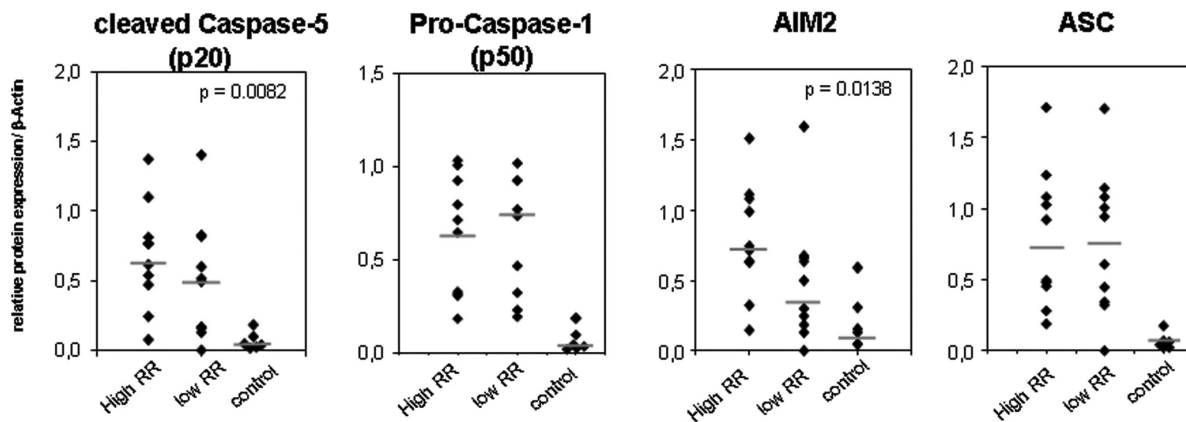


Figure 3. Quantification of ASC, Pro-Caspase-1 and Pro-IL-1 β mRNA expression in AAA versus control aorta. To extract mRNA, samples were processed as described in Materials and Methods. The data represent the relative expression of each mRNA in relation to GAPDH expression, as determined by real-time qPCR. Horizontal bar, median. *p* values are derived from the Mann-Whitney *U* test.

A



B

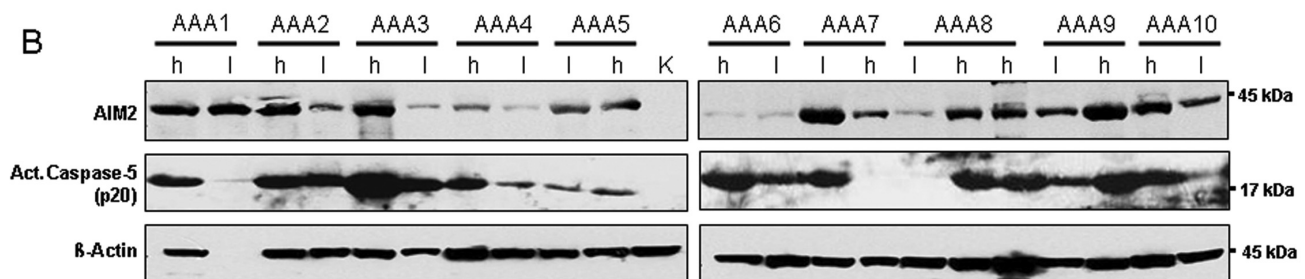


Figure 4. (A) Quantification of cleaved caspase-5 (p20), pro-caspase-1 (p50), AIM2 and ASC protein levels. Tissues were collected from AAA sites with high or low rupture risk (RR) and compared with samples derived from normal aorta (control). Samples were processed for protein analysis as described in Materials and Methods. Horizontal bar, median. (B) Western blot analysis of AIM2 and cleaved caspase-5, derived from 10 AAA lysates (AAA1 to AAA10). AAA sites with high (h) and low (l) rupture risk, derived from the same patient, were compared. K, negative control.

cells as well as in lymphoid tissue located in the outer media and adventitia of AAA. In contrast, inflammasome-positive cells were restricted to cholesterol plaque-associated areas and to single infiltrating cells in control aortas. Analysis of gene expression clearly demonstrated upregulation of the inflammasome core components *ASC*, *Caspase-1* and *IL-1 β* in AAA tissue compared with normal aorta. Moreover, increased amounts of AIM2 protein and mature caspase-5 (p20) point to activation of inflammasomes in inflammatory active areas of AAA tissue. In summary, our data indicate that AAA-associated lymphoid cells are capable of inflammasome signaling and suggest that this inflammasome activation is involved in the chronic inflammatory process driving AAA progression. Sequential staining of the VALT in AAA samples

using markers for different lymphocytes identified the population of inflammasome-positive cells as mostly active B and T cells. However, we cannot exclude the possibility that other lymphoid cells, such as the recently described innate lymphoid cells (ILC), may likewise be represented among the inflammasome-positive cells. Recent evidence suggests that these ILCs, which are characterized by production of different interleukins, can be drivers of various inflammatory disorders in the absence of any overt infection (22). Both promotion and inhibition of inflammasome activity in B cells has thus far been restricted to pathogen infection. Kaposi sarcoma-associated herpesvirus, Vaccinia virus, Epstein-Barr virus and *Salmonella* were identified to target different inflammasome components in B cells (23–25). We cannot con-

clude from our data whether the inflammasome activation in B cells of AAA samples is associated with sterile or with pathogen-induced mechanisms. Thus, future analysis using lymphoid cells that were isolated from AAA tissue is necessary to further characterize the precise identity of the inflammasome-positive cells and their role in AAA progression.

Interestingly, within the VALT, caspase-1 was predominantly detected in cells of the outer compartment, whereas caspase-5 was predominantly found in the germinative center of B cells and in single infiltrating cells. This indicates that inflammasomes composed of different components and with different functions are active in these compartments found in AAA. Whereas caspase-1 is well known to be required for proteolytic activation of IL-1 β and IL-18, little is known about the func-

tion of caspase-5. The latter has little IL-1 β procession activity, is induced by lipopolysaccharide (LPS) and interferon γ (IFN- γ) and appears to cooperate with other inflammatory caspases for full activity (26). Recently, caspase-5 has been found to be upregulated in lesional psoriatic skin (27) and was shown to be involved in inflammatory responses of human retinal pigment epithelial cells (28), which supports a specific role of caspase-5 in sterile chronic inflammation. In line with these findings, we here detected significantly increased amounts of mature, active caspase-5 (p20) in the aortic wall of AAA samples compared with normal aorta. Moreover, more mature caspase-5 was found in AAA areas with a high rupture risk index (according to FE analysis) compared with areas within the same aneurysm that were predicted to have a lower rupture risk (according to FE analysis). It will thus be important to further characterize the regulation of caspase-5 in different inflammatory or lymphoid cells and its role in degradation of the aortic wall in AAA.

A major challenge in diagnostics and therapy of AAA is the prediction of the patient's individual rupture risk as a clinical indication for elective surgical repair. Currently, the maximal AAA diameter (>5.5 cm) and AAA expansion rate are the most frequently used parameters for surgical repair. However, both rupture of smaller AAA (29) and larger aneurysms that remain undetected without rupture have been reported (30). Accordingly, other parameters are needed to identify and focus on true high-risk patients for elective surgery. Along with FE models, identification of inflammatory progression patterns in AAA has recently been suggested as an additional indicator (31). This study demonstrated that inflammatory sites of the aneurysmal wall that were identified by ¹⁸F-FDG uptake through positron emission tomography (PET)/CT were histologically associated with increased amounts of inflammatory infiltrates in the adventitia, wall deterioration and rupture. Our data are in line with these findings, demonstrating that increased inflammasome

activity—as determined by high levels of mature caspase-5—was detected in AAA samples derived from sites that were identified as high rupture risk areas by FE analysis.

We cannot conclude from our data whether inflammasome activation is a bystander of the inflammatory cascade in AAA progression or a driving force of the disease. Neither gene expression nor the protein level of any tested inflammasome component within AAA tissues was significantly correlated with the diameter of the aneurysm or the maximal thickness of the thrombus within the aneurysm (data not shown). Our data rather indicate that the composition and inflammasome activities of lymphoid cells within the aortic wall may be related to rupture risk. Future analysis of larger sample sizes will answer the question of whether inflammasome activation correlates with AAA growth, and whether the inflammasome plays a causative role in disease progression. Unfortunately, we were unable to detect mature caspase-1 and mature IL-1 β in our samples. These proteins, which are clearly associated with inflammasome activity, are usually released from cells upon activation (21) and may thus have been lost during tissue processing. Alternatively, active caspase-5 but not caspase-1 is predominantly active in the AAA wall. Thus, further studies, that is, in animal models, will be important to validate the correlation of inflammasome activity in lymphoid inflammatory infiltrates of the AAA adventitia with rupture risk.

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DISCLOSURE

The authors declare they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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