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# AIM2 levels and DNA-triggered inflammasome response are increased in peripheral leukocytes of patients with abdominal aortic aneurysm

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## Abstract

**Objective and design** Abdominal aortic aneurysm (AAA) is heavily infiltrated with leukocytes, expressing the DNA sensor absent in melanoma 2 (AIM2) and other inflammasome components.

**Methods** Using multicolour flow cytometry, we here compared the expression of the inflammasome components AIM2, NLRP3, and ASC in different peripheral immune cells derived from AAA patients with those from non-AAA patients in a case–control study. In parallel, peripheral blood mononuclear cells (PBMC) of AAA patients and controls were stimulated in vitro with poly-dA:dT or lipopolysaccharide (LPS) to analyze inflammasome activation.

**Results** AIM2 expression was significantly increased in peripheral granulocytes ($P=0.026$), monocytes ($P=0.007$), B lymphocytes ($P<0.0001$), and T lymphocytes ($P=0.004$) of AAA patients. Expression of other inflammasome components did not differ between the groups. Following in vitro stimulation with foreign DNA, PBMC derived from AAA patients released significantly more IL-1β ($P=0.022$) into the supernatant than PBMC from control patients. In contrast, IL-1β release upon LPS stimulation did not differ between the PBMC groups.

**Conclusion** The data indicate the increased activation of an AIM2 inflammasome in peripheral immune cells of AAA patients and point to a systemic AIM2-associated immune response to AAA.

**Keywords** Inflammasome · Innate immunity · Abdominal aortic aneurysm · FACS · Flow cytometry · PBMC

## Introduction

Abdominal aortic aneurysm (AAA), a progressive pathological dilatation of the aortic wall, occurs mostly asymptomatic, but may become life threatening in case of rupture. Despite a global decline, AAA prevalence rates still range between 70 and 350 per 100,000 population in different world regions [1]. Globally, male sex, age, hypertension, and smoking have been identified as major risk factors for AAA. Because of its asymptomatic course, it is often detected only by chance or in screening programs using sonographic imaging. The only parameter used for defining the risk of rupture currently comes from epidemiological observations, suggesting that the rupture risk increases with the diameter from 0% per year in AAA smaller than 40 mm to 3–15% in AAA with a diameter of 50–60 mm and to 30–50% in AAA with a diameter of above 80 mm [2]. Accordingly, most clinical guidelines recommend considering surgery of an AAA in male when the maximum aortic diameter reaches 5–6 cm or more [3]. Patients with higher risk of rupture (i.e., female) are

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considered for surgery when the maximum aortic diameter reaches less than 5 cm [3]. Despite increasing knowledge about the pathophysiology of AAA, there are currently no additional biomarkers to predict individual immunological reactions or rupture risk, and no causative treatment options are available to inhibit growth or rupture of an AAA.

The strong impact of smoking and hypertension as risk factors suggests that the aorta is increasingly damaged by oxidative stress, resulting in progressive deterioration of the aortic wall integrity. Accordingly, chronic inflammation of the aortic wall is a major histopathologic characteristic of AAA, which is accompanied by the loss of contractile smooth muscle cells, fibrosis, and degeneration of elastic fibers [4, 5]. The adventitia and media of AAA are heavily infiltrated with inflammatory and immune cells releasing proinflammatory cytokines [6]. In addition, high amounts of proteases and increased collagen turnover are observed in the AAA wall, all features representing classic inflammatory responses [7–9].

Several recent studies in humans and mouse models provide evidence for an innate immune response in different cell types of the aortic wall during AAA development. In particular, activation of inflammasome signaling and consequential release of active Interleukin1-β (Il-1β) were shown to be associated with AAA [10–12]. Il-1β activation requires a two-step process that is initiated by the expression of Pro-IL-1β mRNA, when cells are exposed to different stimuli, such as pathogens, nanoparticles, crystals, and others [13]. After translation, Pro-Il-1β, a protein of 30 kD, must be enzymatically digested to release its active form, a 17 kD protein. This second step is performed by inflammasomes, which are composed of a core (apoptosis-speck-like protein with a card; ASC), several filaments of Pro-caspase-1, and a sensor protein. The identity of the sensor depends on the pathogenic or danger-associated stimulus. Extracellular stimuli, such as cholesterol crystals, uric acid crystals, fibrils, nanoparticles, or tissue injury, are known to result in the activation of an NLRP3 (nucleotide oligomerization domain-like receptor family; pyrin domain-containing 3) inflammasome [13]. In contrast, dsDNA, i.e., released in response to tissue injury or DNA-damaging agents, results in the activation of an AIM2 (absent in melanoma 2) inflammasome [14]. A causative role in AAA formation was shown for NLRP3, Caspase-1, and IL-1β in animal models. Deficiency in these genes was shown to decrease the incidence, maximal diameter, and severity of murine AAA [11]. In addition, we and others demonstrated high expression of several inflammasome components in human AAA in both, tissue infiltrating immune cells and vascular smooth muscle cells [12, 15, 16].

Besides increased expression of inflammasome components in AAA tissue, we recently reported that AIM2 and NLRP3 inflammasome levels in peripheral blood mononuclear cells (PBMC) increase with age and differ between male and women [17, 18]. Moreover, PBMC extracts derived from patients with AAA displayed more AIM2 protein than age- and sex-matched controls [17]. Given the critical role of inflammasome activity in AAA tissue and its increased detection in blood from AAA patients, we here investigated whether AIM2 and NLRP3 expressions differ between peripheral monocytes, granulocytes, and B cells and T cells in AAA patients and controls (vascular patients without history or current AAA, non-AAA patients). In addition, we examined the hypothesis that PBMC from AAA patients display a stronger inflammasome response to dsDNA as an extracellular stimulus than PBMC from control patients.

## Materials and methods

### Blood samples and patients characteristics

Venous blood was taken from vascular patients on the day of their hospitalization, according to the standard operating procedures of the Vascular Biobank Heidelberg (VBBH). All patients gave their written informed consent to the study, which was approved by the ethical committee of the University of Heidelberg (S-301/2013 and S-412/2013). A total of 149 blood samples were consecutively taken from patients and processed for further analysis within 4 h after venipuncture. The reasons for hospitalization (type of vascular disease), additional previous or chronic diseases, as well as sociodemographic and clinical data of the patients were obtained from the hospital's information system. Patients' characteristics are shown in Table 1. The patient cohort was completely different from the cohort used in our previous analysis [17, 18].

### Study design

To examine the possible relation of AIM2 and NLRP3 activities in peripheral leukocytes with abdominal aortic aneurysm, case–control studies were performed. All patients (AAA and controls) were examined for their aortic diameter by duplex sonography. All AAA patients were additionally examined by preoperative multislice computed tomographic angiography prior to their hospitalization for treatment. For all patients (AAA and controls), the timeframe between their latest aortic imaging and venipuncture for the study was less than 6 months, generally within 1 month. AAA cases were defined as blood samples derived from patients with an aortic diameter of > 50 mm, who were hospitalized for aortic repair. Controls were defined as blood samples from patients who were hospitalized for non-aneurysmal vascular diseases (i.e., peripheral artery disease; carotid stenosis), i.e., without

**Table 1** Patient characteristics

| | FACS analysis | | Poly-dA:dT stimulation | | LPS stimulation | |
|---|---|---|---|---|---|---|
| | AAA patients ($N=25$) | Control patients ($N=31$) | AAA patients ($N=28$) | Control patients ($N=42$) | AAA patients ($N=25$) | Control patients ($N=42$) |
| Age (years ± SD) | 70.2 ± 6.9 | 69.0 ± 7.9 | 71.5 ± 6.5 | 67.9 ± 7.6 | 71.7 ± 7.0 | 68.0 ± 7.7 |
| Male (*n*/%) | 21 (84.0) | 24 (77.4) | 26 (92.9) | 31 (73.8) | 23 (92.0) | 31 (73.8) |
| Smoking (*n*/%) | 19 (76.0) | 22 (71.0) | 21 (75.0) | 26 (61.9) | 18 (72.0) | 24 (57.1) |
| Hypertension (*n*/%) | 23 (92.0) | 31 (100) | 26 (92.9) | 38 (90.5) | 24 (96.0) | 38 (90.5) |
| Diabetes (*n*/%) | 3 (2.0) | 12 (38.7) | 5 (17.9) | 15 (35.7) | 5 (20.0) | 14 (33.3) |
| Hyperlipidaemia (*n*/%) | 22 (88.0) | 26 (83.9) | 23 (82.1) | 34 (81.0) | 20 (80.0) | 35 (83.3) |

any existing or prior abdominal aortic aneurysms (aortic diameter < 30 mm, no previous aortic repair). Because flow cytometry and PBMC isolation both require fresh blood cells for analysis, separation of blood was performed immediately after hospitalization of the initially enrolled 149 individuals, and, thus, before the complete set of clinical data was available from the patients. Depending on the cell number and quality that was available after blood separation, samples were split for parallel analysis by flow cytometry and PBMC stimulation. Before statistical analysis of the data sets, non-eligible samples were excluded according to predefined criteria. To avoid possible interference of medication, samples derived from AAA and control patients with the existing or prior malignant tumor and/or additional aneurysms were excluded from data analysis. In addition, patients receiving medication because of chronic obstructive pulmonary disease (COPD) were excluded from both groups. COPD has been associated with an increased prevalence of AAA [19, 20] and might, therefore, confound our goal to identify AAA-associated inflammasome reactions. Finally, data sets and blood samples of insufficient quality (i.e., hemolysis prior to separation, insufficient cell numbers, and errors in measurement) were excluded from further analysis.

## Combined extracellular and intracellular fluorescent staining and flow cytometry

A multicolor flow cytometric analysis was performed to analyze the expression of inflammasome components in different peripheral leukocyte phenotypes. For each patient, 5 aliquots of 100 µl whole blood were transferred to polystyrene tubes. One aliquot was left unstained to correct for background; four aliquots were simultaneously stained for 15 min at room temperature with mouse anti-human CD3-APC (# 345767, BD Biosciences), mouse anti-human CD14-FITC (clone M5E2 # 555397, BD Biosciences), and mouse anti-human CD19-PerCP-Cy5.5 (# 561295, BD Biosciences). Samples were treated with 2 ml of BD lysing solution (# 349202, BD Biosciences) for 10 min, and centrifuged at 250 g for 10 min. The pelleted cells were washed with FACS buffer (PBS, 5% fetal bovine serum, 0.5% bovine serum albumin, and 0.07% NaN3) and harvested by centrifugation at 250 g for 10 min to remove lysed erythrocytes. Cell pellets were resuspended in 50 µl FACS buffer/0.15% saponin for permeabilization, and the following primary antibodies were added separately to three of the prestained aliquots from each patient/controls: rabbit anti-human AIM2 (# 12948, Cell Signaling Technologies), or rabbit anti-human NLRP3 (# 13158, Cell Signaling Technologies), or rabbit anti-human ASC (#ADI-905-173, ENZO life sciences). Cells were incubated for 30 min, washed with FACS buffer/0.15% saponin, and harvested at 250 g for 10 min. Pellets were resuspended in 50 µl FACS buffer/0.15% saponin and secondary antibody (PE-donkey-anti rabbit Fab2; # 711-116-152, Jackson Immuno Research) was added to the samples for 30 min. After washing twice in 1 ml FACS buffer/0.15% saponin, and centrifugation for 10 min. at 250 g, pellets were resuspended in 150 µl FACS buffer and stored in the dark until analysis, usually within 24–72 h. A multicolor flow cytometric analysis was performed using a BD LSR II flow cytometer (BD Biosciences Immunocytometry Systems) and Cell Quest Software (BD Biosciences Immunocytometry Systems). For cell line acquisition, 10,000 cells, with a scatter gate set on live cells, were collected. Data are presented as mean fluorescence intensity (MFI) after exclusion of the background.

## Gating strategy for identification of different leukocyte phenotypes

Supplementary Figure S1 shows an example of the gating strategy that was used to separate the different phenotypes for further intracellular analysis of fluorescent intensities. In a first step, live CD14-positive cells (identifying monocytes) were separated from CD14-negative cells of similar size (identifying granulocytes). T cells were identified as CD3-positive cells, B cells as CD19-positive cells (supplementary figure S1A). In a second step, the fluorescent intensities of PE (representing NLRP3, AIM2 or ASC, respectively) were plotted against the number of events (CD14-, CD3-,

or CD19-positive cells, respectively) detected in different leukocyte phenotypes (supplementary figure S1B).

### Isolation and in vitro stimulation of PBMC

Separation of plasma and PBMC from blood was performed by Ficoll gradient centrifugation as described previously [18]. After separation, $10^6$ PBMC from each patient/control sample were seeded in 3 wells of a 6-well plate in RPMI-1640 medium with L-Glutamine, without phenol red (Life Technologies), supplemented with 10% fetal bovine serum (Life Technologies), 100 U/ml penicillin, and 100 U/ml streptomycin (Life Technologies). Cells were incubated for 1 h in a humidified atmosphere to allow for attachment. For stimulation, 1 µg/ml Poly(dA:dT)/LyoVec™ (InVivoGen) or 100 ng/ml lipopolysaccharide (LPS; Sigma-Aldrich, Merck) was added to the media. One well was left untreated to serve as a control. Cells were incubated overnight at 37 °C, 5%$CO_2$ in a humidified atmosphere, and supernatants were harvested after 17 h and stored at − 80 °C until analysis.

### Quantification of cytokines from supernatants and patients serum

Active, cleaved IL-1β (p17) derived from PBMC supernatants or patient's serum was quantified by the Duo-Set ELISA Development system for human IL-1β (R&D Systems Europe, Abington, UK) according to the recommendations of the manufacturer. All samples were tested in duplicate. A standard curve constructed with purified human IL-1β of known concentration was used for the calculation of the IL-1β concentration in the samples. Background levels (IL-1β concentration in the supernatant from unstimulated cells of the same patient) were subtracted from concentrations in the supernatants of LPS or poly-dA:dT-stimulated cells, respectively. For detection of human IFN-β in the serum of patients, the Duo-Set ELISA Development system for human IFN-β was used (R&D Systems Europe, Abington, UK) according to the recommendations of the manufacturer, and using a standard curve with purified IFN-β of known concentration.

### Statistical analysis

For flow cytometric analysis, the mean fluorescent intensity (MFI) for AIM2, NLRP3, and ASC was determined in four different leukocyte phenotypes (granulocytes, monocytes, T cells, and B cells), individually from each patient. Background fluorescent intensities were subtracted and the resulting MFIs were used for group analysis (samples from AAA patients versus samples from age-matched controls). For investigation of IL-1β release from PBMC, data derived from ELISA were depicted as pg/ml supernatant. All data were analyzed by IBM SPSS version 21 (IBM Corp, Armonk, NY, USA). Box plots for the figures were prepared using GraphPad Prism (GraphPad, San Diego, CA, USA). For statistical testing of the medians and means, Mann–Whitney *U* test or paired Student's *t* test was applied, when appropriate. *P* values below 0.05 were interpreted as significant.

## Results

### Data acquisition from blood samples

Figure 1a shows the sample distribution before and after exclusion of samples. Complete FACS data sets were obtained from 99 patients, whereas PBMC separation was possible from 118 patients. Complete FACS and PBMC stimulation data were available from 68 patients, 31 samples underwent flow cytometric analysis only, and 50 samples were used for PBMC stimulation only (Fig. 1b). Following the detailed inspection of clinical data, 48 patients (43 patients in the FACS cohort overlapping with 48 patients in the PBMC stimulation cohort) were excluded because of the previous or current comorbidities (malignant tumors, COPD, or additional existing and previous aneurysms). After exclusion of non-eligible data sets, FACS data from 56 samples (25 AAA cases and 31 controls) were used for statistical data analysis. In parallel, 70 samples were eligible for statistical analysis of IL-1β concentrations in the cell supernatant of PBMC by ELISA (28 AAA cases and 42 controls in response to poly-dA:dT stimulation; 25 AAA cases and 42 controls in response to LPS stimulation).

### Intracellular expression of AIM2, NLRP3, and ASC in different peripheral blood cell phenotypes

To investigate the intracellular amounts of the inflammasome components ASC, AIM2, and NLRP3 in different peripheral immune cells, whole blood from patients with AAA and controls was analyzed by multicolor flow cytometry. The percentage of peripheral granulocytes, monocytes, and B and T lymphocytes did not differ between AAA patients and controls (data not shown). We next compared the mean fluorescent intensities (MFI) of the inflammasome sensors AIM2 and NLRP3, as well as the inflammasome core protein ASC in each of the four leukocyte cell types. The highest AIM2 level was detected in granulocytes and was significantly increased in AAA patients compared with controls (median $MFI_{AAA} = 1309$ versus median $MFI_{control} = 920$, $P = 0.026$) (Fig. 2a). In addition, AIM2 levels were significantly increased in monocytes (median $MFI_{AAA} = 668$ versus median $MFI_{control} = 458$, $P = 0.007$), in T lymphocytes (median $MFI_{AAA} = 343$ versus median $MFI_{control} =$

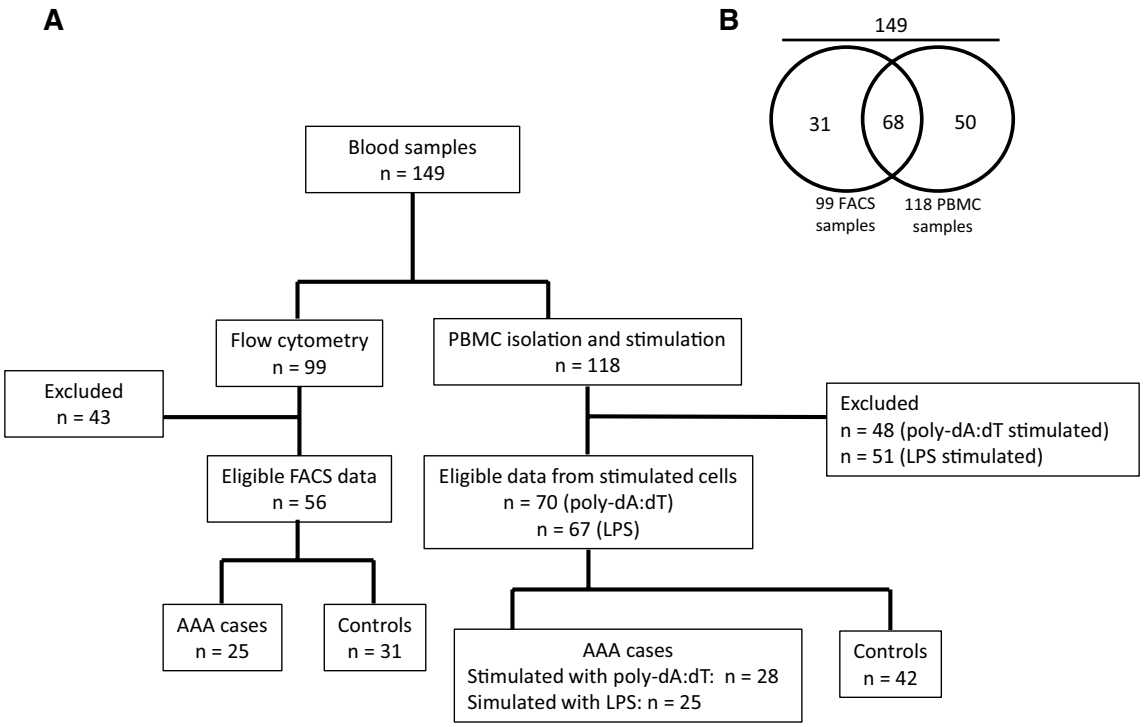


**Fig. 1** **a** Study enrollment, exclusion of samples, and composition of the study groups. **b** Number of blood samples assigned to flow cytometric analysis and PBMC stimulation, before exclusion

225, $P = 0.004$), and in B lymphocytes (median $MFI_{AAA}$ = 297 versus median $MFI_{control}$ = 143, $P < 0.0001$) of AAA patients compared with controls (Fig. 2a). In contrast, the highest NLRP3 level was found in monocytes and the total amount of NLRP3 was higher than that of AIM2 in each of the four leukocyte types. Moreover, there was no significant difference between AAA patients and controls in either of the peripheral cell types (Fig. 2b). Similar to NLRP3, the highest amount of the inflammasome core protein ASC was detected in monocytes in both groups, without showing significant differences (Fig. 2c). In addition, the ASC amounts in peripheral monocytes and B and T lymphocytes did not differ between AAA patients and controls.

## IL-1β release from PBMC after in vitro stimulation with dsDNA (poly-dA:dT) or Lipopolysaccharide (LPS)

To investigate AIM2 and NLRP3 inflammasome activities in peripheral blood cells, we measured IL-1β secretion upon specific stimulation in vitro. In parallel to the flow cytometric analysis, PBMC were isolated from the same whole blood samples and either transfected with poly-dA:dT, known to activate an AIM2 inflammasome, or stimulated with LPS, known to activate an NLRP3 inflammasome. Unstimulated cells of each patient were used as a background control. Upon transfection with poly-dA:dT, PBMC derived from AAA patients ($n = 25$; median $IL\text{-}1\beta_{AAA} = 1.45$ pg/ml) released more active Il-1β into the supernatant than PBMC derived from controls ($n = 42$; median $IL\text{-}1\beta_{non\text{-}AAA} = 1.20$ pg/ml); however, the difference was not significant ($P = 0.371$; Mann–Whitney $U$ test).

In both, the AAA and the non-AAA group, a subset of samples was non-responsive to poly-dA:dT and/or LPS stimulation, resulting in IL-1β concentrations below a threshold of 1 pg/ml. When analyzing only data from responsive samples (IL-1β concentration after stimulation > 1 pg/ml), poly-dA:dT-stimulated PBMC derived from AAA patients ($n = 15$) released significantly more active Il-1β than PBMC derived from controls ($n = 23$); (median $IL\text{-}1\beta_{AAA} = 17.86$ pg/ml versus median $IL\text{-}1\beta_{non\text{-}AAA} = 4.8$ pg/ml; $P = 0.022$; Mann–Whitney $U$ test; Fig. 3a). In contrast to poly-dA:dT stimulation, LPS stimulation resulted in similar levels of active Il-1β in cell supernatants in both PBMC groups, even when non-responsive samples were excluded (median $IL\text{-}1\beta_{AAA} = 1230.0$ pg/ml versus median $IL\text{-}1\beta_{non\text{-}AAA} = 1163.4$ pg/ml; $P = 0.575$; Mann–Whitney $U$ test; Fig. 3b).

## IL-1β and IFN-β serum levels

Because AIM2 is an interferon-inducible gene and because priming of the PBMC might have occurred in vivo by circulating cytokines, we additionally measured the IL-1β and

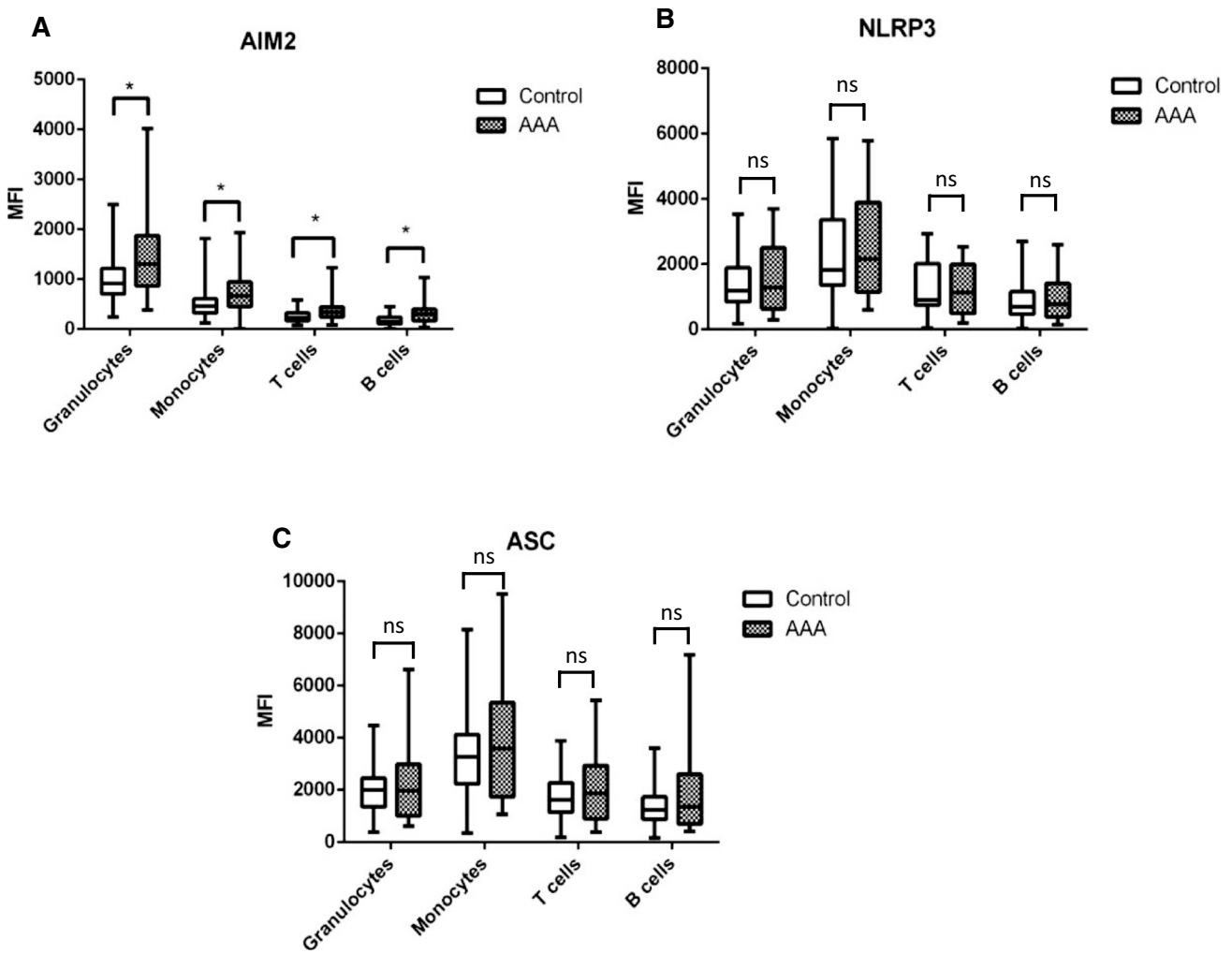


**Fig. 2** Multicolor flow cytometry analysis. Mean fluorescent intensities of PE-labeled AIM2 (**a**), NLRP3 (**b**), and ASC (**c**) in different leukocyte phenotypes of AAA patients and control patients are shown. Boxes represent the range between 25 and 75% quartiles; the whiskers represent the maximum and minimum values. The median is shown as the 50% quartile. The differences between the two groups were statistically analyzed by the Mann–Whitney *U* test. $*P < 0.05$

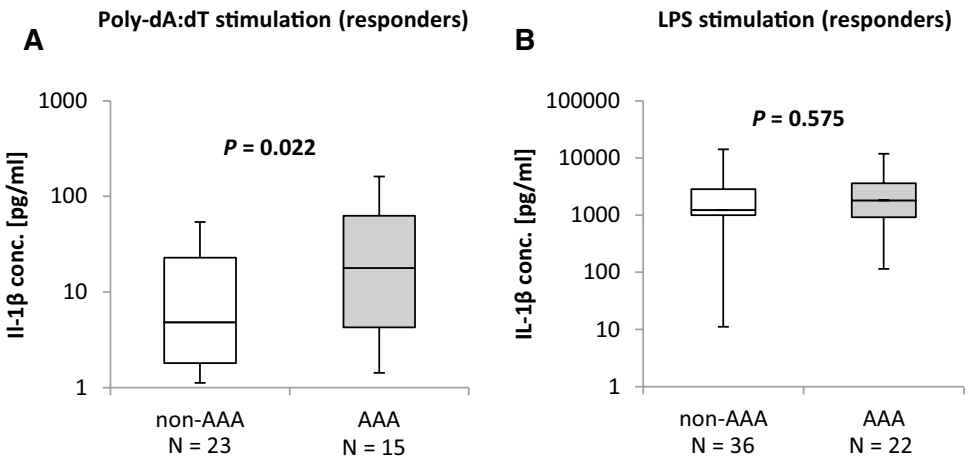


**Fig. 3** IL-1β concentrations [pg/ml] released from PBMC of AAA patients or non-AAA patients, after overnight stimulation of the cells with synthetic ds DNA (poly-dA:dT) (**a**) or LPS (**b**). Data of responders (IL-1β concentration > 1 pg/ml) are shown only. Boxes represent the range between 25 and 75% quartiles; the whiskers represent the maximum and minimum values. The median is shown as the 50% quartile. Differences between the groups were tested by the Mann–Whitney *U* test

IFN-β serum cytokine levels of the corresponding patients (Supplementary Table 1). IL-1β serum concentrations were below detection level in all but one patient. The median levels of IFN-β did not differ between AAA patients and controls (Mann–Whitney *U* test). Moreover, there was no association between IL-1β concentration released from the stimulated PBMC and any of the tested plasma cytokine levels (data not shown).

## Discussion

In the present study, we demonstrated that patients with AAA show increased protein levels of the dsDNA sensor AIM2 in peripheral monocytes, granulocytes, T cells, and B cells, compared with controls. Moreover, our data present evidence that PBMC of AAA patients respond to foreign DNA with higher inflammasome activation and IL-1β release than PBMC from patients without AAA. Using an independent, well-defined sample cohort and multicolor flow cytometry as an alternative methodology for testing, the data thus confirm our previous observation, where AIM2 protein levels were shown to be significantly increased in PBMC cell lysates of AAA patients [17]. In contrast, the protein level of a second inflammasome sensor, NLRP3, which was shown to be reduced in PBMC cell lysates of AAA patients in our previous study [17], was unchanged according to the flow cytometric analysis, here. The reason for this discrepancy might be the selection of patients and controls. Here, we carefully excluded patients with comorbidities such as COPD, malignant tumors, or additional existing and previous aneurysms from both, the AAA group and controls.

Transcript (mRNA) expression levels of inflammasome components have been previously characterized in highly purified neutrophils [21] and B cells [22] from healthy individuals. However, little is known so far about the distribution of corresponding inflammasome proteins in human peripheral blood cells derived from patients. We here compared the protein expressions of AIM2, NLRP3, and the inflammasome core component ASC separately in four different peripheral mononuclear cell types. In addition to our previous finding of increased AIM2 protein levels in PBMC of AAA patients, the present study identified increased AIM2 levels in peripheral granulocytes of AAA patients.

The role of AIM2 and its mechanism of action in sterile inflammation, i.e., in the absence of foreign pathogens, are still incompletely understood. AIM2 induction in peripheral blood cells was previously reported to be associated with the other autoinflammatory diseases. Overexpression of the *AIM2* gene was observed in peripheral macrophages of male patients with systemic lupus erythematosus (SLE), although AIM2 protein levels were not analyzed in that study [23]. In addition, increased AIM2 transcript and protein levels were detected in PBMC from patients with acute pancreatitis, and the activation of the AIM2 inflammasome was shown to correlate with disease severity in these patients [14, 24–27]. Together, this suggests that different peripheral immune cells may upregulate AIM2 in autoinflammatory conditions, or alternatively, the number of AIM2-positive immune cells increases under these circumstances. Our flow cytometric data, based on the MFIs, are in favor of a general upregulation of the AIM2 protein amount in different peripheral immune cells, because the percentage of AIM2-positive granulocytes, macrophages, and T and B cells did not differ between AAA patients and controls (data not shown).

Consistent with the elevated AIM2 protein levels in peripheral immune cells of AAA patients, we found increased release of mature IL-1β from poly-dA:dT-stimulated but not from LPS-stimulated PBMC from AAA-patients. Being a DNA sensor molecule, interaction of AIM2 with cytosolic DNA results in the activation of an inflammasome and release of mature IL-1β in different cell types in pathological and inflammatory conditions [14]. Although we did not analyze the activation of the AIM2 inflammasome itself, our data indicate that PBMC derived from AAA patients are more sensitive towards cytoplasmic DNA than PBMC of control patients. Together with the finding of AIM2 overexpression in different peripheral blood cell types of AAA patients, this suggests a role of AIM2 and its inflammasome in an inflammatory or immune response towards the patient's AAA. Obviously, PBMC of AAA patients are already primed for AIM2 inflammasome activation, which results in boosted IL-1β release. Since release of mature IL-1β from LPS-stimulated PBMC was equally high in the AAA patients and controls, here, we conclude that priming of the NLRP3 inflammasome, which is triggered by LPS [28], does not differ between the two groups. The precise cell type responsible for the AIM2-mediated release of mature IL-1β cannot be identified from our stimulation experiments, because PBMC consist of a mix of monocytes, B cells, T cells, and NK cells. Moreover, stimulation of granulocytes by foreign DNA and LPS was not possible in our study. Given the high AIM2 protein level in peripheral granulocytes, we suggest additional studies investigating the granulocyte response to foreign DNA in AAA patients in more detail.

In addition, we cannot deduce from our study, why peripheral blood cells from AAA patients are more sensitive to cytoplasmic DNA than controls. The major physiological role of AIM2 is innate immune response and defence against infections by intracellular pathogens, such as certain DNA viruses and bacteria. More recently, a role of AIM2 in sterile inflammation and autoimmunity has been suggested [14, 24–27]. Host DNA was shown to accumulate in the cytosol of different cell types, due to impaired degradation or clearance from dying neighbouring cells [29]. Thus, peripheral

blood cells of AAA patients might be activated by DNA released from apoptotic or necrotic cells when passing the aneurysm. This hypothesis is supported by the observation that AAA-infiltrating B and T lymphocytes mainly consist of activated memory cells [30]. In addition, the aneurysm wall is known to be heavily infiltrated with different inflammatory cell phenotypes [6] including neutrophils, which particularly reside in the intraluminal thrombus and the adventitial layer of human AAA [31]. We, therefore, suggest further investigations in cell culture and animal experiments to demonstrate whether the AIM2-mediated inflammatory response contributes to AAA progression or whether it rather counteracts and decelerates AAA progression.

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## Compliance with ethical standards

**Conflict of interest** Xianghui Xiao has received financial support from the German Society of Vascular Surgery and Vascular Medicine for attending the annual meeting of the society in 2017. Maani Hakimi is CEO of CODE Medical Frankfurt GbR, Germany. Dittmar Böckler is consultant for Medtronic, W.L Gore & Ass. Endologix. The authors have no competing interests. MW, GW, YS, and SD have no conflict of interest for this publication.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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