Acta Neuropathol
DOI 10.1007/s00401-011-0939-3

REVIEW ARTICLE

Saccular intracranial aneurysm: pathology and mechanisms

Juhana Frösen · Riikka Tulamo · Anders Paetau · Elisa Laaksamo · Miikka Korja · Aki Laakso · Mika Niemelä · Juha Hernesniemi

Received: 24 August 2011 / Revised: 22 December 2011 / Accepted: 31 December 2011

Abstract Saccular intracranial aneurysms (sIA) are pouch-like pathological dilatations of intracranial arteries that develop when the cerebral artery wall becomes too weak to resist hemodynamic pressure and distends. Some sIAs remain stable over time, but in others mural cells die, the matrix degenerates, and eventually the wall ruptures, causing life-threatening hemorrhage. The wall of unruptured sIAs is characterized by myointimal hyperplasia and organizing thrombus, whereas that of ruptured sIAs is characterized by a decellularized, degenerated matrix and a poorly organized luminal thrombus. Cell-mediated and humoral inflammatory reaction is seen in both, but inflammation is clearly associated with degenerated and ruptured walls. Inflammation, however, seems to be a reaction to the ongoing degenerative processes, rather than the cause. Current data suggest that the loss of mural cells and wall degeneration are related to impaired endothelial function and high oxidative stress, caused in part by luminal thrombosis. The aberrant flow conditions caused by sIA geometry are the likely cause of the endothelial dysfunction, which results in accumulation of cytotoxic and pro-inflammatory substances into the sIA wall, as well as thrombus formation. This may start the processes that eventually can lead to the decellularized and degenerated sIA wall that is prone to rupture.

Keywords Aneurysm · Intracranial · Histopathology · Pathophysiology · Inflammation

J. Frösen (✉) · M. Korja · A. Laakso · M. Niemelä · J. Hernesniemi
Department of Neurosurgery, Helsinki University Central Hospital, Topeliuksenkatu 5, 00260 Helsinki, Finland
e-mail: juhana.frosen@hus.fi

J. Frösen · R. Tulamo · E. Laaksamo · M. Korja · A. Laakso · M. Niemelä · J. Hernesniemi
Neurosurgery Research Group, Biomedicum Helsinki, Helsinki, Finland

A. Paetau
Department of Pathology, Helsinki University Central Hospital, Helsinki, Finland

Introduction

Intracranial aneurysms are pathological dilatations of intracranial vessels that may have an abnormally weak wall prone to rupture (Fig. 1). Aneurysm rupture causes hemorrhage to the subarachnoid space surrounding the brain, and sometimes into the brain parenchyme. Most intracranial aneurysms occur in the cerebral arteries, in which both saccular and fusiform (Fig. 1) aneurysms are found. Saccular intracranial aneurysm (sIA) is the most common type of all intracranial aneurysms and also the most frequent cause of aneurysmal or non-traumatic subarachnoid hemorrhage (SAH).

Saccular intracranial aneurysms as a clinical problem

The incidence of aneurysmal SAH is approximately 10–11/100,000 in most Western populations, but twice as high in Finland and Japan [33]. Since the mortality of aneurysmal SAH is around 30–40% despite modern neurosurgical intensive care and almost half of survivors are left disabled [58, 79], the burden of mortality and morbidity caused by the disease is significant. Aneurysmal SAH affects younger patients than most other forms of stroke or cardiovascular diseases; in a global meta-analysis the median age for SAH is 60 years [58]. Moreover, a significant proportion of aneurysmal SAH patients are of working age [31].

Published online: 17 January 2012

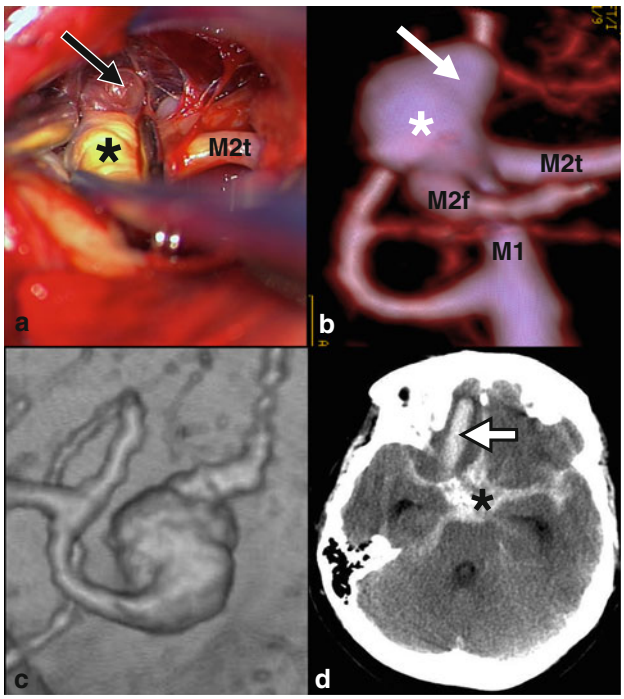

Fig. 1 **a** Fundus of a ruptured aneurysm at the middle cerebral artery (exposed via partial opening of the fissura Sylvi in a patient with SAH) and **b** computed tomography angiogram (CTA) of the same aneurysm. This aneurysm has a thick-walled part (*asterisk*) and a thin-walled secondary protrusion (*arrow*) that is the most likely bleeding site. M2f refers to the frontal branch and M2t to the temporal branch of the middle cerebral artery. **c** A fusiform aneurysm, a segmental dilatation of a vessel, at the distal posterior cerebral artery (CT angiogram). **d** Rupture of a saccular or fusiform intracranial aneurysm can cause subarachnoid hemorrhage (*asterisk*) and intracerebral hemorrhage (*arrow*) (computed tomography, blood in *white*)

Due to the sinister outcome of aneurysmal SAH, many unruptured sIAs are treated prophylactically before they rupture. Currently this can be done by isolation of the sIA from the cerebral circulation by either endovascular occlusion of the aneurysm sac or by microsurgical methods, most commonly ligation of the aneurysm neck. Sometimes ligation of the parent artery combined with a bypass to the distal arterial tree can be considered, or in rare cases even direct resection of the aneurysm followed by microsurgical reconstruction of the arterial vessels. All the current methods to prevent sIA rupture are associated with the risk of morbidity and even mortality. To assess the risk of rupture of individual sIAs with current methods is demanding and inaccurate, but of utmost clinical significance because of the risks involved in both the operative and the conservative management.

Clinical risk factors for aneurysmal SAH

The overall risk of sIA rupture is approximately 1% per year in the Finnish population [38]. Although a large, multinational prospective follow-up study reported significantly lower risk of rupture of sIAs in patients without prior SAH (especially in small <7 mm sIAs) [93], careful meta-analysis of other published series estimated the overall risk of rupture to be around 1.2% in Western populations [92]. In Japanese series, the annual rupture risk has been even higher, up to 2.3% [94].

Geometry of the sIA (e.g., shape and size), anatomical location (e.g., anterior communicans artery) and patient-related factors significantly modify the risk of rupture [4, 37, 64, 89, 91, 93]. In prospective population-based follow-up cohorts, smoking, untreated hypertension and female gender have been shown to be the most important risk factors for aneurysmal SAH [34, 70].

Studies that have followed and determined risk factors for rupture in patient cohorts with unruptured sIAs are few. Juvela et al. [38] followed a Finnish patient cohort ($n = 142$) with 181 unruptured sIAs diagnosed with angiography (performed because of SAH from another sIA or other causes) at a time when unruptured sIAs were not treated surgically at his department (before 1979). The patients were followed until SAH, death or the years 1997–1998, resulting altogether in 2,575 person-years of follow-up.

Juvela showed that sIA size, smoking and younger age at the beginning of the follow-up were risk factors for sIA rupture [38]. However, in this series of unruptured sIAs, hypertension and female gender were not associated with increased risk of rupture, although they are known risk factors for aneurysmal SAH [38]. In another study, Juvela et al. [37] followed a cohort ($n = 94$) of patients diagnosed with either ruptured or unruptured sIAs, and determined that independent risk factors for the formation of new sIAs were current smoking and female gender.

According to the series by Juvela et al., smoking is a risk factor for both the formation of sIAs and the rupture of already formed sIAs. Female gender seems to be a risk factor for the formation of sIAs, but does not seem to affect the risk of rupture once the sIA has been formed. Since hypertension did not increase the risk of rupture in already formed sIAs, but is associated with increased risk of aneurysmal SAH, it seems that hypertension is a risk factor for sIA formation but does not seem to affect the risk of rupture significantly once the sIA has been formed.

Genetics of sIA formation and SAH

Approximately 10% of SAH patients have two or more family members also affected by aneurysmal SAH or unruptured sIAs [67, 75]. Among these families, the prevalence of unruptured sIAs is approximately 10% [66], which is significantly higher than estimated in the average

population [65]. This suggests that family history is a risk factor for sIA formation and aneurysmal SAH. Indeed, in a Swedish nationwide population-based case control study, more than two affected relatives significantly increased the risk of aneurysmal SAH [5].

Although a family history of SAH or sIAs is a risk factor for aneurysmal SAH, the largest twin study to date with 79,664 twin pairs (0.29% monozygotic) and 509 SAH cases during a follow-up of 6.01 million person-years did not show a significant degree of genetic contribution to SAH [44]. This demonstrates that modifiable risk factors determine the risk of aneurysmal SAH more than genetic background [44]. It seems that familial accumulation of SAH cases is probably due to the familial clustering of environmental risk factors (e.g., hypertension and smoking).

There are, however, some autosomal hereditary diseases, namely polycystic kidney disease, Ehler-Danlos type IV and fibromuscular dysplasia, that are associated with sIAs, increased risk of aneurysmal SAH or aneurysmal SAH [8, 11, 21, 68, 74]. Unlike previously thought, the hereditary Marfan syndrome, which causes defective elastin fibers, does not cause an increased risk of sIA formation [12]. The predisposition to sIAs caused by these genetic diseases explains a very small number of unruptured sIAs and aneurysmal SAHs.

Several studies of genetic linkage and single nucleotide polymorphism (SNPs) associations with sIAs have been made in Japanese, Finnish and other populations [55]. In most studies, association to a specific gene has not been found, although associations to several genetic loci have been reported. Very few of them have been replicated in other populations. These genetic polymorphisms are likely to be high in number and very variable among different populations. It can be speculated that any genetic polymorphism that affects the control of cerebral artery wall structure, of cerebral blood pressure or response of the cerebral artery to increased stress may increase the risk of sIA formation and rupture. However, even the strongest reported genetic associations to non-familial aneurysmal SAH or sIA formation are low in comparison to smoking, hypertension and female gender.

IA formation—lessons from experimental models and observational data

Intracranial aneurysms and aneurysmal SAH are exceedingly rare in children, whereas the incidence of sIAs and aneurysmal SAH increases with age [27, 28, 33]. Unlike previously thought, sIAs are not congenital (with rare exceptions) but acquired lesions that develop and grow during life [81].

The formation of true aneurysms of the cerebral arteries can be induced in many animals by a combination of hypertension and disruption of collagen synthesis [24, 25, 53]. In the induced sIAs, the first step seems to be disruption of elastic laminae and death of medial smooth muscle cells [40, 43], followed by aneurysmal outbulging of the arterial wall and inflammatory cell (macrophage) infiltration [35]. Several studies by Aoki et al. [2] and others have demonstrated the important role of inflammatory reaction of the cerebral artery wall in predisposing to the formation of induced sIAs in murine models.

Observations from experimental models suggest that the formation of sIAs is caused by a mismatch in the tensile strength of the cerebral artery wall and the hemodynamic stress to which it is exposed. This interpretation is in line with the observations from patient series that hypertension and mechanical weakness of the cerebral artery wall matrix (e.g., Ehler-Danlos IV patients) predispose to sIA formation [34, 63, 68, 70].

It seems likely that all the factors that affect either the strength of the cerebral artery wall or the hemodynamic stress in the cerebral vasculature are potential risk factors for sIA formation. Inflammation of the cerebral artery wall may decrease its tensile strength and thus predispose patients to sIA formation. Smoking, which is an independent risk factor for sIA formation, is known to cause inflammation in the arterial walls.

Not all sIAs rupture—why some do

Although cerebral artery aneurysms can be induced in laboratory animals, these aneurysms do not spontaneously rupture. This suggests that the formation of an aneurysm is a separate process from the rupture of an existing aneurysm, and that the pathobiology leading to formation of an aneurysm or its rupture is not entirely the same.

Meta-analysis of prospective autopsy series reported approximately 3.6% prevalence of sIAs that had never ruptured [65], whereas a meta-analysis of prospective angiography series reported a 6.0% prevalence of unruptured sIAs [65]. Also all clinical series report sIA rupture rates that are far from 100%. It is clear that only some sIAs rupture. This also suggests that the pathophysiology of the sIAs that rupture is somehow different from those that never rupture.

In order to decipher why some of the sIAs rupture, their histopathology and molecular biology have to be studied thoroughly. The comparison of ruptured and unruptured human sIAs can identify factors that associate with rupture in sIAs. The role of these factors in sIA biology should then be studied in experimental models to establish which of them cause sIA wall degeneration and rupture.

Why do sIAs rupture? Observations from classical histological stainings

Early histopathology studies using classical histological staining techniques have shown that the sIA wall is characterized by lack of internal elastic lamina and normal intima-media-adventitia layers [26, 72]. Already these studies reported that some of the sIA walls had plenty of disorganized mural cells (vascular smooth muscle cells, myofibroblasts, and fibroblasts), whereas some of the walls had lost most of them [26, 69, 72, 73, 80]. These studies also showed that the endothelial layer lining the luminal surface of the sIA is often irregularly arranged and there are visible gaps in between the endothelial cells [73].

In addition, these studies already reported the presence of inflammatory cells identified as polymorphonuclear leukocytes, plasma cells and small round cells (lymphocytes) [10, 80]. However, these inflammatory cells were not further characterized until the availability of modern immunohistochemical staining techniques.

Why do sIAs rupture? Observations from immunohistochemistry series

Only a few large histopathological series that use immunostaining techniques to compare specific cell populations in ruptured and unruptured human sIAs have been published. The Japanese series by Kataoka et al. [39] reported both structural degeneration and infiltration of inflammatory cells in the wall of ruptured sIAs. The later Finnish series by Frösen et al. [16] characterized rupture-associated sIA wall degeneration further. As in the prior classical histology series, both the Japanese and Finnish series described loss of endothelium, loss of mural cells, breakdown of the collagen matrix and partial hyalinization of the wall in association with rupture [16, 39] (Fig. 2). Moreover, in the Finnish series, loss of endothelia and subsequent thrombus formation on the luminal surface of the sIA wall were clearly associated with both the degeneration and rupture of the wall (Fig. 2) [16]. Whereas ruptured sIA walls were characterized by the above-mentioned degenerative changes, unruptured sIA walls resembled myointimal hyperplasia or neointima (Fig. 2) [16] that is part of the wound-healing process of arteries.

In these immunohistochemistry series, the degree of inflammatory cell infiltration was for the first time quantitated and shown to be higher in ruptured than unruptured sIAs [16, 39]. Many of the samples studied in the Finnish series were resected after a very short time interval from sIA rupture (less than 8 h, some 4 h) [16], and in both the Japanese and Finnish series some inflammatory cells were also present in some unruptured sIA walls [16, 39]. These findings suggest that the inflammatory cell infiltration observed in the sIA walls (Fig. 2) is present already before the rupture.

In addition to the cell-mediated immune reaction, also the humoral immune response is active in the sIA wall. Chyatte et al. [10] first reported the deposition of complement components and antibodies in the walls of a few unruptured and ruptured sIAs. Later, Tulamo et al. demonstrated in the Finnish series that complement activation was associated with the sIA rupture and wall degeneration described previously by Frosen et al. [85] (Fig. 2). The complement activation in the sIA wall seems to be of a more chronic than acute nature, since the activation is predominantly by classical pathway activation with alternative pathway amplification and significant accumulation of C3d is detected [86]. C3d is a component of the complement cascade, and accumulation of C3d occurs in chronic inflammatory states [54].

Why do sIAs rupture? Observations from gene expression studies

Four studies comparing the gene expression of ruptured and unruptured sIAs with genome-wide microarrays have been published [46, 47, 49, 62]. The first microarray study comparing ruptured and unruptured sIA walls was by Krischek et al. [46] who compared six ruptured and four unruptured sIAs and found an association of antigen presenting inflammatory cells with rupture.

The largest microarray study compared gene expression in 12 ruptured sIAs with 10 unruptured sIAs, as well as with 26 samples from intact middle meningeal artery or superficial temporal artery [49]. This study reported overexpression of collagenases (matrix metalloproteinase 2 and 9), pro-apoptotic genes and inducible nitric oxide synthetase in ruptured sIAs, as well as downregulation of anti-apoptotic genes. The second largest study compared 11 ruptured and 8 unruptured MCA aneurysms, and identified 1,426 differently regulated genes, among which signaling pathways associated with inflammatory cell infiltration, oxidative stress, disturbed cell homeostasis and dysfunctional endothelium were upregulated in ruptured sIAs [47]. The third largest study compared eight ruptured and six unruptured sIAs [62]. Contrary to the two larger microarray studies and the prior histological series, this study reported downregulation of inflammation in ruptured sIAs.

Except the findings of one study, these studies at the transcriptome level are in accordance with the histopathological series that associated endothelial dysfunction, loss of mural cells, inflammatory cell infiltration and degradation of the matrix with sIA wall rupture.

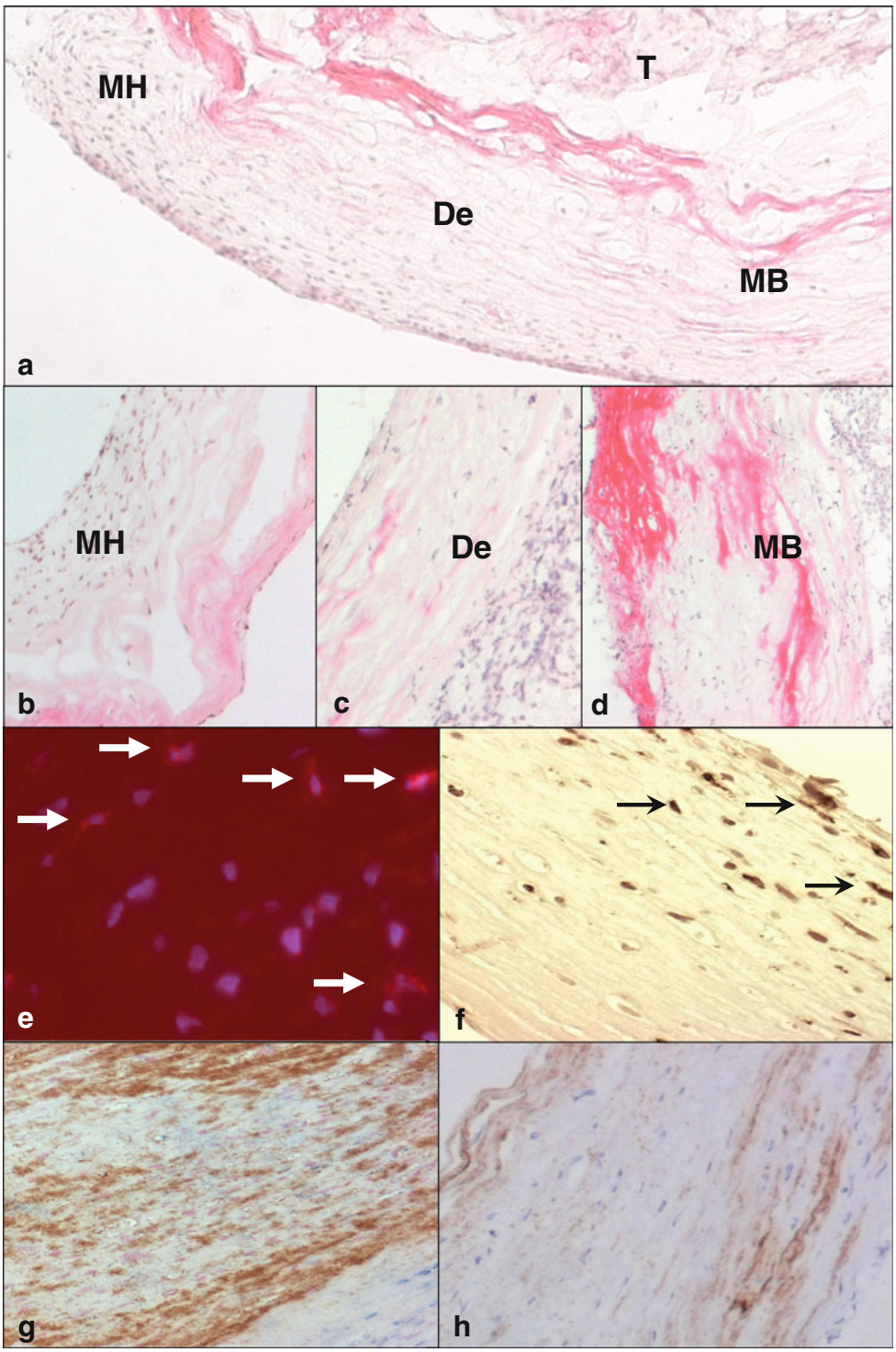

Fig. 2 **a** The wall of an aneurysm that is undergoing degeneration often shows a gradual change from well-cellularized areas with myointimal hyperplasia (MH) to decellularized areas that have lost mural cells (De) (HE). Matrix breakdown (MB) and hyalinization are seen in decellularized areas of the wall. In these parts, the luminal surface is often covered by a thrombus (T). Other examples of wall degeneration are shown in **b**, **c** and **d**, where the hyalinization resembles fibrinoid necrosis (HE). Inflammatory cells, such as **e** T-cell immunofluorescence staining for CD45RO, a marker for T-memory cells (antibody clone UCHL1 in red and DAPI stained nuclei in blue) and **f** macrophages (CD68 immunostaining, antibody clone PG-M1) are detected in aneurysm walls. **g** Antibodies (double immunostaining with IgG in *brown*, alpha-smooth muscle actin in *blue*, nuclei *pale red*) and **h** complement activation (SC5b-9 immunostaining, antibody clone A239) are also detected in aneurysm walls

Why does the sIA wall rupture? Chronic proteolytic injury and loss of mural cells

The aneurysm wall ruptures when the matrix of the sIA wall has degenerated sufficiently to become too fragile to resist the hemodynamic pressure of the cerebral arteries. Collagen fibers exposed to mechanical stress eventually degenerate [71], and therefore need to be repaired and maintained by continuous synthesis of new collagen. In the sIA wall, the collagen fibers are also exposed to degradation by active collagenases [7], in addition to the "wear and tear" caused by mechanical stress. Collagenases are synthesized by mural cells in the sIA walls [19, 41] but may also be derived from the circulation [84].

Under normal circumstances degeneration or injury of the artery wall induces the mural smooth muscle cells to proliferate and synthesize a new matrix [56]. This occurs also as an initial response to many of the degenerative changes occurring during aging, although in most of these situations the actual disease is related to subsequent loss of medial smooth muscle cells [71].

Injury to the media layer or to the endothelium leads to migration of smooth muscle cells to the intima [56]. In the intima these cells proliferate and synthesize new matrix, forming fibrous tissue commonly referred to as intimal hyperplasia [56] (or in animal models and in some human vascular pathologies as neointimal hyperplasia or neointima). A similar tissue remodeling process is observed also in experimental saccular aneurysm models in which a saccular pouch is constructed with an arterial transplant [18] (Fig. 3a, b).

A tissue-remodeling process similar to the "healing" of damaged artery walls seems to be ongoing also in the sIA wall. In many sIA walls, luminal thrombus is infiltrated by smooth muscle cells (SMC) [16] (Fig. 3c), and in many sIA walls mural cells proliferate and synthesize new collagen matrix [16] (Fig. 3d, e). This proliferation of mural cells, synthesis of new matrix, and organization of mural thrombus by SMCs is likely to increase the tensile strength of the sIA wall and hence protect from sIA rupture.

Ruptured sIA walls are characterized by loss of mural cells [16, 39] (Fig. 3f). In sIA walls that have lost their mural cells, the "repair and maintenance" process maintained by matrix synthesis and proliferation of mural SMCs is disrupted. Loss of mural cells together with the "wear and tear" to which collagen fibers are exposed and the proteolytic injury ongoing in some sIA walls predisposes the sIA wall to rupture. Hence, the loss of mural cells is a key event that leads to the degeneration and eventual rupture of the sIA wall.

Mechanisms of cell death in the sIA wall—programmed cell death or uncontrolled necrosis?

In order to deduce why the cells of the sIA wall die, it is important to know how they die. Programmed cell death or apoptosis has been reported to be associated with sIA wall rupture first in a Japanese series by Sakaki et al. [69] who used terminal deoxynucleotidyl transferase dUTP nick end labeling (Tunel) staining in 13 ruptured and 14 unruptured sIA samples. Later, the finding was replicated using Tunel and electron microscopy in an Italian series [61] of 17 ruptured and 10 unruptured sIAs. However, in two larger Finnish studies (42 ruptured and 24 unruptured [16], and 31 ruptured and 22 unruptured [85]) the ratio of Tunel + cells was higher in ruptured sIA walls in wall areas outside the myointimal hyperplasia, but the overall difference of Tunel + cells in ruptured and unruptured sIA walls did not reach statistical significance.

Tunel-staining is not specific for apoptosis, but may also stain cells undergoing necrosis. Activation of cysteine-dependent aspartate-directed proteases (caspases), the effector enzymes of apoptosis, is an alternative marker for apoptosis. Guo et al. [23] showed in a Chinese series of 15 ruptured sIA that caspase activity is found in the sIA wall in addition to Tunel positivity. In addition, Pentimalli et al. [61] and Tulamo et al. [85] showed apoptotic cells in the sIA walls with electron microscopy.

All these reports agree that some degree of apoptosis is ongoing in the sIA wall. Tunel-positive cells are, however, found also in unruptured sIA walls [16, 85], and it is not completely evident that ruptured and more degenerate sIA walls have significantly higher Tunel positivity than unruptured ones. Given also that in most sIA walls the degree of apoptosis (ratio of Tunel-positive cells) is relatively low compared to the degree of mural cell loss, it seems likely that also uncontrolled necrotic cell death occurs in the wall of many sIAs and has a very significant role. This interpretation is supported by findings in sIA wall histopathology, where scattered debris is often seen in the extracellular matrix in areas with few mural cells, and some areas resemble fibrinoid necrosis in histology (Fig. 2d).

Humoral inflammatory reaction—cause or co-variate of cell death in the sIA wall?

Inflammation was observed in cerebral aneurysms already by Virchow, and has been speculated ever since by many authors to be the cause of wall degeneration and rupture [90]. However, although the inflammation does not seem to be a response to rupture (see above) [16], it is not necessarily the cause of degeneration and rupture either.

Antibodies and the complement system are the mediators of the humoral immune response. Antibodies (IgM and IgG) are found in most sIA walls, bound primarily to the wall matrix but also to the mural cells in some sIA walls [10, 86]. Complement activation does not, however, correspond to the accumulation of these antibodies [86], and inflammatory cell infiltration does not either (unpublished observation). This suggests that an antibody-mediated inflammatory response is not the primary cause of the inflammation in the sIA wall.

Activation of the complement system leads eventually to the formation of membrane attack complexes (MAC) on the surface of cells, if the whole cascade is fully activated (terminal activation). MAC causes cell damage and may lead to programmed or necrotic cell death. The terminal

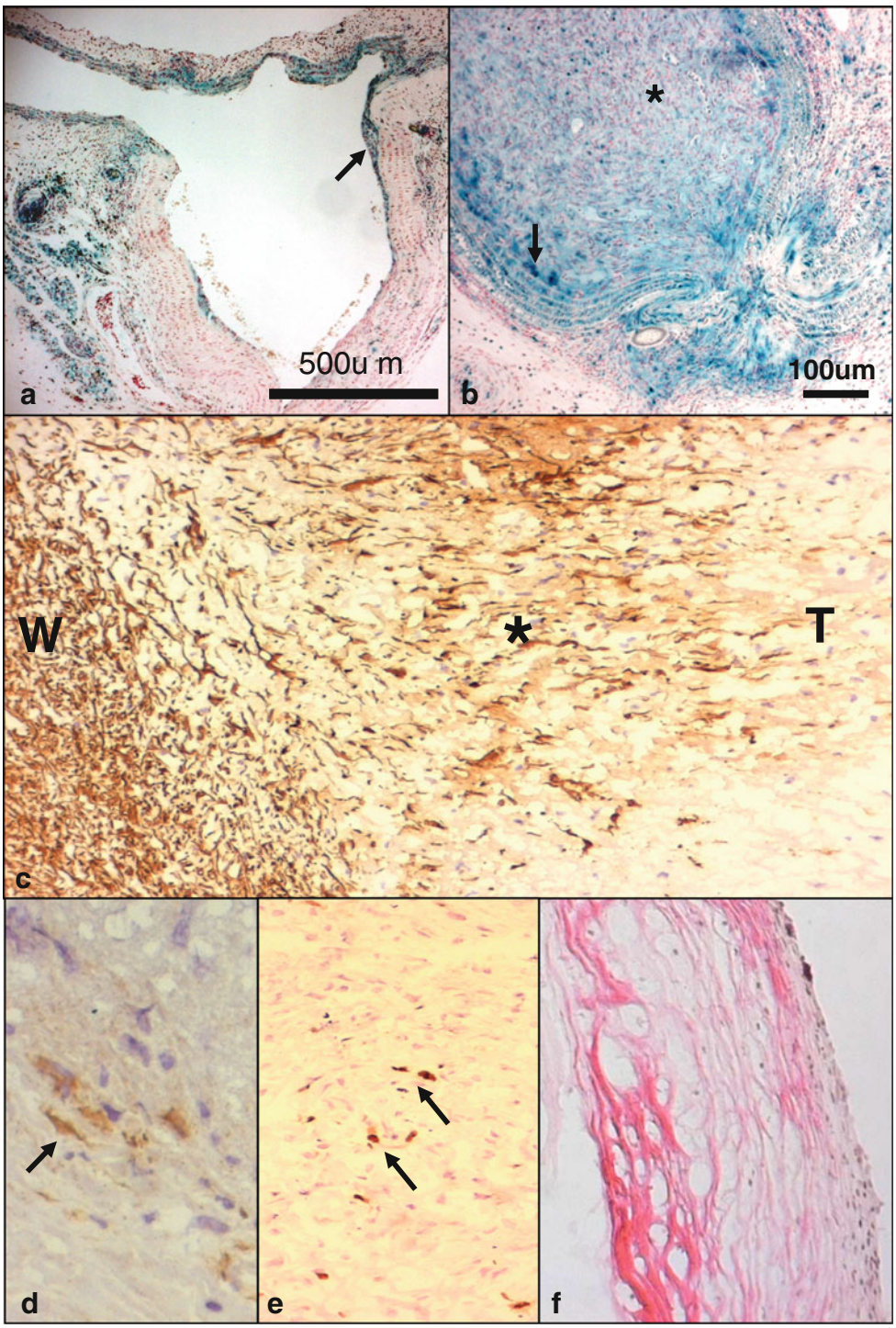

Fig. 3 Surgically constructed saccular arterial pouches develop in **a** 1 week myointimal hyperplasia (*arrow*) that is derived in part from the parent artery and circulating cells. After 1 month, **b** myointimal hyperplasia has filled the whole lumen but is mostly derived from the aneurysm wall. In order to identify the origin of the myointimal hyperplasia, transplants were made from genetically labeled ROSA mice (ROSA mice cells are blue in X-gal staining) to normal mice and vice versa. Migration of smooth muscle cells (**c**, *asterisk*) from the pouch wall (W) into the luminal thrombus (T) is also seen in human aneurysms (immunostaining for smooth muscle myosin heavy chain, antibody clone: SMMS-1). **d** Mural cells of the human aneurysm wall also synthesize new matrix (immunostaining for hydroxyproline esterase, antibody clone: 5B5) and **e** proliferate (immunostaining for Ki67, antibody clone: MIB-1). A similar wound-healing reaction as in aneurysms with myointimal hyperplasia and in saccular pouches in animal models is not seen in **f** decellularized aneurysm walls

complement activation in the sIA wall is associated with wall degeneration and rupture [85]. However, in the sIA wall, MAC formation is rarely observed on the cell surface, but rather the terminal complement complexes are found mostly in the matrix and cellular debris in decellularized regions [85]. This suggests that complement activation might rather be a reaction to necrotic cell death than the cause of it.

The complement system is activated in the sIA wall by a classical pathway with an alternative pathway amplification [86, 87]. During the activation of the complement cascade, pro-inflammatory chemotactic chemokines are released, which recruits macrophages and T cells [42]. It is possible that complement activation caused by wall degeneration is one of the mechanisms by which inflammatory cells are recruited into the degenerating sIA wall.

Cell-mediated inflammatory reaction—cause or co-variate of cell death in the sIA wall?

Cell-mediated immune response can induce cell death via T-cell or macrophage activation. T cells are found in the sIA wall [10, 16], but no study of the type or activity of T cells in the sIA wall has been published thus far. The microarray studies do not suggest association of cytotoxic T-cell response, nor Th1 or Th2 type cytokine profile with sIA wall degeneration or rupture [46, 47, 49, 62].

Macrophages can induce programmed cell death using Fas-ligand and tumor necrosis factor alpha (TNFα) [6, 88]. Whereas studies of Fas-ligand in the sIA wall have not been published, Jayaraman et al. [36] have shown TNFα mRNA and protein in a small series of ruptured sIA walls. Later, a Finnish microarray study showed higher TNFα mRNA expression, as well as changes in the expression of TNFα signaling-related genes in association with sIA rupture [47]. TNFα activation induces apoptosis via activation of caspase-8 [1]. Thus far, caspase-8 has not been shown in the sIA wall, so definitive proof that TNFα induces cell death in the sIA wall is still missing.

What can be deduced from the inflammatory reaction in the sIA wall?

Although several studies confirm at the mRNA, protein, and cellular levels the association of inflammation and rupture of the sIA wall, the currently available data do not demonstrate inflammation-induced cell death in the sIA wall—rather the rarity of it. Regardless of whether inflammation is a cause or reaction to the degeneration of the sIA wall, it is certainly a significant modulator of the pathobiology of the sIA wall. Furthermore, the study of the type of the inflammatory response in the sIA wall may reveal clues of what causes the wall degeneration.

The majority of macrophages in the sIA wall are CD163 positive (CD163+) [16]. CD163+ macrophages are associated with so-called alternative macrophage response [13, 22, 51, 57]. CD163 is a scavenger receptor for breakdown products of hemoglobin [13, 51]. Although intramural dissections and intramural thrombus are rarely seen in sIA walls [16, 60], hemosiderin deposits and cells that have phagocytosed iron are detected in some sIA walls (Fig. 4a, b). However, in most sIA walls that are infiltrated with CD163+ macrophages, no iron phagocytosing cells or hemosiderin deposits are seen (unpublished observation). Although breakdown of red blood cells and subsequent accumulation of heme deposits and iron might induce inflammatory cell infiltration in some sIAs, this does not seem to be the cause of inflammatory cell infiltration in most inflamed sIA walls.

CD163+ macrophages are part of an antioxidant defence response triggered by excessive oxidative stress [57]. Response to excessive oxidative stress was associated with sIA wall rupture in the Finnish microarray study [47]. Increased expression of hemeoxygenase 1, an enzyme that detoxifies oxygen radicals, is associated with sIA wall degeneration and rupture also at the protein level (unpublished).

Taken together these findings suggest that the macrophage infiltration in the sIA wall is at least in part a reaction to the high oxidative stress that likely has a significant role in sIA wall degeneration and loss of mural cells.

Effects of oxidative stress—genesis of cytotoxic and immunogenic oxidated lipids

Luminal thrombosis that is associated with sIA wall degeneration and rupture [16] is a potent source of oxidative stress caused by peroxidases of neutrophils trapped in the thrombus [30]. Indeed, very high endogenous peroxidase activity is observed in peroxidase stainings in the sIA walls with luminal thrombus (unpublished) (Fig. 4c, d).

High oxidative stress causes oxidation of extracellular and intracellular lipids, damages cell membranes and triggers activation of caspase-9 that leads to downstream activation of other caspases and eventual apoptosis of the cell [88]. Caspase-9 activity is detected in sIA walls and is associated with rupture (unpublished). Lipids modified by oxidation are also observed in the sIA wall, both in the extracellular matrix and in the mural cells [20, 86]. Oxidated lipids are cytotoxic and can trigger both apoptotic and necrotic cell death [29].

Accumulation of lipids in the sIA wall has been reported by Stehbens and later by Kosierkiewicz et al. [45, 81]. In our series, intramural lipid accumulation (Oil-Red-O staining) is associated with degeneration of the sIA wall (unpublished). Cells filled with intracellular lipid (foam cells) are observed in sIA walls (Fig. 4f, g), mostly in those areas that resemble early atherosclerotic lesions. In atherosclerotic lesions, foam cells are typically macrophages that have infiltrated the artery wall and phagocytose the accumulated extracellular lipids [52]. In atherosclerotic lesions, some of these macrophages also act as antigen-presenting cells, and trigger an immune response against the oxidated and otherwise modified lipids that have accumulated in the artery wall [52].

Upregulation of genes related to antigen presentation was observed in ruptured sIAs in two microarray studies [46, 47]. In addition, patients with ruptured or unruptured sIAs have differences in their humoral immune response against oxidated lipid epitopes [20].

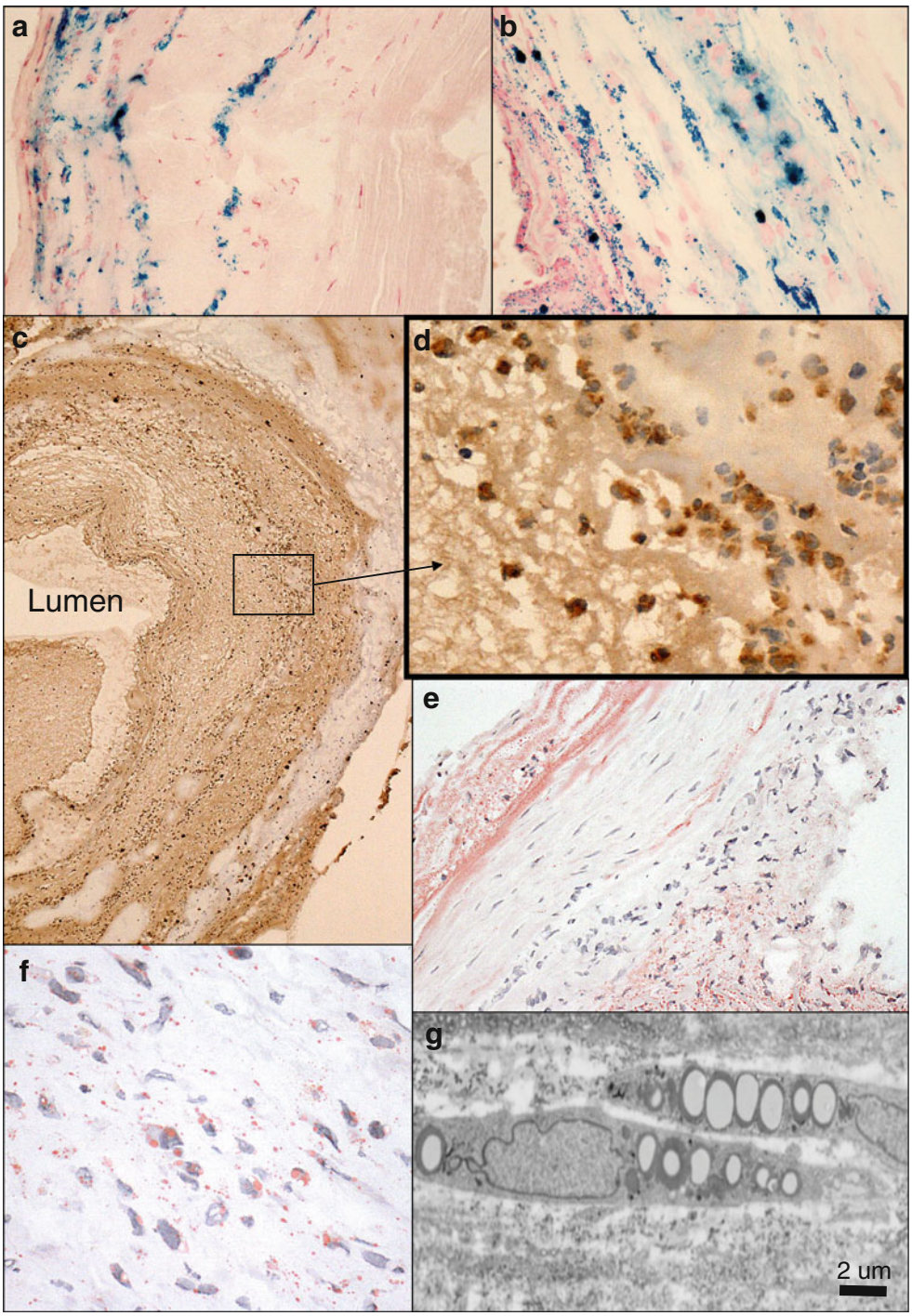

Fig. 4 **a** Iron accumulates in the aneurysm wall (Prussian blue staining) and **b** is phagocytosed by mural cells (Prussian blue staining). **c** The luminal thrombus, as well as **d** especially the polymorphonuclear leukocytes trapped in it, is a major source of peroxidase activity in the aneurysm wall (endogenous peroxidase activity stained with diaminobenzidine). **e** Lipids accumulate to the aneurysm wall matrix (Oil-Red-O staining) and are **f** phagocytosed by cells in the aneurysm wall (Oil-Red-O). This leads to **g** foam cells formation (transmission electron microscopy)

These observations suggest that oxidative stress can cause cell death in the sIA wall by both direct damage to mural cells and indirectly via accumulation of cytotoxic oxidated lipids in the sIA wall. Moreover, oxidative stress can indirectly induce inflammation in the sIA wall by oxidative modification of intramural lipids, which then turn immunogenic and trigger inflammation.

The effects of luminal thrombus on the sIA wall

The histopathological series clearly demonstrate that thrombus formation on the luminal surface of the sIA wall is part of the wall degeneration [16]. Probably the first degenerative change of the sIA wall is the loss of the intact endothelial layer, which leads to formation of the fibrin network and thrombus on the exposed collagen surface.

Thrombocytes get trapped to the fibrin network of the thrombus. The degranulation of thrombocytes leads to the release of angiogenic and vascular growth factors [59]. The mural cells in the sIA wall express receptors for several thrombocyte-released growth factors [16]. Several of these growth factors (e.g., TGF-beta) can modulate mural cell survival, proliferation and matrix metabolism [17, 59]. The thrombocyte-derived growth factors also affect the remaining endothelial cells [59]. In addition to the similar effects as on the mural cells, angiogenic growth factors (e.g., VEGF) also increase the permeability of the endothelial layer [59]. This increases transendothelial diffusion, which significantly facilitates the accumulation of lipids and plasma proteins (including antibodies and complement components) to the sIA wall. This is likely to trigger cell death and inflammation.

Neutrophils are also trapped in the fibrin networks [15, 30]. In abdominal aortic aneurysms (AAA), the degranulation of entrapped neutrophils leads to chronic proteolytic injury that degrades the AAA wall [15, 30]. In addition to matrix-degrading proteases, neutrophils also release highly active peroxidases that cause increased oxidative stress [15, 30]. Also other cytotoxic (e.g., granzymes) and pro-inflammatory substances can be released from neutrophils [78]. As in AAA walls, neutrophils are also trapped in the luminal thrombus of the sIA wall (Fig. 4). This likely leads to similar chronic proteolytic injury as in AAA walls, as well as causes death of mural cells via the effects of increased oxidative stress (Fig. 5).

In addition to the "active" biological effects caused by thrombus released factors, the luminal thrombus may also "passively" affect the sIA wall by limiting diffusion of oxygen and nutrients from the lumen and thus promote cell death in the sIA wall [32].

When an aneurysm is coiled or embolized by other means, luminal thrombus formation is induced in the sIA fundus. This can prevent later rupture if the thrombus becomes stable and organizes into a myointimal hyperplasia-like fibrous tissue [3, 82]. However, in up to 20% of embolized sIAs the sIA recanalizes during follow-up, and may regrow and rupture [14]. This is likely due to failed thrombus organization. The effect of luminal thrombus on the sIA wall seems to be dependent on whether the thrombus is organized by infiltrating smooth muscle cells or myofibroblasts, or whether it remains as an unorganized fibrin network that attracts new neutrophils and other inflammatory cells that release their cytotoxic and proteolytic contents and maintain a state of chronic injury. Again, the role of mural cells is paramount.

Thrombus formation—link between aneurysm biology and geometry

Intact endothelium prevents luminal thrombus formation, as well as limits diffusion from the circulation in the vascular wall [50, 83]. Non-physiological flow conditions can cause endothelial dysfunction or complete loss of endothelium, which then leads to exposure of collagen and other matrix proteins that activate the coagulation cascade, with subsequent thrombus formation and the wall remodeling described above.

Endothelium is sensitive to sharp gradients in wall shear stress (WSS) as well as to non-physiologic WSS levels [9]. Shojima et al. [76] have shown that WSS conditions are high in the sIA neck region but significantly lower in the fundus where rupture most often occurs. Our preliminary results suggest that aberrant WSS conditions are associated with de-endothelialization and degeneration of the sIA wall (unpublished).

Flow condition and WSS, as well as pressure conditions, are determined by the sIA geometry [76, 77]. When the sIA develops and grows, its geometry changes and subsequently also the flow conditions in the sIA fundus. In addition, the previously formed local thrombus and its growth further changes the luminal shape and the flow conditions.

Clinical series clearly demonstrate that sIA geometry (e.g., shape, size, aspect ratio) is associated with the risk of rupture [4, 37, 38, 91, 93]. WSS changes that cause endothelial dysfunction, thrombus formation and the subsequent wall degeneration or remodeling may explain why.

Molecular biology of aneurysm growth

Geometry of the sIA is often not stable. Many sIAs grow or change in shape during follow-up [37]. This is very significant in clinical practice, since the risk of rupture increases with the size of the aneurysm [38, 93], and multilobular or tubular shape (high aspect ratio) is associated with sIA rupture [4, 64, 91].

The shape and size of the sIA can change either by expansion of the wall due to proliferation of mural cells, or by distension due to hemodynamic pressure or by a combination of these two mechanisms. Cell proliferation has been detected in the sIA wall in two Finnish series [16, 85]. Mural cells capable of synthesis of a new collagen matrix are also found in sIA walls [16]. These observations show that some sIA walls are capable of enlargement by growth. Since many sIA walls are very degenerated, it seems likely that they enlarge by mechanical distension as well. However, no clear correlation with wall degeneration and sIA size or shape has been observed in large histopathological series. Shape and size, however, are associated with activation of the intracellular signaling cascades controlling cell death and proliferation, among other things [48].

Cell proliferation can be induced by various stimuli, which activate a signaling cascade of mitogen-activated

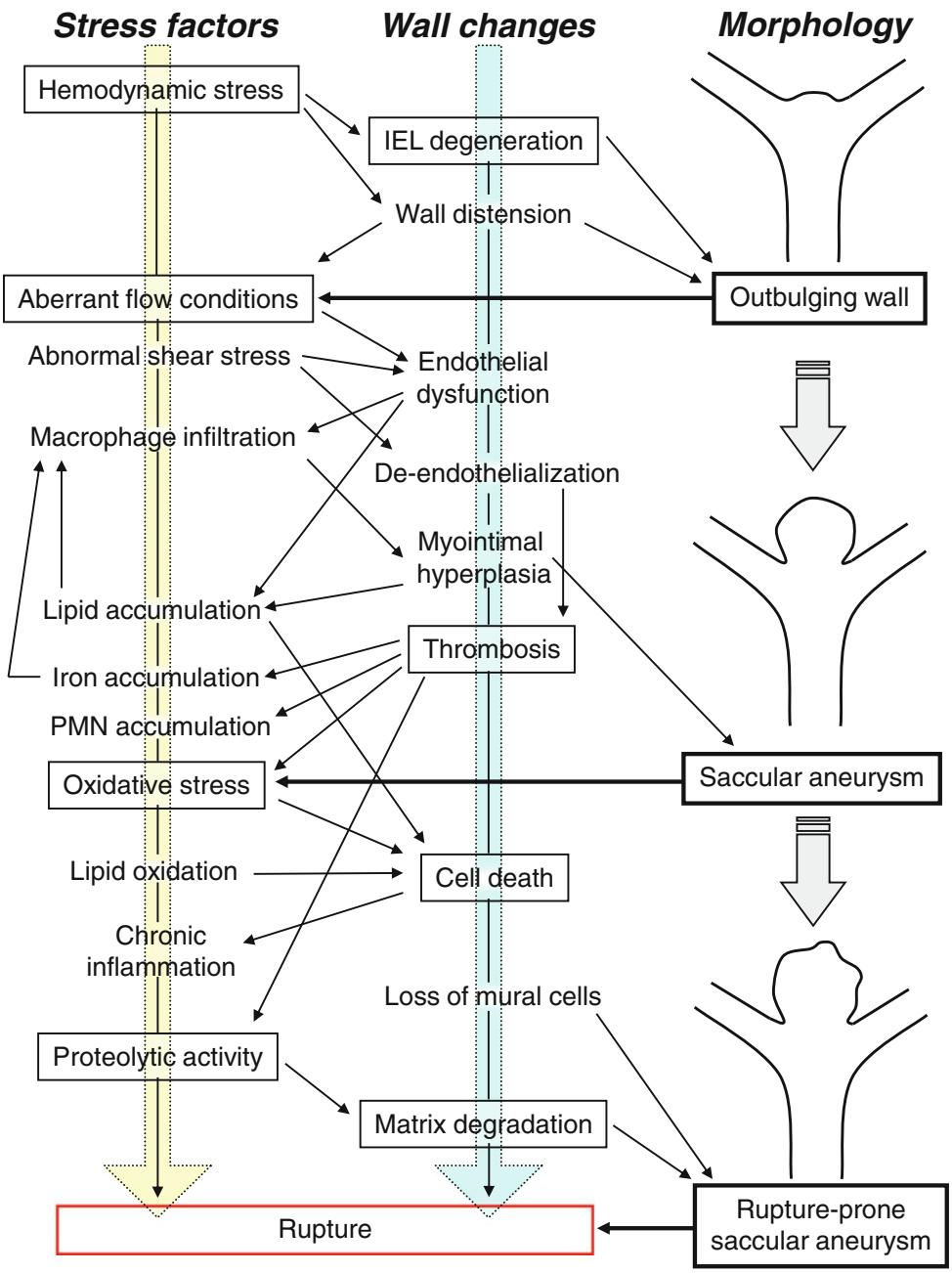

Fig. 5 Mechanistic flow chart of sIA pathobiology showing in parallel the stress factors and wall changes and their interactions, which gradually lead to changes in the sIA morphology. The *long arrows* (*yellow and blue*) represent both the causality and progression within the process, which ultimately leads to the rupture of an sIA. The key pathobiological elements are shown in *rectangles*. It is notable that this is a rough representation as many of the processes occur simultaneously. *IEL* internal elastic lamina, *PMN* polymorphonuclear leukocyte

protein kinases (MAPK). Of the MAPKs, the phosphorylated active form of c-p54 Jun N-terminal kinase (JNK) and p38 is associated with sIA growth [48]. The MAPKs are activated in response to inflammatory cytokines, growth factors, mechanical stretch or cellular stress. It seems possible that the inflammation of the wall, luminal thrombus-derived growth factors, wall degeneration and hemodynamic stress could all trigger MAPK activity, which leads to cell proliferation and possible growth of the sIA. Not only does growth of the sIA affect the biology of its wall, but growth of the sIA results from the biology of the wall.

Summary

Aneurysm ruptures when blood pressure-induced tension of the wall exceeds its strength. The sIA wall is not an inert collagen meshwork, but instead has several active cell populations that can both "repair and maintain" the wall under stress, or can "degrade and destroy" the cells and matrix of the sIA wall.

Loss of mural cells is a key event that shifts the balance between "repair and maintenance" and wall degeneration, and eventually leads to a rupture-prone wall. Death of sIA

wall cells can be caused by many factors, of which excessive oxidative stress seems the most dominant based on current data. A key event in the processes that lead to cell death and subsequent matrix degeneration in the sIA wall is the loss of functioning endothelium and subsequent thrombus formation on the luminal surface of the sIA wall. Dysfunction of the endothelium can be caused by the aberrant flow conditions in the sIA lumen, which in turn are a result of the geometry of the lesion. The fate of an unruptured sIA depends on a balance of hemodynamic forces and wall biology that interact and guide the remodeling of the aneurysm geometry as well as wall structure.

It seems likely that novel imaging methods that can show loss of endothelial function, luminal thrombus formation, death of mural cells or inflammation could be used to detect rupture-prone sIAs, given that these findings are associated with a degenerate rupture-prone walls in histopathology. Moreover, since these changes seem to be triggered by aberrant flow conditions that are geometry dependent, it might be possible to use sIA geometry to screen unruptured sIAs for those that need to be more carefully studied for possible degenerative changes.

In addition to the possible significant improvements in the diagnostics of sIAs at risk of rupture, in depth knowledge of the pathobiology of the sIA wall may allow the development of novel pharmaceutical or other biological therapies that would shift the balance between "repair and maintenance" and "cell death and wall degradation." This would hopefully reduce the risk of sIA rupture without the need of surgery or other invasive interventions.

Acknowledgments This work was supported by the research funds of The Helsinki University Central Hospital (EVO Grant TYH 2010209) and research grants from The Sigrid Juselius Foundation, Helsinki, Finland; The Maire Taponen Foundation, Helsinki, Finland; and The Biomedicum Helsinki Foundation, Helsinki, Finland.

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