Mast Cells, Neovascularization, and Microhemorrhages are Associated With Saccular Intracranial Artery Aneurysm Wall Remodeling

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Abstract
Chronic inflammation contributes to remodeling, degeneration, and rupture of saccular intracranial artery aneurysms. Mast cells are important proinflammatory and proangiogenic cells in chronic inflammatory vascular diseases. Here we studied mast cells and neovascularization in 36 intraoperatively resected aneurysms using histology and immunohistochemistry and analyzed the clinical characteristics of the aneurysms according to bleeding status (unruptured vs ruptured). Among the 36 aneurysms, 9 contained mast cells (tryptase-positive cells) and 15 contained neovessels (CD34- and CD31-positive capillary-like structures). The density of neovessels was significantly higher in aneurysm walls containing mast cells than in walls not containing them. In particular, wall areas with abundant mast cells and neovessels also contained iron deposits, indicating damage of newly formed endothelium with ensuing microhemorrhages. Walls with the highest neovessel density and the greatest iron deposition also showed evidence of degeneration. Finally, none of the mast cell-containing aneurysms showed an intact luminal endothelium. Thus, mast cells may adversely affect both neovascular and luminal endothelia. The novel association of mast cells with neovessels and injurious microhemorrhages, as well as with luminal endothelial erosion, suggests that mast cells contribute to remodeling and degeneration of saccular intracranial artery aneurysms.

Key Words: Atherosclerosis, Inflammation, Intracranial aneurysm, Lipid, Mast cell, Microhemorrhage, Neovascularization.

INTRODUCTION
Rupture of a saccular intracranial artery aneurysm (sIA) causes subarachnoid hemorrhage (SAH), which has a highly adverse outcome and is associated with a mortality of approximately 50% (1). Subarachnoid hemorrhage affects mainly the working-age population (2), and many of those who survive are left with significant deficits (1). Aneurysmal SAH can be prevented by microsurgical clipping or endovascular embolization of the unruptured sIA; however, both procedures have significant risks (3).

The pathogenesis of sIA wall rupture is not understood. Chronic inflammation is associated with sIA wall remodeling and rupture (4–9). Lack of internal elastic lamina, erosion of luminal endothelium, infiltration of inflammatory cells, apoptosis of smooth muscle cells (SMCs), and presence of myointimal hyperplasia, fibrosis, and thrombus are characteristic of sIA wall remodeling (4, 5). Activation of the complement system, an important part of innate immunity, is also associated with sIA remodeling and rupture (7). In addition, calcification (5) and lipid accumulation may be present (6, 8, 10). Of particular significance is the recent observation that accumulation of lipids and oxidized lipids is also associated with sIA wall degeneration and rupture (10). Accordingly, the histopathologic changes in sIA walls resemble those in atherosclerotic plaques (11, 12), where low-density lipoprotein (LDL) particles accumulate in the inner layer of the wall (the intima), become oxidized or otherwise modified, and trigger local chronic inflammation and inflammatory cell infiltration (13).

Mast cells (MCs) are essential regulators of both innate and adaptive immune systems and play an important role in the pathogenesis of chronic inflammatory diseases (13–15). Upon activation, MCs immediately degranulate (i.e. exocytose) a fraction of their cytoplasmic secretory granules, which contain histamine, heparin, neutral proteases, and some cytokines (16). After they are activated, they rapidly generate and secrete prostaglandins and leukotrienes, and then begin to slowly secrete a variety of growth factors and proinflammatory cytokines. With respect to neutral granule proteases, all MCs contain tryptase, and a fraction of them also contain chymase. Mast cells degrade LDL and high-density lipoprotein particles, thus contributing to lipid accumulation in the arterial wall (13). They also activate matrix metalloproteinases, which degrade various components of the
extracellular matrix (ECM) (13), and thus may contribute to the loss of tensile strength of the arterial wall. Moreover, MC proteases directly degrade the pericellular matrices of SMCs and endothelial cells, thereby causing their apoptotic death. Mast cells are suggested to contribute to the pathogenesis of human abdominal aortic aneurysm via similar mechanisms (17, 18). Mast cells have been reported in intracranial arteries adjacent to ruptured sIAs (19) and, more recently in a preliminary report, also in sIAs (20). Most interestingly, in a rat model of experimental sIA, the numbers of MCs increased during sIA formation, whereas pharmacologic inhibition of MC degranulation decreased the size of the sIA and prevented thinning of the sIA wall (21).

Mast cells are also powerful angiogenic cells, accompanying neovessels in human coronary (22) and carotid (23, 24) atherosclerotic plaques, in coronary artery aneurysms (25), in aortic valve stenosis (26), and in abdominal aortic aneurysms (17). Neovessels in advanced atherosclerotic plaques are fragile, rupture easily, and cause intraplaque microhemorrhages (22, 27–29). The extravasation of red blood cells in the arterial wall may lead to proinflammatory iron deposition (28–30). In atherosclerotic plaques, neovessels and iron deposits appear in association with MCs (30). Interestingly, recent studies have described neovascularization in sIAs in association with inflammatory cell infiltration (31). To our knowledge, however, no previous data on microhemorrhages in the sIA wall exist.

In view of the findings summarized previously, MCs seem to contribute to different types of chronic inflammatory vascular diseases. Thus, we wanted to study their role in the pathobiology of human sIAs. Because MCs are strongly related to neovascularization and the associated proinflammatory microhemorrhages, we investigated these pathologic changes in sIAs and their potential associations with sIA wall remodeling.

MATERIALS AND METHODS

Sample Collection and Clinical Data

We studied a series of 36 patients with 16 unruptured and 20 ruptured sIAs. The sIA samples were resected intraoperatively after the sIA neck had been clipped at the Department of Neurosurgery of Helsinki University Central Hospital (Helsinki, Finland). The specimens were immediately snap-frozen in liquid nitrogen and stored at −80°C. Clinical data were collected from medical records, and sIA dimensions were obtained from preoperative computed tomography angiography images. The appropriate departmental Hospital Ethics Committee approved the study protocol. The frozen sIA samples were embedded in Tissue-Tek (Sakura, Alphen aan den Rijn, the Netherlands) and snap-frozen in liquid nitrogen, and the blocks were cryosectioned at 4 μm for histologic and immunohistochemical staining.

Histology

The sections were stained with Gill hematoxylin and eosin G. Lipids were stained with Oil Red O (ORO) in unfixed cryosections that were first incubated in absolute propylene glycol (Sigma, St Louis, MO) for 5 minutes then in 0.5% ORO solution in propylene glycol for 7 minutes and finally washed twice in 85% propylene glycol, running tap water, and aqua. Iron deposits were detected by Prussian blue staining for ferric iron using a modification of the Perl method (Artisan Iron Stain Kit; Dako, Glostrup, Denmark), according to the manufacturer’s protocol. The nuclei were background-stained with Mayer hematoxylin (Sigma).

Immunohistochemistry

Table 1 shows the monoclonal mouse anti-human primary antibodies, their concentrations, and the fixatives used. After fixation, endogenous peroxidase was blocked with 0.9% H2O2 (Merck, Espoo, Finland) in phosphate-buffered saline (PBS) at room temperature (RT) for 20 minutes. Nonspecific binding was blocked with either 5% normal horse serum (Vector Laboratories, Burlingame, CA) or 4% normal horse serum and 1% fish skin gelatin (fatty acid–free; Sigma) in buffer solution (3% bovine serum albumin [Sigma] and 0.1% Tween [Sigma] in PBS) at RT for 30 minutes. The primary antibodies were diluted in the buffer solution and incubated for 60 minutes at RT or overnight at 4°C. For secondary detection, we used either Vectastain Elite ABC kit (Vector Laboratories) or EnVision kit (Dako) according to the manufacturers protocols. The signal was visualized with diaminobenzidine (Vector Laboratories). For background staining, the sections were incubated in Mayer hematoxylin (Sigma) and embedded in J.T. Baker UltraKitt mounting medium (Mallinkrodt Baker, Deventer, the Netherlands). An irrelevant IgG class–specific monoclonal antibody (IgG1 or IgG2a; Serotec, Oxford, United Kingdom) served as a substitute for secondary detection.
for primary antibody in sIA-negative controls. Human tonsils served as both positive and negative controls.

**Histologic Analysis**

The stained slides were photomicrographed with a Zeiss Axioplan 2 imaging microscope (Flukoal Bv Micro-optik, Deurnen, the Netherlands) and a Progres C7 USB digital camera (Jenoptik, Jena, Germany). For quantitative analysis, the cells were counted in 3 photomicrographed standard-sized areas, each 0.613 mm², which represented the areas most intensely stained for the cells of interest (hot spots). In a few cases, the number of analyzed areas had to be restricted because of the small sample size. Thrombus, if present, was excluded from cell count analysis.

The presence or absence of myointimal hyperplasia, decellularization, fibrosis, thrombosis, and atheroma was evaluated from hematoxylin and eosin and α-smooth muscle actin (αSMA) stainings. The endothelium was considered intact if a continuous monolayer of CD31-positive endothelial cells lined the lumen. The sIA wall structure was analyzed for remodeling changes by 3 blinded observers (Eliisa Ollikainen, Riikka Tulamo, and Juhana Frösen) and classified by the dominant wall type either as type A, B, C, or D (5). Type A represents a wall with intact endothelium and in which SMCs are linearly organized; type B represents a thickened wall with disorganized SMCs; type C represents a hypocellular wall with myointimal hyperplasia or organizing luminal thrombus; and type D represents an extremely thin hypocellular wall with organized luminal thrombus. Total numbers of CD68-, CD163-, and CD3-positive leukocytes were counted in the standard-sized areas of the sIA walls. The presence or absence and the localization of ORO-positive lipids (either intracellular or extracellular) and the staining pattern of lipid accumulation were analyzed. The ROI-positive area was measured and expressed as a percentage of the total surface area of an sIA wall section (ImageJ; NIH Software). For detection of neovascularization, the sections were stained for CD34 or CD31. Because CD31 is expressed also by leucocytes and platelets (32) and CD34 is predominantly a cell surface marker of hematopoietic multipotential progenitor cells (33), we defined neovessels only as CD31- or CD34-positive structures with capillarylike morphology (24). To obtain the distribution density of neovessels (number of neovessels per square millimeter), we counted the total number of CD34-positive neovessels in a section and then divided it by the whole surface area of the section, which had been measured with ImageJ. For identification of iron deposits, Prussian blue–positive signals were detected in serial sections. Samples that contained strong iron deposition with large deposits were analyzed separately from those that contained only weak positive staining.

To quantify MCs in sIAs, we stained 7 sections of each sIA sample for tryptase. To define the proportion of MCs positive also for chymase, we stained 4 additional sections for chymase. The distribution densities of MCs (MCs per square millimeter) in the sIA walls were calculated similarly to those of neovessels, and the total numbers of MCs were calculated similarly to those of other inflammatory cells, as described previously. We also counted activated (degranulated) MCs (i.e., those showing diffuse pericellular tryptase staining) and expressed their numbers as percentages of the total number of MCs (34, 35).

**Statistics**

Data analyses were performed using the IBM SPSS Statistics Software, version 21 (IBM Corp). For categorical variables, proportions were calculated and Fisher exact test was used. For continuous variables, median and range were calculated, and Mann-Whitney U test, Kruskal-Wallis multiple comparison test, and Spearman correlation test were used. The α level was set at 0.05.

**RESULTS**

**Basic Characteristics of sIAs**

Patients with unruptured (n = 16; 44%) or ruptured (n = 20; 56%) sIAs did not differ in age, sex, presence of previous SAHs, or dimensions of the sIA. As in our previous studies, the numbers of inflammatory cells and the degenerative changes by 3 blinded observers (Eliisa Ollikainen, Riikka Tulamo, and Juhana Frösen) and classified by the dominant wall type either as type A, B, C, or D (5). Type A represents a wall with intact endothelium and in which SMCs are linearly organized; type B represents a thickened wall with disorganized SMCs; type C represents a hypocellular wall with myointimal hyperplasia or organizing luminal thrombus; and type D represents an extremely thin hypocellular wall with organized luminal thrombus. Total numbers of CD68-, CD163-, and CD3-positive leukocytes were counted in the standard-sized areas of the sIA walls.

| Variable | Unruptured sIAs (n = 16) | Ruptured sIAs (n = 20) | p    |
|----------|--------------------------|------------------------|------|
| Patient  |                          |                        |      |
| Female, n (%)* | 11/16 (69) | 14/20 (70) | 1.000 |
| Age, median (range), years† | 55 (36–67) | 52 (24–87) | 0.962 |
| Patient with multiple (≥2) sIAs, n (%) | 10/16 (63) | 4/20 (20) | 0.016‡ |
| Patient with previous aneurysmal SAHs, n (%)* | 2/16 (13) | 0/20 (0) | 0.190 |

**Aneurysm**

| Variable | 3 photomicrographed standard-sized areas, each 0.613 mm² |
|----------|--------------------------------------------------------|
| Fundus width, median (range), mm† | 4.8 (3–13) | 4.8 (3–12) | 0.396 |
| Fundus length, median (range), mm‡ | 5.8 (2–18) | 7.2 (3–15) | 0.191 |
| Histology of sIA wall |                          |                        |      |
| Endothelium not intact, n (%)* | 9/16 (56) | 17/20 (85) | 0.073 |
| SMC disorganization present, n (%)* | 4/16 (25) | 12/20 (60) | 0.049‡ |
| Intraluminal fresh thrombosis present, n (%)* | 0/15 (0) | 10/18 (56) | 0.001‡ |
| Intraluminal organizing thrombosis present, n (%)* | 1/15 (7) | 10/18 (56) | 0.004‡ |
| Lipid present in the ECM, n (%)* | 14/16 (88) | 14/20 (70) | 0.257 |
| Neovessels present, n (%)* | 7/16 (44) | 8/20 (40) | 1.000 |
| Iron deposition present, n (%)* | 6/16 (38) | 3/20 (15) | 0.146 |
| MCs present, n (%)* | 6/16 (38) | 3/20 (15) | 0.146 |
| Total number of inflammatory cells |                          |                        |      |
| Tryptase-positive MCs, median (range)†§ | 0 (0–24) | 0 (0–32) | 0.262 |
| CD68-positive macrophages, median (range)†§ | 7 (0–228) | 67 (0–527) | 0.042‡ |
| CD163-positive macrophages, median (range)†§ | 33 (5–185) | 132 (28–443) | 0.005‡ |
| CD3-positive T lymphocytes, median (range)†§ | 8 (0–137) | 30 (2–357) | 0.058 |

* Fisher exact test.
† Mann-Whitney U test. Note that medians for MCs are 0 because the number of sIAs without MCs is higher than the number of sIAs with MCs.
‡ p ≤ 0.05 is considered significant.
§ In standard-sized areas of 0.613 mm².
and remodeling changes in the sIA wall were associated with sIA rupture (Table 2).

Among the samples, 9 of 36 (25%) were classified as type A (i.e. they displayed a wall with an intact endothelium [Fig. 1F] and linearly organized SMCs); 12 of 36 (33%) were classified as type B (i.e. they displayed a thickened wall with myointimal hyperplasia and disorganized SMCs); 11 of 36 (31%) were classified as type C (i.e. they displayed a hypocellular wall in addition to either myointimal hyperplasia or organizing luminal thrombus; Fig. 1); and 2 of 36 (6%) were classified as type D (i.e. they displayed an extremely thin thrombosis-lined hypocellular wall) (5). Owing to the small number of type D sIAs, we excluded this group from further analysis. In addition, 2 samples could not be analyzed for wall type because of an unfavorable orientation in the tissue block. The distribution of different wall types among unruptured and ruptured sIAs is shown in Table 3. Wall type was not associated with patients’ age, sex, smoking history, hypertension, presence of previous SAHs, sIA multiplicity, or sIA dimensions. Table 3 shows significant associations of CD68- and CD163-positive macrophages and CD3-positive T lymphocytes with different wall types.

Lipids in sIAs
Oil Red O–positive lipids (i.e. neutral lipids; cholesterol esters and triglycerides) were present in 33 of 36 (92%) sIAs, without showing any association with sIA rupture (Table 2). Among these 33 sIAs, 28 contained extracellular lipids in the ECM, and 5 contained only foam cells (i.e. intracellular lipids). The extracellular lipids displayed multiple patterns of accumulation: a lakelike pattern in 10 sIAs, a single strand in 6 sIAs, and multiple strands in 12 sIAs (Fig. 2). The sIA wall type was associated with the presence of lipids in the ECM. Oil Red O–positive lipids showed the smallest degree of accumulation in the thick-walled and highly cellularized type B sIAs (Table 3); instead, lipids had often accumulated in the hypocellular wall areas (Figs. 2E, F).

Neovascularization and IronDeposition in sIAs
The distribution density of neovessels was highest in the degenerated sIA wall type C (Table 3), but it did not show an association with sIA rupture (Table 2). Either CD31- or CD34-positive neovessels (Figs. 3H, 4A, D, G) were present in 15 of 36 sIAs (42%); 1 sIA contained only a single CD31-positive neovessel and was therefore excluded from further analysis. We also found a number of CD34-positive continuous structures without a capillarylike morphology, which were considered to represent early-stage neovascularization (36). Among the sIAs, 8 of 36 (22%) were completely negative for CD34.

Iron deposition was present in 9 of 36 sIAs (25%); among these, 6 contained strong iron deposition with large
iron deposits (Figs. 4C, F, I), and 3 contained only a few weakly stained iron deposits. Neovessels were present in all 6 slAs with strong iron deposition; this type of iron deposition colocalized with neovessels (Fig. 4) and was also associated with the distribution density of neovessels ($p < 0.001$). In addition, marked iron deposition was associated with infiltration of CD163- and CD68-positive macrophages ($p = 0.016$ and $p = 0.017$, respectively) and slA wall degeneration (Table 3). Iron deposition, whether strong or weak, was not associated with slA wall rupture (Table 2).

**MCs in slAs**

Tryptase-positive MCs were found in 9 of 36 (25%) slAs; 6 of these were also chymase-positive. Further analysis of MC-positive slAs is shown in Table 4. Among the MC-positive slAs, 3 were ruptured (Table 2). Mast cells were

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**FIGURE 2.** Different ORO lipid staining patterns in slAs and lipid accumulation in hypocellular slA wall areas. (A-E) Variable staining patterns of ORO-positive lipids (red) in 5 slAs as follows: cytoplasmic (arrowhead) ORO-positive lipids (A); lakelike accumulation (arrowheads) of ORO-positive lipids in the ECM (B); a single layer (between arrowheads) of ORO-positive lipids in the ECM adjacent to a thrombus (T) (C); 2 layers (between pointing arrowheads) of ORO-positive lipids in the ECM (D, E). (F) α-Smooth muscle actin staining (adjacent section of the sample shown in E) demonstrates a lack of αSMA-positive cells (brown; pointing arrowheads) in 2 layerlike areas corresponding to the ORO-positive layers in (E). (G) Negative control (Neg; irrelevant primary antibody, adjacent section of the sample shown in E and F). Thin arrows show the sample orientation of the slA wall from the adventitial side to the luminal side. Scale bars = (A) 10 μm; (B-G) 100 μm.
present only in type B and type C sIAs (Table 3). Owing to an unfavorable sample orientation, 1 MC-positive sIA remained unclassified for wall type. Mast cells were located in variable parts of the sIA wall. A thrombus was present in 2 MC-positive sIAs, but the thrombus itself did not contain MCs. The presence of MCs was not associated with the following clinical characteristics: age, sex, smoking history, hypertension, previous SAHs, or sIA size or multiplicity.

None of the 9 MC-positive sIAs had an intact endothelium (Table 4): 2 of 9 had some endothelial cells left, and the endothelium was totally absent in 7 of 9. In serial sections, the MC-positive areas also contained macrophages (CD68- and CD163-positive cells) and T lymphocytes (CD3-positive cells) (Figs. 3C–G), and the numbers of CD68-positive macrophages and T lymphocytes were significantly higher in MC-positive sIAs than in MC-negative sIAs (Table 4). Lipids were found in 8 of 9 MC-positive sIAs, and the degree of lipid accumulation was greater in MC-positive sIAs than in MC-negative sIAs (Table 4).

In all 9 MC-positive sIAs, neovessels were present and colocalized with MCs. Moreover, the distribution density of neovessels was higher in MC-positive sIAs than in MC-negative sIAs (Table 4). Marked iron deposition was present in most MC-positive sIAs (Table 4) and, importantly, it was associated with the distribution density of MCs (p = 0.001). Figure 4 provides a typical example of an sIA wall, in which the neovessels and iron colocalize with MCs.

**DISCUSSION**

We demonstrate that the presence of MCs in the human sIA wall is associated with histopathologic changes in the sIA, such as wall remodeling, lipid accumulation, and inflammatory cell infiltration. We also describe the appearance of MCs, particularly in wall areas with abundant neovascularization and iron deposition, indicative of microhemorrhages.

Mast cells are strong proangiogenic cells found in several vascular diseases (17, 18, 22–26). Indeed, MCs release various factors, such as vascular endothelial growth factor, basic fibroblast growth factor (30), heparin, histamine, tryptase, chymase, and tumor necrosis factor, and other cytokines with angiogenic potential (26, 28). Vascular endothelial growth factor, basic fibroblast growth factor, and their receptors are expressed in sIAs (37, 38), and the expression of the latter is associated with sIA wall remodeling and rupture (38). Interestingly, we found a strong correlation between MCs and neovascularization. Furthermore, neovascularization was associated with sIA wall remodeling and rupture (38). Taken together, our findings underline the significant angiogenic potential of the sIA wall.

Newly formed neovessels are leaky (40), and MC-derived histamine can increase such leakiness (14). Moreover, the proteases released by MCs and other leukocytes have the potential to trigger neovessel rupture with ensuing microhemorrhage (22, 28, 41). Once extravasated, erythrocytes become phagocytozed by tissue macrophages (42). Erythrocytes may also lyse

**FIGURE 3.** Mast cells, other inflammatory cells, and neovessels colocalize in an unruptured sIA wall. The panels show immunohistochemistry of inflammatory cells and neovessels (brown) in the same sIA wall area (rectangle in A). (A) A tryptase (Try)-stained unruptured sIA with a large luminal thrombus. W and T stand for wall and thrombus, respectively; these are separated by a dashed line. (B–H) Close-up images of different stainings performed in parallel sections of the sample as follows: negative control (Neg; irrelevant primary antibody; B), CD68-positive macrophages (C), Try-positive MCs (D, E; arrowheads), CD163-positive macrophages (F), CD3-positive T lymphocytes (G), and CD34-positive neovessels (H) (see also the double-lined close-up image and the endothelial cell indicated with an arrowhead). Thin arrows show the sample orientation of the sIA wall from the adventitial side to the luminal side. Scale bars = (A) 300 μm; (B–H) 50 μm; (high-power image of H) 10 μm.
and release hemoglobin, which, when complexed with haptoglobin, becomes phagocytozed particularly by CD163-positive macrophages (42–44). In each case, macrophages convert ingested hemoglobin into hemosiderin (e.g., as illustrated in Figs. 4H, K) (45). If not phagocytozed or if released by macrophages (46), iron, originated from hemoglobin, can form extracellular deposits and trigger local inflammation and oxidative stress (29, 42, 46). Oxidized LDL has been found in sIA walls, reflecting oxidative stress, (6, 8, 10). We found that the presence of marked iron deposition in sIAs was associated with the presence of both CD163 and CD68-positive macrophages, which may indicate a role for macrophages in the clearance of iron in sIAs. Moreover, as in the present and previous studies, the presence of CD163-positive macrophages is strongly associated with sIA wall remodeling and rupture (5–7). The presence of abundant iron deposition was detected particularly in areas in which numerous neovessels and MCs were also present. This implicates a role for MCs in triggering leakage and rupture of neovessels in the sIA wall. The iron deposits, which reflect microhemorrhages, were also associated with wall degeneration. Thus, iron may have a role in sIA wall remodeling, probably by enhancing inflammation and making the wall fragile.

Inflammation induces vascular wall remodeling (47) and is associated with sIA rupture (4–9). Here, we show a correlation between the infiltration of macrophages and T lymphocytes and sIA wall degeneration (wall type), which emphasizes the role of these inflammatory cells in walls that are more prone to rupture. Mast cells appeared in close proximity to infiltrates of other

FIGURE 4. Neovessels, MCs, and iron colocalize in a ruptured sIA wall. Adjacent sections of a ruptured sIA stained for CD34 (A; neovessels) (brown), tryptase (Try) (B; MCs) (brown), and iron (C) (blue). W and T stand for wall and thrombus, respectively, separated by a dashed line in each panel. (D–I) Close-up images show the intensely stained representative wall areas (referring to the rectangular areas shown in A–C). (J, K) Negative controls (irrelevant primary antibodies) are adjacent sections to those shown in (G) and (H). The iron-containing hemosiderin deposits are responsible for the yellowish-brown staining seen in negative controls. Notably, true positive staining (arrowheads in G and H) is absent in the corresponding negative controls (arrowheads in J and K). Thin arrows show the sample orientation of the sIA wall from the adventitial side to the luminal side. Scale bars = (A–C) 300 µm; (D–K) 50 µm.
TABLE 4. Association of Histologic Characteristics of sIAs With MCs

| Variable                                | Absent (n = 27) | Present (n = 9) | p      |
|-----------------------------------------|-----------------|----------------|--------|
| Histology of sIA wall                   |                 |                |        |
| Endothelium not intact, n (%)*          | 17/27 (63)      | 9/9 (100)      | 0.039† |
| Intraluminal fresh thrombosis present, n (%)* | 10/26 (38)     | 0/7 (0)        | 0.073  |
| Intraluminal organizing thrombosis present, n (%)* | 9/26 (35)      | 2/7 (29)       | 1.000  |
| Lipid present in the ECM, n (%)*        | 21/27 (78)      | 7/9 (78)       | 1.000  |
| Ratio of lipid-rich area, median (range), %† | 9 (0–37)        | 37 (0–69)      | 0.022† |
| Neovessels present, n (%)*              | 6/27 (22)       | 9/9 (100)      | <0.001† |
| Distribution density of neovessels, median (range), neovessels/mm²‡ | 0 (0–15)        | 2 (0–50)       | <0.001† |
| Iron deposition present, n (%)*         | 3/27 (11)       | 6/9 (67)       | 0.003† |
| Strong iron deposition present, n (%)*  | 1/27 (4)        | 5/9 (56)       | 0.002‡ |
| Total number of inflammatory cells      |                 |                |        |
| CD68-positive macrophages, median (range)§ | 15 (0–527)      | 124 (42–297)   | 0.012† |
| CD163-positive macrophages, median (range)§ | 72 (5–417)      | 158 (35–443)   | 0.110  |
| CD3-positive T lymphocytes, median (range)§ | 11 (0–357)     | 52 (25–137)    | 0.010† |
| Further analysis of MC-positive sIAs     |                 |                |        |
| Total number of MCs, median (range)¶    | 23 (3–170)      |                |        |
| Total number of degranulated MCs, median (range)¶ | 15 (2–110)     |                |        |
| Ratio of degranulated MCs to all MCs, median (range), %¶ | 67 (43–96)      |                |        |
| Distribution density of MCs, median (range), MCs/mm²¶ | 4 (1–69)        |                |        |
| Total number of MCs in standard-sized areas, median (range)§ | 7 (2–32)        |                |        |

* Fisher exact test.  
† p ≤ 0.05 is considered significant.  
‡ Mann-Whitney U test.  
§ In standard-sized areas of 0.613 mm².  
¶ In sample section area.

inflammatory cells, and their presence was associated with high numbers of CD68-positive macrophages and CD3-positive T lymphocytes, a finding in accordance with earlier observations on atherosclerotic lesions (13). Thus, MCs might regulate inflammatory reactions in the sIA wall together with other inflammatory cells. By releasing the proteases tryptase and chymase, MCs degrade pericellular matrices and thus may cause apoptotic death of SMCs and detachment of endothelial cells (13, 18). Mast cells also activate the matrix metalloproteinases secreted by tissue macrophages (13) and may release matrix metalloproteinase-9 on contact with activated T lymphocytes (48). Of note, we found a trend between the presence of MCs and sIA wall degeneration, a process characterized by SMC and endothelial cell death (6). Moreover, the secreted proteases released by activated MCs avidly degrade high-density lipoprotein, thereby preventing cholesterol efflux from foam cells (49). When cholesterol-filled foam cells ultimately die, their intracellular lipids are liberated into the extracellular space, where they appear as ORO-positive accumulation, which may become oxidized. We postulate that analogous mechanisms operate also in the sIA wall.

In conclusion, MCs may contribute to the pathogenesis of the sIA wall via induction of its remodeling, inflammation, and neovascularization. Particularly, the observed associations of MCs with erosion of luminal endothelium and microhemorrhages define endothelial cells as a potential target of MCs, whether they cover the luminal surface of the sIA wall or fragile neovessels. Thus, this work describes a novel potential mechanism to explain the connection between inflammation and sIA wall degeneration. Elucidation of the clinical significance of MCs in sIA wall remodeling is warranted.

ACKNOWLEDGMENTS

We are grateful to neuropathologist Anders Paetau, MD, PhD, at the Department of Pathology. We also thank Suvi Sokolnicki, Mari Jokinen, and Nancy Lim for excellent technical assistance.

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