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Role of inflammation (leukocyte-endothelial cell interactions) in vasospasm after subarachnoid hemorrhage

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Abstract

Background: Delayed vasospasm is the leading cause of morbidity and mortality after aneurysmal subarachnoid hemorrhage (aSAH). This phenomenon was first described more than 50 years ago, but only recently has the role of inflammation in this condition become better understood.

Methods: The literature was reviewed for studies on delayed vasospasm and inflammation.

Results: There is increasing evidence that inflammation and, more specifically, leukocyte-endothelial cell interactions play a critical role in the pathogenesis of vasospasm after aSAH, as well as in other conditions including meningitis and traumatic brain injury. Although earlier clinical observations and indirect experimental evidence suggested an association between inflammation and chronic vasospasm, recently direct molecular evidence demonstrates the central role of leukocyte-endothelial cell interactions in the development of chronic vasospasm. This evidence shows in both clinical and experimental studies that cell adhesion molecules (CAMs) are up-regulated in the perivasospasm period. Moreover, the use of monoclonal antibodies against these CAMs, as well as drugs that decrease the expression of CAMs, decreases vasospasm in experimental studies. It also appears that certain individuals are genetically predisposed to a severe inflammatory response after aSAH based on their haptoglobin genotype, which in turn predisposes them to develop clinically symptomatic vasospasm.

Conclusion: Based on this evidence, leukocyte-endothelial cell interactions appear to be the root cause of chronic vasospasm. This hypothesis predicts many surprising features of vasospasm and explains apparently unrelated phenomena observed in aSAH patients. Therapies aimed at preventing inflammation may prevent and/or reverse arterial narrowing in patients with aSAH and result in improved outcomes.

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Keywords: Endothelial cells; Haptoglobin; Hemoglobin; Inflammation; Leukocytes; Subarachnoid hemorrhage; Vasospasm

Abbreviations: aSAH, aneurysmal subarachnoid hemorrhage; CAM, cell adhesion molecule; CRP, C-reactive protein; CSF, cerebrospinal fluid; E-selectin, endothelial selectin; ELAM-1, endothelial leukocyte adhesion molecule; eNOS, endothelial nitric oxide synthase; ET, endothelin; GMP-140, granule membrane protein-140; Hp, haptoglobin; Hgb, hemoglobin; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; JNK, c-Jun N-terminal kinase; LAM-1, leukocyte adhesion molecule-1; LECAM, leukocyte/endothelial cell adhesion molecule; L-selectin, leukocyte selectin; LFA-1, lymphocyte function-associated antigen-1; LPS, lipopolysaccharide; Mac-1, macrophage antigen-1; MEL, murine erthroleukemia; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; NSAID, nonsteroid antiinflammatory drug; PADGEM, platelet activation dependent granule-external membrane; PARP, poly ADP-ribose polymerase; PECAM-1, platelet-endothelial cell adhesion molecule-1; P-selectin, platelet selectin; RBC, red blood cell; TCD, transcranial Doppler; TNFα, tumor necrosis factor-α; TQ, thymocyte-Q; WBC, white blood cell; VCAM-1, vascular cell adhesion molecule-1

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1. Introduction

Delayed or chronic vasospasm is the leading cause of morbidity and mortality after aneurysmal subarachnoid hemorrhage (aSAH) [104,105,228,234]. This phenomenon was first documented angiographically by Ecker and Riemenschneider [46] in 1951 and was subsequently correlated to the development of focal neurologic deficits by Fisher and colleagues [63] in 1977. Subsequent to these initial findings in patients with aSAH, cerebral vasospasm has also been observed in other conditions, including meningitis [18,59,177,197,257], traumatic brain injuries [139,140,214,237,265], and after craniotomies [10,51,258]. As a result, there has been an increased interest in understanding the molecular mechanisms responsible for the development of vasospasm.

Vasospasm after aSAH in humans is biphasic, with an acute and a chronic phase [251]. The acute phase typically occurs 3 to 4 hours after the hemorrhage and generally resolves rapidly, whereas the chronic phase typically occurs 3 to 14 days later [251]. This chronic or delayed phase is characterized by the sustained narrowing of the arteries, which can lead to permanent deficits and death in 20% to 40% of patients [76,104,105,228].

The chronic phase of vasospasm can be defined either angiographically or clinically. Angiographic vasospasm is arterial narrowing seen on angiography, whereas clinical vasospasm is the development of focal neurologic deficits presumably as a result of this arterial narrowing. After aSAH, patients can fall into 1 of 3 categories: (1) 33% develop both angiographic vasospasm and clinical signs of ischemia; (2) 43% develop angiographic vasospasm, but no clinical symptoms; and (3) the remaining 24% have neither angiographic nor clinical vasospasm [44]. The ability to predict which patients will develop clinical vasospasm, however, remains limited primarily because the pathophysiology of vasospasm remains unclear.

Inflammation and, more specifically, leukocyte-endothelial cell interactions appear to play a critical role in vasospasm (Table 1) [68]. In this review, we discuss the progress that has been made in understanding the role of inflammation in vasospasm. These advances may have potential implications for prospectively identifying and treating patients at greatest risk of developing chronic vasospasm.

2. Overview of the inflammatory hypothesis of vasospasm after SAH

Hemoglobin (Hgb), which is the iron-containing oxygen-transport protein present within red blood cells (RBCs), is the most abundant protein present in the blood [148]. This protein is extremely toxic when released from RBCs during hemorrhagic and/or hemolytic events, as well as after RBC senescence and breakdown [2,47,72,74,203,211,245]. The immune system prevents this toxicity by efficiently scavenging free Hgb molecules [5]. This is accomplished when macrophages bind to and clear this extracorpuscular Hgb [5]. This binding and clearance, however, relies on the identification of Hgb only when it is conjugated with the haptoglobin (Hp) protein [5]. Haptoglobin is a serum protein that binds to free Hgb with high affinity [5]. Of particular interest is that humans are the only species with 2 forms of haptoglobin (Hp 1 and Hp 2) and that individuals with the Hp 2-2 genotype develop particularly severe inflammatory reactions [70,143,252,253].

During SAH, blood spills into the subarachnoid space. This stimulates the rapid expression of specific cell adhesion molecules (CAMs) on the luminal surface of endothelial cells [68]. This allows macrophages and neutrophils to bind to the endothelial cells and enter the subarachnoid space, where they phagocytose extravasated RBCs and process Hgb via Hp-Hgb complexes [19]. Macrophages and neutrophils, however, remain trapped in the subarachnoid space due to the absence of lymphatics in the central nervous system and impaired cerebrospinal fluid (CSF) flow caused by the SAH [68]. These macrophages and neutrophils then start to die and degranulate 2 to 4 days after their entry into the subarachnoid space [28,41,62,84,160,193]. This results in the release of massive quantities of intracellular endothelins and oxygen free radicals, which results in inflammatory-induced arterial vasoconstriction [28,41,62,84,160,193]. Arterial narrowing, however, is only one manifestation of the inflammatory response to the RBCs in the subarachnoid space. The clinical deterioration of patients "in vasospasm" is because of the extensive inflammatory components leading to meningitis and cerebritis. This explains why other conditions such as bacterial meningitis are also associated with both the meningitic syndrome and arterial narrowing seen after aSAH [18].

3. Hematologic processes

3.1. Hemoglobin, haptoglobin, and the haptoglobin polymorphism in humans

Erythrocytes, or RBCs, are the most common type of blood cell [5]. Red blood cells are continuously produced in the bone marrow at a rate of approximately 2 million cells/s and remain viable for up to 120 days [5]. The major protein component of RBCs is Hgb [180]. Hemoglobin is a complex molecule containing heme groups with iron atoms that temporarily link to oxygen molecules [1]. Intracorpuscular Hgb is usually degraded by macrophages in the reticuloendothelial system, which phagocytoses senescent erythrocytes and degrades Hgb into bile and bilirubin [180]. Bilirubin is less toxic to the body than extracorpuscular Hgb [2,47,72,74,203,211,245].

Extracorpuscular Hgb is extremely toxic but is released into the bloodstream and interstitium during hemolytic and hemorrhagic conditions, respectively [2,47,72,74,203,211,245]. It is a proinflammatory stimulus that up-regulates the expression of endothelial and leukocyte adhesion molecules,

Table 1
Molecular and cellular components in delayed vasospasm

Molecule	Description	Role in vasospasm after aSAH
Erythrocytes	These cells are the most common type of blood cell. There are approximately 2-3 × 10^{13} circulating RBCs in an adult at any given.	These cells are released into the subarachnoid space after an aneurysmal rupture [9].
Hgb	Major protein present in RBCs, which functions to transport oxygen molecules by forming transient linkages with oxygen via iron atoms in its heme complex.	RBCs degrade in the subarachnoid space, releasing Hgb. Extracorpuscular Hgb is a potent proinflammatory stimulus, which also increases oxygen radical formation, decreases nitric oxide concentrations, and increases prostaglandin synthesis. Increases up-regulation of leukocyte and endothelial cell adhesion molecules thereby recruiting leukocytes to the site of hemorrhage [59,237,246].
Selectins		
E-selectin (CD62E, ELAM-1, LECAM-2)	Endothelial cell protein that recognizes and binds to sialyl Lewis^x on leukocytes.	Expression is up-regulated by the presence of inflammatory cytokines. Binding of E-selectin on endothelial cells to sialyl Lewis^x on leukocytes allows flowing leukocytes to roll along the endothelial surface, bringing them near the site of inflammation/hemorrhage. This allows the leukocytes to hone into the site of inflammation caused by the hemorrhage [219].
Sialyl Lewis^x	Leukocyte carbohydrate that is constitutively expressed on granulocytes and monocytes.	See above [122].
Integrins		
LFA-1 (CD11a/CD18) Mac-1 (complement receptor 3, CD11b/CD18)	Heterodimeric proteins on granulocytes and macrophages that recognize and bind to immunoglobulin superfamily proteins.	Expression is up-regulated in response to inflammatory cytokines. Binding of LFA-1 and Mac-1 to ICAM-1 allows rolling leukocytes to adhere to the endothelial surface, bringing them in close contact to chemoattractants. This allows the leukocytes to tether to the endothelial cell surface and undergo eventual transendothelial migration to the site of hemorrhage [38,39,224].
Immunoglobulin superfamily		
ICAM-1 (CD54)	Cell adhesion protein present on endothelial cells that recognizes and binds to leukocyte integrins. Expression is up-regulated in response to inflammatory cytokines.	See above [199,261].
Proinflammatory cytokines		
IL-1, IL-6, IL-8, TNFα	Proteins secreted by a variety of cell types including leukocytes and lymphocytes.	Secretion of these proteins increases the expression of adhesion molecules (selectins, integrins, immunoglobulin superfamily protein) on leukocytes and endothelial cells, enabling honing, recruitment, and transmigration of leukocytes to the site of inflammation/hemorrhage [4,67,80].
Leukocytes		
Granulocytes (neutrophils) Monocytes (macrophages)	Cells of the immune system that respond to inflammatory signals.	These cells are recruited to the site of hemorrhage, where they phagocytose RBCs and Hp-Hgb complexes. They become trapped in the extravascular spaces, where they die and degranulate, releasing oxygen radicals, endothelins, and proinflammatory mediators [81]. This results in the additional recruitment of more leukocytes.
Hp	Abundant serum protein produced by hepatocytes. Forms high affinity bonds with free Hgb. The Hp-Hgb complex is recognized and degraded by macrophages, neutralizing the toxic effects of free Hgb.	Individuals with the Hp 2-2 genotype will have more severe Hgb-induced inflammation and leukocyte recruitment as their Hp is less effective at neutralizing free Hgb [26,154,175].
NO	Chemical compound primarily produced by endothelial (eNOS) and nNOS in the intima and adventitia of the cerebral vessels, which acts to dilate arterial vessels in response to stress.	Hgb scavenges free NO, destroys nNOS-producing neurons, and causes eNOS dysfunction, which decreases the cerebral vessel-dilating capacity [58,59,78].
ETs		
ET-1	Proteins that are primarily produced and released by vascular endothelial cells and act as potent vasoconstrictors.	After an SAH, concentration levels of ETs are increased and may potentiate inflammation-induced vasoconstriction [119,166,253].

The major molecules that have been implicated in the pathogenesis of chronic vasospasm after aSAH are as follows: cluster of differentiation (CD), endothelial leukocyte adhesion molecule-1 (ELAM-1), ETs, Hp, Hgb, ICAM-1, LECAm-2, LFA-1, Mac-1, NO, and RBC.

thereby, recruiting leukocytes to the site of hemorrhage [203]. Free Hgb also decreases nitric oxide–related vasodilation [48] and stimulates the oxidation of arachidonic acid, leading to increased prostaglandin synthesis [212]. Moreover, the iron component of the heme complex increases free radical and lipid peroxide formation, which causes cell damage by peroxidation of cell membrane lipids [2,74,211]. The collective proinflammatory and vasoconstrictive effects of extracorpuscular Hgb appear to play a critical role in the pathogenesis of vasospasm [41,160]. In fact, the concentration and evolution of Hgb in the subarachnoid space parallels the increased risk and time course of vasospasm after aSAH [28,41,62,84,160,193]. Furthermore, in experimental models, Hgb alone causes prolonged contraction when applied to the cerebral arteries, and the use of Hgb antagonists reduces this contraction [41,160].

Haptoglobin is an abundant serum protein that is primarily produced by hepatocytes [88]. It functions to attenuate the toxic effects of free extracorpuscular Hgb by forming a stable high-affinity bond with the Hgb molecule [70,143,252,253]. This binding leads to the formation of a Hp-Hgb complex [143], which is then broken down by either parenchymal cells in the liver [110,111] or macrophages in the reticuloendothelial system [148]. The latter is the only mechanism that exists for removing free Hgb released at extravascular sites [120,148,170]. This mechanism involves the endocytosis of the Hp-Hgb complex by macrophages with the CD163 scavenger receptor [120]. This internalized complex is then degraded by lysosomal enzymes, and the heme group is ultimately converted into a less toxic bilirubin [120,148]. Bilirubin is transported by albumin to the liver, where it is conjugated and excreted in the bile [148].

Interestingly, humans—unlike other mammalian species—have 2 alleles for the Hp gene, which have been designated Hp 1 and Hp 2 [13,122,227]. Although all other mammalian species possess only the Hp 1 allele, the Hp 2 allele is unique to humans [122]. This allele is thought to have been created by an intragenic duplication of exons in the Hp 1 allele approximately 2 million years ago [13]. Therefore, the Hp genotype of a human individual may be Hp 1-1, Hp 2-1, or Hp 2-2 [13,227].

This polymorphism in the Hp allele has important structural and functional consequences. The Hp 1 protein product is univalent, whereas the Hp 2 protein product, as a result of the genetic duplication, is bivalent [252,253]. This causes the Hp protein to be a linear dimer in Hp 1-1 individuals, a linear polymer in Hp 2-1 individuals, but a cyclical polymer in Hp 2-2 individuals [129,252,253]. These structural distinctions confer different functional advantages. The Hp 1-1 protein, primarily because of it smaller size, can bind with and promote the clearance of Hgb molecules with greater efficiency than the larger cyclical Hp 2-2 protein [96]. In fact, the Hp 1-1 dimeric protein is noted to have superior antiinflammatory, immunomodulatory, antioxidant, and vasodilatory effects as compared to the Hp 2-2 cyclical protein in in vitro and animal studies [7,11,96,121,146]. In addition, the Hp 2-2 genotype is overrepresented in diseases with inflammatory components including rheumatoid arthritis [37], systemic lupus erythematosus [194], coronary artery disease [21,34,128], and diabetes [11,34,224]. The Hp 2 allele, however, has managed to spread in the human gene pool because the Hp 2-2 protein provides protection against infectious diseases including Streptococcus pyogenes–related illnesses and Plasmodium falciparum malaria, among others [8,117,122]. The Hp 2-2 protein, unlike the Hp 1-1 protein, has bacteriostatic properties that allows it to agglutinate the T4 antigen of Streptococcus pyogenes [117], as well as present intracellular Plasmodium falciparum antigens on the cell surface to increase host antibody acquisition [8]. Therefore, although the Hp 1-1 protein has increased antiinflammatory and antioxidant capabilities [7,11,96,121,146], the Hp 2-2 protein provides a selective advantage against certain infections with high prevalence and mortality worldwide [8,117,122]. The disadvantage of Hp 2-2, however, is that it has poor antiinflammatory properties or, phrased differently, Hp 2-2 is proinflammatory.

3.2. Acute and chronic components of the inflammatory response

The inflammatory response can be divided into an acute and a chronic component. These components are distinguished by their time course as well as their cell mediators. The acute component is a short-term process that has an immediate onset of action (within a few hours), with the primary effector cells being polymorphonuclear neutrophils, macrophages, and monocytes [219,220]. The chronic component, on the other hand, has a delayed onset of action (over days to weeks), with the primary effector cells being lymphocytes and plasma cells [60,165].

The key events in acute inflammation consist of both vascular and cellular phenomena [219,220]. The vascular events include relaxation of the terminal arterioles and contraction of the capillary endothelial cells in the inflammatory region [219,220]. The relaxation of the terminal arterioles increases blood flow to the site of inflammation, whereas contraction of the capillary endothelial cells increases vascular permeability [219,220]. Neutrophils, macrophages, and monocytes are the main effector cells of the acute inflammatory reaction. They pass through the dilated vascular beds, form transient attachments with the vascular walls, and roll along the surface of the endothelial cells [231]. These leukocytes then migrate between the endothelial cells to the site of inflammation [231]. Once in the extravascular space, these leukocytes phagocytose the foreign particle stimulating the inflammatory response and eventually degranulate, releasing enzymes and other toxic intermediates into the extravascular space [231]. Each of the cellular processes involved in the migration of leukocytes from the intravascular to the extravascular space requires specific interactions between different CAMs on the

leukocytes and endothelial cells [231]. These processes and CAMs are described in more detail in Section 5.4.

Chronic inflammation is caused by a persistent inflammatory stimulus [60,165]. This process is primarily mediated by lymphocytes and long-lived monocytes [60,165]. Lymphocytes invade the affected tissue, where T lymphocytes and B lymphocytes play a role in cell-mediated and humoral-mediated immunity, respectively [60,165]. Long-lived monocytes also invade the affected tissue and mature into macrophages, where they become tethered into place [60,165]. These lymphocytes and macrophages release cytokines (eg, interferon-γ), reactive oxygen species, and hydrolytic enzymes that maintain the inflammatory response [60,165]. This chronic component develops over days to weeks and can persist for many months or even years [60,165].

4. Inflammation-based diseases

Inflammation and leukocyte-endothelial cell interactions play a critical role in the pathophysiology of many diseases, in addition to vasospasm after aSAH. These conditions include asthma [95,256], ischemic stroke [90,91], and atherosclerosis [248] among others. Studying the immunobiology of these diseases has provided useful insights into the importance of inflammatory mechanisms in vasospasm, especially asthma.

An asthmatic attack, like vasospasm after aSAH, is biphasic. The acute phase is triggered by inhaled irritants that cause the release of histamine and leukotrienes from mast cells and usually resolves after a few hours [126]. The chronic phase develops 4 to 6 hours later and is mediated by recruitment of circulating inflammatory cells to the airways via CAMs [256].

Initial therapies focused on muscle relaxation with the use of bronchodilators and were only partially effective because asthma attacks often recurred [206]. The addition of antiinflammatory therapies aimed at inhibiting CAMs and subsequent neutrophil infiltration, however, led to improved outcomes with decreased recurrence rates [206,256]. This recruitment of circulating inflammatory cells via CAMs therefore appears to play a pivotal role in both asthma and chronic vasospasm [68].

5. Critical role of inflammation in chronic vasospasm

5.1. History of chronic vasospasm

Although angiographic evidence supporting the presence of vasospasm was first reported in 1951 [46], awareness of this phenomenon originated almost 100 years earlier. In 1859, William Gull [73] from England reported a case consistent with vasospasm. In this case, a female patient had an ischemic insult with acute neurologic deterioration [73]. Her neurologic symptoms gradually improved for the next 4 days; however, on the fifth day, her condition rapidly deteriorated, and she died [73]. An autopsy demonstrated a ruptured middle cerebral artery aneurysm, and the surrounding brain parenchyma had notable softening consistent with an ischemic event [73].

After this sentinel case, several experimental models in the early half of the 20th century provided evidence that the cerebral arteries were capable of becoming "spastic" [64,94,201,268]. Florey [64] in 1925 observed that feline cerebral arteries underwent local spasm when subjected to mechanical and electrical stimulation. Zucker [268] in 1944 reported that lysed RBCs could stimulate smooth muscle contraction in cerebral vessels. Ecker and Riemenschneider [46] later reported in 1951 the presence of angiographically confirmed cerebral vasospasm in 6 patients with aSAH. They found that the degree of spasm closely correlated with the size of the blood clot, and this process was self-limited because none of the patients had vasospasm 26 days after aSAH [46]. Later in 1977, Fisher and colleagues [63] correlated the development of vasospasm with the appearance of focal neurologic deficits. These findings were later corroborated by Takemae et al [236] in 1978.

Despite these findings, the pathophysiology of vasospasm remained poorly understood, which hindered the development of measures to successfully prevent and/or reverse vasospasm. Currently, the only treatments that are partially effective in blunting the ischemic injury caused by vasospasm are hypertensive-hypervolemic-hemodilutional ("triple H") therapy [50,127,198] and angioplasty [87,152]. Clinical trials with nicardipine [106] and clazosentan [243], however, have effectively prevented arterial narrowing but have not resulted in improved neurologic outcomes. Understanding the molecular mechanisms underlying cerebral vasospasm may allow identification of a new class of drugs with the ability to reduce its risk and severity after aSAH. Inflammation and, more specifically, leukocyte-endothelial cell interactions appear to play a critical role.

5.2. Early clinical evidence of inflammation in vasospasm

5.2.1. Elevated body temperature and vasospasm

The earliest clinical sign suggesting the role of inflammation in chronic vasospasm was elevated body temperature or fever, which is an indirect indicator of inflammation [83]. The association of fever with aSAH was first recognized in 1955 by Walton [246]. Walton [246] noted that patients who presented with aSAH and fever had worse outcomes than those of patients without elevated temperatures. However, it was not until 1980 that body temperature was correlated with the development of vasospasm [205]. Rousseaux and colleagues [205] studied the fever curves in patients with aSAH. They found that 88% of patients with aSAH and temperatures greater than 38°C developed angiographic evidence of vasospasm with delayed ischemic signs, whereas only 18% of patients without vasospasm exhibited the same fever curves [205]. The temperatures in these vasospasm patients typically peaked between 38°C and 39°C

by the fifth day after aSAH and lasted an average of 9 days [205], consistent with the time course of vasospasm [251].

This association between pyrexia and vasospasm has been confirmed in other studies [167,251]. Weir et al [250] found that 60% of patients with temperatures greater than 37.5°C 6 days after having an aSAH developed clinical vasospasm. This was double the rate for patients with lower body temperatures [250]. Oliveira-Filho and colleagues [167] reported similar findings and found that symptomatic vasospasm was independently associated with temperatures greater than 38.3°C in multivariate analysis. The presence of fever in this study was associated with poor outcomes [167].

5.2.2. Leukocytosis and vasospasm

An elevated white blood cell (WBC) count has also been associated with poor outcomes and an increased risk of symptomatic vasospasm [136,145,157,158,229,250]. The association between leukocytosis and poor outcomes was first reported by Neil-Dwyer and Cruikshank [157] in 1974, who found increased mortality rates for patients who had elevated WBC counts after aSAH. Maiuri and colleagues [136] reported similar results where leukocytosis was associated with a 90% rate of poor outcome, even in patients with good neurologic function on admission.

The correlation between leukocytosis and vasospasm, however, was not made until the late 1980s [229]. Spallone et al [229] found that increased WBC count was linked to the development of ischemia after aSAH. Niikawa et al [158] later demonstrated that the rise in WBC count followed the time course of chronic vasospasm development. Finally, McGirt et al [145] performed a multivariate analysis and found that peak leukocyte counts were independently associated with the development of cerebral vasospasm, where patients with leukocyte counts greater than 15×10^9 cells/L had a 3-fold increased risk of vasospasm.

5.2.3. Other markers of inflammation and vasospasm

"Immune complexes," or antibody-epitope combinations, are seen in patients with clinical evidence of vasospasm after aSAH [169,172,173]. Pellettieri et al [172] were the first to report that patients with SAH and angiographic and/or clinical vasospasm had significantly higher levels of circulating immune complexes (52%) as compared to patients with SAH and no evidence of vasospasm (9%). This same group later observed that high immune complex levels preceded the onset of vasospasm, whereas low levels preceded clinical improvement [173].

Complement proteins are activated under inflammatory conditions [38] and are also present in higher levels in patients with vasospasm [107,169]. Ostergaard et al [169] showed that patients with vasospasm had a 2-fold increase in plasma C3d levels at the time when spasm occurred as compared to aneurysm patients without vasospasm [107]. Kasuya and Shimizu [107] also found that other activated complement proteins including C3a and C4a were higher in the CSF of patients who developed delayed ischemic neurologic deficits as compared to patients without deficits. Mack et al [134] have shown that elevated levels of complement C3a (the critical component of the alternative complement pathway) in patients with aSAH correlate with poor outcomes.

Not surprisingly, increased levels of C-reactive protein (CRP) have also been seen in patients with vasospasm [204]. C-reactive protein is an acute phase protein that is a sensitive indicator of systemic inflammation [174]. In a recent study, Rothoerl et al [204] found that CRP levels were not only significantly higher in patients who developed symptomatic vasospasm but also correlated with poor 1-year neurologic outcomes.

5.2.4. Histopathologic changes with vasospasm

Histopathologic examination of the spastic cerebral arteries also supports an inflammatory role underlying vasospasm [35,89,92,208,222]. Crompton [35] was the first to document the association of inflammation with aSAH in 1964. After examining the cerebral arteries in patients who died after aSAH, he found intense collections of mononuclear leukocytes below the endothelium of the arteries in closest proximity to the aneurysm [35]. Hughes and Schianchi [92] in 1978 later demonstrated the presence of macrophages in the tunica media and adventitia of the vessels formerly shown to be in vasospasm on angiography. Furthermore, Hoshi et al [89] and Ryba et al [208] independently used immunofluorescence to show the presence of immunologic and inflammatory proteins including IgM and C3 in the endothelium of spastic arteries in aSAH patients [89,208].

5.3. Early experimental evidence of inflammation in vasospasm

5.3.1. Inducing vasospasm with inflammatory agents

Although early clinical evidence suggested that inflammation plays a role in the development of vasospasm [136,145,157,158,167,172,173,205,229,246,250], a definitive causative link between inflammation and vasospasm had been difficult to elucidate. It has been argued that fever, leukocytosis, immune complexes, and arterial histologic changes were merely secondary phenomena in vasospasm after aSAH. To address this question, several groups have induced vasospasm with inflammatory agents in the absence of blood products and other processes associated with SAH [149,155,175,195,260]. Peterson and colleagues [175] injected latex and dextran beads into the cistern magna of dogs. These foreign bodies led to 35% to 40% vasoconstriction on angiography, which was significantly greater than cell-free plasma injections [175]. Moreover, this spasm was correlated to the infiltration of lymphocytes, neutrophils, and macrophages into the subarachnoid space [175]. Yanamoto et al [260] found the same results after injecting polystyrene latex beads in rabbits. These beads caused a dose-dependent effect on arterial narrowing after cisternal injection [260]. Mori et al [149] and Nagata et al [155]

reported similar results after the injection of talc (crystallized hydrous magnesium sulfate) into the cisterna magna of dogs. The introduction of this foreign material induced a delayed and prolonged constriction of the basilar artery in the absence of immediate changes [149,155]. Histologically, this arterial constriction was also correlated with leukocyte infiltration into the adventitia of the basilar artery, as well as marked constrictive and degenerative changes including myonecrosis and subintimal proliferation [149,155]. These pathologic changes were closely analogous to the changes observed in human autopsy cases with chronic cerebral vasospasm [149,155].

More recently, we evaluated the effect of lipopolysaccharide (LPS) on the development of chronic vasospasm in the absence of blood products in the subarachnoid space in rabbits [195]. Lipopolysaccharide is a purely inflammatory protein that is present on the outer membranes of gram-negative bacteria [199]. We found that the controlled release of LPS into the subarachnoid space of rabbits produced chronic vasospasm in a dose-dependent manner analogous to that induced by SAH in this model [195]. This study further supports a primary role of inflammation in the development of chronic vasospasm. The key feature of all these studies is that chronic vasospasm was elicited in the absence of RBCs or Hgb in the subarachnoid space.

5.3.2. Inhibiting vasospasm with immunosuppressive and/or antiinflammatory agents

It has been hypothesized that if inflammation plays a critical role in the development of vasospasm, then the use of immunosuppressive and/or antiinflammatory agents should attenuate the development of vasospasm. This hypothesis has been tested in several animal [24,26,27,78,150,154,161,176,255] and human clinical studies [25,81,138,207,209] with mixed results. These studies evaluated the efficacy of various agents including corticosteroids [24-27,81], cyclosporine [78,138,175,207,209], tacrolimus (FK-506) [150,154,161], and nonsteroidal antiinflammatory drugs (NSAIDs) [26,255].

Corticosteroids are a class of steroid hormones with potent immunosuppressive and antiinflammatory effects [188]. They function by suppressing cytokine production, inhibiting leukocyte function, and decreasing prostaglandin synthesis [188]. Corticosteroids tend to suppress chronic inflammation better than acute inflammation and thus have a more pronounced effect on lymphocytes instead of macrophages/neutrophils [52]. Chyatte and colleagues [26,27] evaluated the effects of high-dose methylprednisolone (15 mg/kg every 8 hours) on vasospasm in the double-hemorrhage canine model. They found that the use of high-dose steroids decreased the extent of vascular contraction and structural derangements in the walls of the cerebral vessels as compared to placebo and even low-dose steroids [26,27]. They also found that the administration of high-dose steroids, as compared to low-dose steroids, effectively suppressed prostaglandin E2 synthesis after experimental hemorrhage [24].

Corticosteroids have also shown promising results in human clinical studies [25,81]. Chyatte and colleagues [25] treated 21 patients judged to be at high risk of vasospasm based on poor neurologic grade on admission and/or a large amount of subarachnoid blood on computed tomographic scans. The treated patients had improved neurologic outcomes, decreased mortality rates, and reduced incidences of delayed cerebral ischemia as compared to matched controls [25]. Hashi and colleagues [81] evaluated the effects of corticosteroids after the onset of vasospasm in a multicenter study. Patients treated with hydrocortisone, regardless of neurologic grade on admission, demonstrated improved 1-month neurologic findings including mental status, speech, and motor function [81].

Cyclosporine is an immunosuppressant that inhibits the transcription of interleukin-2 (IL-2), causing effector T-cell dysfunction [192]. It is commonly used in patients with allogeneic organ transplants to prevent host-vs-graft response [192]. Its efficacy in preventing vasospasm has been evaluated in both experimental and clinical studies with mixed results [79,138,176,207,209,210]. Peterson et al [176] evaluated the combinatorial effects of cyclosporine and steroids in dogs after experimental SAH. In the group that received combined therapy, there was less arterial narrowing as compared to the nontreated group [176]. Handa et al [79] found similar results, where the average reduction in vessel caliber was significantly less in the cyclosporine-treated group than in the nontreated group. However, they found no significant difference between the 2 groups in the amount of IgG deposition and inflammatory reaction in the spastic arterial wall [79]. Nagata et al [154], on the other hand, failed to demonstrate any difference in vessel caliber between the cyclosporine-treated and non-treated groups in dogs.

Clinical studies with cyclosporine have failed to demonstrate a therapeutic effect [138,207,209]. Manno et al [138] found that the use of cyclosporine, despite its safe pharmacologic profile, failed to prevent the development of chronic vasospasm in patients with Fisher grade 3 SAH. Ryba et al [207,209], however, found that the use of cyclosporine only in combination with nimodipine significantly improved neurologic outcome in patients who underwent early aneurysmal clipping (<72 hours) after an SAH.

Tacrolimus (FK-106) is another immunosuppressive agent that has a similar mechanism of action to cyclosporine [112]. It binds to different protein targets but ultimately inhibits the transcription of IL-2 leading to decreased effector T-cell function [112]. This drug should therefore have similar results to cyclosporine; however, experimental models have failed to demonstrate any therapeutic efficacy [150,154,161]. These studies have not demonstrated any significant difference in vessel caliber and pathologic changes between the tacrolimus-treated and control groups after experimental SAH in dogs [150,154,161]. As a result of these experimental findings, clinical trials evaluating the efficacy of tacrolimus have not been conducted.

Nonsteroidal antiinflammatory drugs have antiinflammatory, antipyretic, and analgesic effects [71]. These drugs function by nonselectively inhibiting cyclooxygenase, thereby, reducing prostaglandin synthesis [71]. In addition, certain NSAIDs such as ibuprofen inhibit intercellular adhesion molecule-1 (ICAM-1; CD54) expression and thus prevent leukocyte migration into the periadventitial space [101,189,238]. White and Robertson [255] found that, as a group, intravenously administered NSAIDs significantly reduced the occurrence of vasospasm in a canine model of SAH. The effects of piroxicam were found to be more effective than other NSAIDs, including meclofenamate, ibuprofen, and aspirin, presumably due to its longer half-life [255]. However, the NSAIDs in this study were administered 30 minutes before and 3 hours after intracisternal injection of blood, which would limit its use in clinical settings [255].

The effects of systemic complement depletion have also been studied in both experimental [69,260,261] and clinical settings [99,262]. In both types of studies, the use of various agents that decrease serum levels of activated complement proteins have resulted in decreased incidences of vasospasm [69,99,260-262]. German and colleagues [69] studied the effects of cobra venom factor, which consumes complement proteins. Pretreatment with cobra venom factor 24 hours before experimental SAH reduced the extent of arterial narrowing in rabbits [69]. Yanamoto and colleagues [260,262] studied the effects of nafamostat mesilate, which is a serine protease inhibitor that prevents activation of the complement system. The use of this drug reduced angiographic arterial narrowing in both a hemorrhagic [262] and latex bead model of vasospasm in rabbits [260]. Moreover, the use of this drug alone [262] and in combination with a thromboxane synthetase inhibitor [99] reduced the incidence of vasospasm in small clinical trials.

5.4. Recent scientific developments that have led to an understanding of the molecular mechanisms underlying vasospasm

The early clinical and experimental studies reviewed above provided only indirect evidence that inflammation is a critical component in the pathogenesis of vasospasm. The clinical studies demonstrated that inflammatory markers including pyrexia [167,205,246,250], leukocytosis [136,145,157,158,229,250], and circulating immune complexes [169,172,173] were more commonly present in patients who developed chronic vasospasm. The experimental studies provided evidence that vasospasm could be induced with proinflammatory agents in the absence of blood products [149,151,155,175,195,260] and that the use of general immunosuppressants and antiinflammatory agents could decrease the incidence of vasospasm [24-27,69,78,81,99,138,176,207,209,210,260-262]. However, the molecular mechanisms underlying the development of vasospasm after aSAH remained poorly understood. Insights into this process were gained through several discoveries, which included the technique for creating monoclonal antibodies, the characterization of CAMs, and the molecular 3-step hypothesis of leukocyte-endothelial cell interactions in inflammation [231,232].

5.4.1. Advent of molecular immunology

The advent of molecular immunology has led to an improved understanding of the direct role of leukocyte-endothelial cell interactions in chronic vasospasm. Before the discovery by Kohler and Milstein [116] of the hybridoma technique for generating monoclonal antibodies, the scientific community relied on polyclonal antibodies obtained from the serum of immunized animals to study protein interactions [164]. These polyclonal antibodies would bind to several epitopes on the same antigen, making the study of a specific ligand virtually impossible [164]. However, Kohler and Milstein [116] in 1975 discovered how to produce monoclonal antibodies from hybridomas, which were formed by fusing malignant myeloma cells with antibody-producing B cells. The myeloma cells were clonogenic with the ability to reproduce indefinitely, whereas the selected B cells produced a specific antibody [116]. By fusing these 2 cell types, Kohler and Milstein [116] were able to obtain cells selected for both immortality and specific antibody production. This discovery, for which they won the Nobel Prize in 1984, has allowed the purification, identification, and tracing of a vast variety of ligands, including those potentially involved in the development of vasospasm [116].

5.4.2. Recognition of CAMs

The use of monoclonal antibodies led to the recognition of CAMs, which was another pivotal discovery that had implications for understanding leukocyte-endothelial cell interactions. Early evidence of the importance of cell adhesion was derived from patients who were genetically deficient in leukocyte integrins [4]. These patients with leukocyte adhesion deficiencies failed to develop erythema, swelling, and pus after local bacterial infections [4]. This was due to an inability of their neutrophils to bind to and cross the endothelium and subsequently accumulate at inflammatory sites [4].

The 3 classes of CAMs that mediate leukocyte-endothelial cell interactions are selectins, integrins, and immunoglobulin superfamily proteins. The cadherin family of CAMs is important in embryology and oncogenesis but less relevant in inflammation [235]. Selectins are a class of proteins expressed by a variety of cells including leukocytes, endothelial cells, and platelets [125,130]. The different types of selectins include leukocyte (L)-selectin (CD62L, leukocyte adhesion molecule-1 [LAM-1], leukocyte/endothelial cell adhesion molecule-1 [LECAM-1], Leu-8, murine erthroleukemia-14 [MEL-14], and thymocyte-Q [TQ-1]), endothelial (E)-selectin (CD62E, endothelial leukocyte adhesion molecule-1 [ELAM-1], and LECAM-2), and platelet (P)-selectin (CD62P, granule membrane protein-

140 [GMP-140] and platelet activation dependent granule-external membrane protein [PADGEM]) [125,130]. These proteins bind to sialylated carbohydrates and are necessary for leukocyte rolling, whereby labile adhesions occur between the flowing leukocyte and endothelial cells [124,202]. This process is critical for leukocyte homing and recruitment [124,202].

Integrins on leukocytes recognize and form strong adhesions to immunoglobulin superfamily proteins on endothelial cells [39,230]. Integrins are unique among CAMs in that they are made up of 2 subunits. The most commonly studied leukocyte integrins include lymphocyte function-associated antigen-1 (LFA-1, CD11a/CD18) and macrophage antigen-1 (Mac-1, complement receptor 3, CD11b/CD18) [39,123,132]. The immunoglobulin superfamily proteins include ICAM-1 (CD54), vascular-cell adhesion molecule 1 (VCAM-1, CD106), and platelet-endothelial cell adhesion molecule-1 (PECAM-1, CD31) [40]. The strong adhesions that occur between these 2 types of CAMs are necessary for leukocyte extravasation and emigration [39,230].

5.4.3. Understanding leukocyte migration

Recognition of the differential expression and location of CAMs provided insight into the multistep process required for leukocyte extravasation and emigration. This multistep process has essentially been simplified into 3 critical molecular events as follows: selectin-mediated rolling, chemokine-triggered activation, and integrin-dependent arrest [231]. Under this paradigm, sialylated carbohydrates on leukocytes interacts with endothelial selectins, which creates the initial tethering of leukocytes to the vessel walls and causes them to roll along the endothelium [100]. This tethering brings the leukocytes near chemoattractants released from the local tissue [17]. The chemoattractants then bind receptors on the leukocyte surface, leading to the activation of integrins [17]. The integrins can then bind to members of the immunoglobulin superfamily expressed on the endothelium [17]. This increases leukocyte adhesiveness and results in the arrest of rolling leukocytes [20]. The leukocytes then undergo transendothelial migration or diapedesis, whereby intravascular leukocytes enter extravascular tissue and migrate to sites of inflammation [216].

5.5. Current molecular evidence of inflammation in vasospasm

These advances in molecular immunology have been vital to understanding the molecular mechanisms underlying chronic vasospasm. The discovery of monoclonal antibodies has allowed identification of the ligands that are present after aSAH in both clinical and experimental studies. These ligands include selectins, integrins, and immunoglobulin superfamily proteins. These findings provide direct evidence that inflammation and, more specifically, leukocyte-endothelial cell interactions play a central role in the development of vasospasm.

5.5.1. Selectins in chronic vasospasm

The expression of selectins allows leukocytes to form labile adhesions with endothelial cells, which is critical for leukocyte homing and recruitment [124,202]. The presence of selectins is therefore a direct indicator of leukocyte-endothelial cell interactions and thus inflammation [124,202].

Clinical studies on selectin adhesion molecules in aSAH have not been consistent. Polin et al [186] performed a prospective case-control study with aSAH and non-aSAH control patients. The levels of E-selectin were found to be elevated in patients after SAH as compared to control patients and were highest in patients who developed moderate or severe vasospasm [186]. Nissen et al [162,163] also conducted a prospective study comparing serum concentrations of E-, P-, and L-selectins between patients who did and did not develop delayed cerebral ischemia after grade 1 or 2 SAH. Serum P-selectins were higher in patients who developed ischemia, whereas serum L-selectins were higher in patients who did not develop ischemia [162,163]. There was, however, no significant difference in serum E-selectin levels between the 2 cohorts [162,163].

It has been postulated that if selectins are critical to the development of vasospasm, then selectin inhibition should decrease the severity of vasospasm. Lin et al [131] tested this hypothesis with a monoclonal anti–E-selectin antibody in a murine SAH model. The use of this monoclonal antibody improved lumen patency from 78% to 91% and decreased peripheral WBC counts when compared to SAH controls [131].

5.5.2. Integrins in chronic vasospasm

Integrins, namely LFA-1 and Mac-1, are another regulator of leukocyte adhesion and migration [39,230]. We have evaluated the effects of monoclonal antibodies against LFA-1 and Mac-1 in rat [30], rabbit [191], and monkey vasospasm/SAH models [29]. In the rat femoral artery model, we systemically administered anti–LFA-1 monoclonal antibody [30]. This antibody significantly decreased the extent of femoral artery spasm and the amount of granulocyte/macrophage infiltration as compared to the that in the control group that received isotype-matched antibodies [30]. In the rabbit SAH model, we showed that systemic administration of anti-CD11/CD18 monoclonal antibodies against both LFA-1 and Mac-1 increased basilar lumen patency by 25% as compared to placebo controls [191]. These results in the rabbit model have also been supported by Bavbek et al [9] using an intracisternally administered monoclonal antibody. Moreover, we showed that the use of this antibody prevented experimental cerebral vasospasm in nonhuman primates, despite the unaltered presence of Hgb in the subarachnoid space [29].

Statins provide another avenue to study the effects of integrins. This class of drugs exerts pleiotropic effects beyond lipid lowering [53]. These actions include up-regulation of endothelial nitric oxide synthase (eNOS) and

antiinflammatory activity [53,98,144,249]. This antiinflammatory activity includes the inhibition of leukocyte integrins including LFA-1 and Mac-1 [53,98,144,249]. This inhibition prevents leukocytes from adhering to endothelial cells, hindering leukocyte emigration [53,98,144,249]. Moreover, statins also reduce the expression of proinflammatory cytokines (tumor necrosis factor-α, IL-1, and IL-16) [6,115]. The use of statins in preventing vasospasm in clinical studies after aSAH, however, has shown mixed results [119,133,171,239,240].

5.5.3. Immunoglobulin superfamily proteins in chronic vasospasm

Leukocyte integrins bind to immunoglobulin superfamily proteins on endothelial cells [39,123,132]. The immunoglobulin superfamily proteins are necessary for leukocyte-endothelial cell adhesion and subsequently leukocyte emigration [39,123,132]. The presence of immunoglobulin superfamily proteins, namely ICAM-1, is therefore an indicator of inflammation [39,123,132].

The immunoglobulin superfamily proteins are up-regulated in patients who develop clinical vasospasm. Polin et al [186] found that the levels of ICAM-1 and VCAM-1 were elevated in the CSF of aSAH patients as compared to non-aSAH patients. Connolly and colleagues later correlated this rise in serum ICAM-1 levels with poor neurologic outcomes after aSAH [135]. In a follow-up study, this same group demonstrated that an increase in serum ICAM-1 levels during the perivasospasm period was associated with the development of angiographic vasospasm [147]. Kaynar et al [108] and Cheong et al [23] have reported similar results.

Animal models of SAH have also demonstrated the presence of ICAM-1 up-regulation [3,80,226]. We have shown that ICAM-1 immunoreactivity was only extensively increased in the blood-exposed vessels beginning 3 hours after clot placement and persisted for 24 hours, which corresponds to the time of peak vasospasm in the rat femoral artery model [226]. Handa et al [80] found that ICAM-1 was up-regulated in the endothelial and medial layers of the basilar artery after experimental SAH in rats. These same findings have also been reproduced in a canine model of SAH [3].

The ability of anti–ICAM-1 monoclonal antibodies to decrease vasospasm provides direct evidence that leukocyte-endothelial cell interactions play a critical role in vasospasm. In fact, we have shown that the use of anti–ICAM-1 monoclonal antibodies decreases the extent of vasospasm and inflammatory cell infiltrates as compared to isotype-matched controls in a rat femoral artery model [168]. In a follow-up study, we demonstrated that this antibody has a similar effect in preventing chronic vasospasm and inflammatory cell infiltrates as an anti–LFA-1 monoclonal antibody [30]. In addition, Bavbek et al [9] showed that the use of both of these monoclonal antibodies (anti–ICAM-1 and anti–LFA-1) have an additive effect on inhibiting vasospasm in a rabbit model. These studies support the role that both immunoglobulin superfamily proteins and integrins are required for leukocyte-endothelial cell interaction and subsequent vasospasm development [9,30,168].

The use of drugs such as statins and ibuprofen, which decrease immunoglobulin superfamily up-regulation, also decreases vasospasm in experimental studies. Statins, in addition to decreasing leukocyte integrin function, may also reduce immunoglobulin superfamily expression. In fact, several clinical studies have shown that statin use decreases serum ICAM-1 levels in hypercholesterolemic patients [6,22,43,115,196] and may contribute to the beneficial effects of statins in reducing the incidence of vasospasm in clinical trials [119,133,171,239,240]. Ibuprofen is another antiinflammatory agent that inhibits ICAM-1 and VCAM-1 up-regulation [86,101]. We have demonstrated that the use of controlled-release polymers containing ibuprofen significantly inhibited vasospasm when administered at 0 and 6 hours after blood exposure in a rat femoral artery model [238]. Moreover, this correlates with a decrease in the number of monocytes and macrophages in the periadventitial space [238]. We have confirmed these results in both a rabbit [65] and monkey SAH model [189]. Furthermore, Chyatte et al [26] demonstrated that ibuprofen prevented ultrastructural changes from occurring in the cerebral vessel walls after blood injection in dogs. The limitation of ibuprofen, however, is that it is only effective at preventing vasospasm when given within 6 hours of hemorrhage [65,189,238]. This time limitation would hinder its efficacy in clinical trials.

The experiments described above in which monoclonal antibodies against integrins and immunoglobulin superfamily CAMs prevent vasospasm provide the most convincing experimental evidence of the critical role of leukocytes in producing chronic vasospasm. In the rabbit and primate SAH models, despite the presence of RBCs and Hgb in the subarachnoid space, treatment with monoclonal antibodies against CAMs prevents leukocyte migration and abolishes vasospasm. This shows that in the absence of leukocytes, and despite the presence of RBCs and Hgb in the subarachnoid space, chronic vasospasm does not occur.

5.5.4. Molecular detection of other proinflammatory proteins

The advent of molecular immunology has allowed the detection of other proinflammatory proteins in patients with aSAH and vasospasm besides CAMs. These proteins include cytokines, c-Jun N-terminal kinase (JNK), and poly (ADP-ribose) polymerase (PARP). The presence of these proteins further supports the inflammatory etiology underlying posthemorrhagic vasospasm.

Cytokines are signaling proteins secreted by a variety of cell types including leukocytes and lymphocytes [200]. These proteins play a role in both innate and adaptive immunity [200]. As with other CAMs, the up-regulation of cytokines is an indicator of inflammation [200]. The main proinflammatory cytokines that have been demonstrated to be elevated in patients with vasospasm include IL-1, IL-6,

IL-8, and TNFα [3,55,67,82,156,217,247]. Drugs that inhibit cytokine production have been shown to attenuate vasospasm in animal models [14,15].

c-Jun N-terminal kinase is a mitogen-activated kinase that is involved in several physiologic and pathologic functions including inflammation [137]. Yatsushige et al [263] found that this pathway is activated after intracisternal injection of autologous blood in dogs. In addition, the use of a JNK inhibitor attenuated angiographic vasospasm, improved neurologic function, and reduced the number of infiltrated leukocytes and IL-6 production after blood injection [263].

Poly (ADP-ribose) polymerase is a nuclear enzyme that modulates the expression of CAMs and neutrophil recruitment during several physiologic and pathologic processes including inflammation [36]. Satoh et el [215] demonstrated that PARP activation was present in the smooth muscles and adventitia of blood-exposed vessels in a rabbit model of SAH and that use of a PARP inhibitor attenuated the degree of arterial narrowing. The attenuating effects of this inhibitor have also been observed in a canine model of SAH [264].

6. Other potential contributors to the development of chronic vasospasm

6.1. Nitric oxide depletion

Nitric oxide (NO), or endothelium-derived relaxing factor, is produced primarily by endothelial (eNOS) and neuronal nitric oxide synthase (nNOS) in the intima and adventitia of the cerebral vessels, respectively [182]. This molecule acts to dilate the arteries in response to shear stress and/or metabolic demands [182]. However, after a hemorrhagic event, free Hgb disrupts many components of NO-mediated vasodilation. Hemoglobin scavenges free NO, destroys nNOS-producing neurons, and causes eNOS dysfunction [47].

The role of NO, such as inflammation, has been a target of interest in many clinical and experimental studies. Nitrites, which are a major source of endogenous NO [32,254], have been shown to be decreased in the CSF of patients who develop vasospasm after aSAH [181,184]. We have also shown that intracranial delivery of controlled released polymers containing NO prevented the development of vasospasm in both a rat and rabbit SAH model [66,190]. In fact, delayed implantation of the polymers at either 24 or 48 hours after hemorrhage also prevented the development of vasospasm [190]. Furthermore, the use of regional NO delivery via direct intracarotid or intracerebral arterial infusion [103,185], local NO delivery via intrathecal or intraventricular injection [49], systemic NO delivery [49], and systemic infusion of nitrites decreased the extent and/or incidence of vasospasm in both experimental and clinical studies [184]. The clinical use of repleting NO or repairing NO dysfunction has yet to be elucidated [182].

6.2. Endothelins

Endothelins (ETs) are potent vasoconstrictors that are expressed by a variety of cell types, although the most common source is vascular endothelial cells [93,213]. Endothelins have been implicated in a variety of diseases including hypertension, pulmonary hypertension, acute renal failure, cardiac hypertrophy, and chronic heart failure, among others [54,225]. Clinical and animal studies on ETs have had conflicting findings. Several clinical studies have demonstrated that the rises in serum and CSF levels of ET-1 correspond to the time course of vasospasm [141,218]. Other clinical studies, however, have failed to demonstrate a significant change in ET levels during vasospasm [77]. Likewise, intracisternal administration of ET-1 in some animal studies resulted in arterial narrowing consistent with vasospasm [223,266], whereas other animal studies failed to demonstrate a significant change [85,159,183]. Furthermore, the use of monoclonal antibodies against ET-1 [259] and ET receptors [31,267], as well as ET activation enzyme inhibitors [142], attenuated vasospasm in some [31,267] but not all studies [33]. Additional studies are therefore needed to clarify the role of ET in chronic vasospasm.

7. Vasospasm in the absence of aSAH

There is increasing evidence that cerebral vasospasm is not unique to aSAH and that it occurs in other conditions affecting the central nervous system. These conditions include infectious meningitis [18,59,177,197,257], traumatic brain injuries [139,140,265], and craniotomies [10,51,258], among others. The development of vasospasm in these other conditions can be predicted and explained by the inflammatory hypothesis.

There has been an increasing number of reports documenting the occurrence of vasospasm in patients with meningitis [18,59,177,197,257]. We have shown that the occurrence of vasospasm after meningitis closely resembles the time course present in vasospasm after aSAH [18]. Others have reported that the development of vasospasm in patients with meningitis is associated with poor outcomes [197]. Cerebral vasospasm after meningitis, as with hemorrhage-associated vasospasm, can also be explained by the inflammatory hypothesis. Bacterial meningitis is associated with the entry of leukocytes into the subarachnoid space and subsequent cytokine release [45,56,57,153,221,244]. This release of cytokines, through intracellular signaling events, augments the inflammatory response [57,179]. This includes the up-regulation of CAMs [57,179], which play a critical role in vasospasm after aSAH.

Vasospasm also occurs in 25% to 40% of head trauma patients [139]. It is seen in both penetrating [118] and nonpenetrating [140] head injuries. Posttraumatic vasospasm resembles aSAH-associated vasospasm [118,265]. In fact, the incidence of vasospasm after traumatic brain injury is similar to that after aSAH [166]. Moreover, the vasospasm is also biphasic with an acute and chronic phase [265], and the

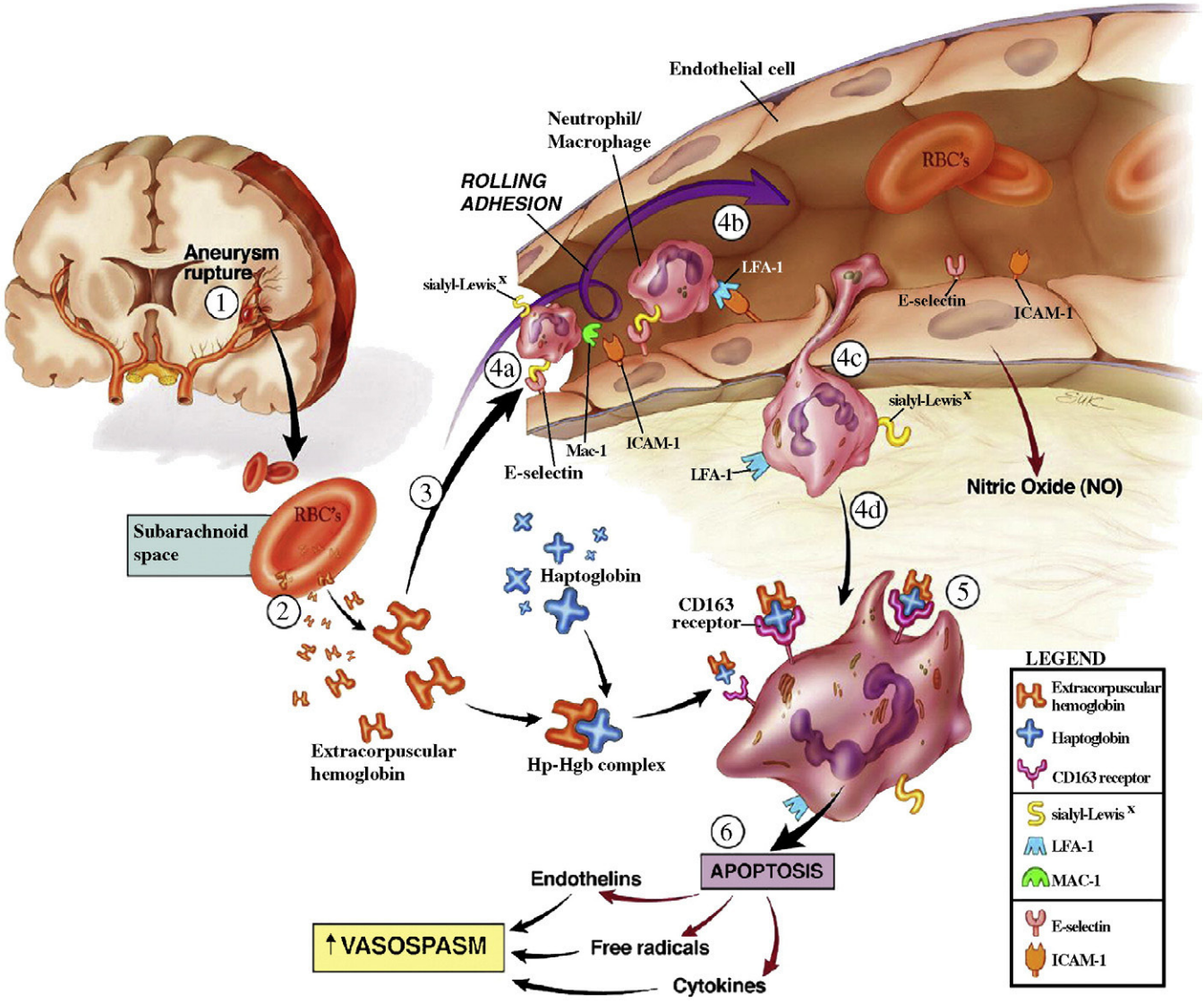

Fig. 1. Molecular events leading to the development of chronic vasospasm after aSAH. 1: The rupture of a cerebral artery aneurysm introduces blood into the subarachnoid space. 2: These RBCs degrade and release Hgb. 3: Extracorpuscular Hgb released from the subarachnoid RBCs induces the expression of CAMs on leukocytes and endothelial cells. 4A: Sialylated carbohydrates on flowing leukocytes (granulocytes and neutrophils) form transient linkages with selectins on endothelial cells (E-selectins), which allows the leukocytes to roll along the endothelial surface. 4B: Leukocyte integrins (LFA-1 and Mac-1) then form strong bonds with endothelial immunoglobulin superfamily proteins (ICAM-1), tethering the leukocytes to the endothelial cells. 4C: The leukocytes travel across the endothelial cells into the subarachnoid space. 4D: The leukocytes migrate within the subarachnoid space, where they phagocytose free Hgb molecules. 5: Haptoglobin (Hp) typically binds to extracorpuscular Hgb, forming an Hp-Hgb complex. This complex is recognized and phagocytosed via the CD163 scavenger receptor on macrophages, which degrade Hgb into a less toxic bilirubin. Individuals with the Hp 2-2 genotype have Hp 2-2 proteins that bind with less affinity than Hp 1-1 protein. 6: The leukocytes die within a few days, releasing proinflammatory cytokines, oxygen radicals, and ETs. These effects increase leukocyte migration, deplete NO-related vasodilation, and compound inflammation-induced arterial contraction and chronic vasospasm.

time course mirrors aSAH-associated vasospasm [118]. In addition to posttraumatic vasospasm, there have been case reports of vasospasm occurring in patients undergoing craniotomy for nonvascular reasons [10,51,258]. Although the clinical and basic science studies on posttraumatic and postcraniotomy vasospasm are limited, the process appears to be initiated by a traumatic and/or hemorrhagic event, which leads to the development of inflammation and subsequently vasospasm.

8. Future implications on the treatment of patients with vasospasm

8.1. Current treatment limitations

Several different physiologic and pharmacologic treatment strategies have been used to prevent or relieve chronic vasospasm after aSAH [18,50,58,233]. Mainstay current treatment is hypertensive-hypervolemic-hemodilutional ("triple H") therapy [109], where the physiologic basis is

to increase blood flow to areas vulnerable to ischemia [97]. However, prophylactic hypertensive-hypervolemic-hemodilutional therapy in large prospective controlled trials has failed to reduce the incidence of clinical and/or angiographic vasospasm, improve cerebral blood flow, or improve outcomes, and additionally is associated with increased costs and complications [50,198]. Other physiologic treatments including transluminal balloon angioplasty [87,152], lumbar drainage of CSF [113,114], and the use of intracisternal thrombolysis [61] have also been associated with minimal benefits and increased complications. Likewise, the use of pharmacologic treatments including nimodipine, a calcium channel blocker [58,178]; tirilazad mesylate, a nonglucocorticoid free radical scavenger [75,102]; and intraarterial vasodilators [187,242] have had these same limitations. Clinical trials with nicardipine [106] and clazosentan [243] have unquestionably and effectively prevented arterial narrowing but have not resulted in improved outcomes.

8.2. Prospective identification of patients at risk for developing vasospasm after aSAH

Currently, the ability to prospectively determine which patients with aSAH will develop vasospasm is extremely limited. Prospective identification of patients at greater risk for developing vasospasm may allow for selective early administration of potentially harmful therapies. It may also lead to novel therapeutic approaches aimed at reducing the incidence and severity of vasospasm after aSAH.

Interestingly, we have found that the Hp genotype may serve as a molecular marker for predicting which patients will develop clinical vasospasm after aSAH [19]. We genetically engineered mice to express the Hp 2-2 genotype, which is only present in humans [13,122,227]. Mice with the Hp 2-2 genotype, as compared to wild-type Hp 1-1 mice, developed more severe arterial narrowing and became clinically symptomatic after experimental SAH [19]. Moreover, this vasospasm was correlated with a greater concentration of neutrophils and macrophages in the subarachnoid space, which supports the inflammatory hypothesis [19]. Of note, the distribution of patients who have symptomatic vasospasm (30%), angiographic vasospasm without symptoms (50%), and no angiographic or symptomatic vasospasm (20%) in the western world approximates the distribution of these Hp genotypes in this same area [44]. In western populations, 36% of individuals are Hp 2-2, 48% are Hp 2-1, and 16% are Hp 1-1 [13]. In addition, a recent clinical study found that patients with the Hp 2 allele (Hp 2-2 and Hp 2-1) had an increased incidence of TCD-vasospasm as compared to Hp 1-1 patients (87% vs 22%) [12]. This study, however, was limited by its small patient numbers (n = 32), its failure to elucidate the risk of patients homozygous for the Hp 2 allele (Hp 2-2), and its use of TCD, which is not a specific indicator of vasospasm [16,42,241]. Prospective clinical studies are therefore necessary to clarify the role the Hp genotype plays in the development of vasospasm.

8.3. Possible molecular mechanisms underlying the development of vasospasm

A growing body of evidence supports the central role of inflammation in the development of vasospasm. Inflammation and, more specifically, leukocyte-endothelial cell interactions explain the development of vasospasm in experimental and clinical studies. In fact, it explains the pathogenesis of vasospasm across diverse pathologic conditions including aSAH, traumatic SAH, traumatic non-SAH brain injuries, and bacterial meningitis.

On the basis of current evidence for the inflammatory mechanisms underlying vasospasm, we have formulated a hypothesis to explain the association between these various pathologic conditions and chronic vasospasm. In this hypothesis, the presence of erythrocytes in the subarachnoid space causes the up-regulation of CAMs, namely ICAM-1, on cerebral endothelial cells. Up-regulation of ICAM-1 or similar molecules is also caused by the presence of bacteria in patients with bacterial meningitis and of subarachnoid erythrocytes in patients with aSAH or traumatic SAH. The up-regulation of ICAM-1 allows the leukocytes with LFA-1 or Mac-1 on their surface to bind to the endothelial cells and undergo transendothelial cell migration into the periadventitial space. These subarachnoid leukocytes phagocytose erythrocytes in SAH and bacteria in bacterial meningitis. The leukocytes, which are trapped in the subarachnoid space, die and degranulate after 2 to 4 days, which corresponds to the time course and onset of chronic vasospasm (Fig. 1). The death of the leukocytes results in the release of ET and oxygen free radicals, as well as the consumption of NO. These molecular mechanisms are strongly and moderately augmented in Hp 2-2 and 2-1 patients, respectively. These events lead to clinical vasospasm in Hp 2-2 patients, angiographic vasospasm in Hp 2-1 patients, and no signs of vasospasm in Hp 1-1 patients. Obviously, future studies are needed, to test this hypothesis.

9. Conclusion

Delayed or chronic vasospasm is the leading cause of morbidity and mortality after aSAH. It is a phenomenon that has been described and studied for decades, but the pathophysiology remains unclear. It is poorly understood why some patients develop clinical vasospasm after aSAH, whereas others remain unaffected. A growing body of evidence supports the role of inflammation and, more specifically, leukocyte-endothelial cell interactions in the development of vasospasm. Further understanding of this inflammation-based disease is required to formulate effective treatment strategies for preventing or reversing the development of this devastating condition.

References

[1] Agarwal N, Gordeuk RV, Prchal JT. Genetic mechanisms underlying regulation of hemoglobin mass. Adv Exp Med Biol 2007;618: 195-210.
[2] Agil A, Fuller CJ, Jialal I. Susceptibility of plasma to ferrous iron/ hydrogen peroxide-mediated oxidation: demonstration of a possible Fenton reaction. Clin Chem 1995;41:220-5.
[3] Aihara Y, Kasuya H, Onda H, et al. Quantitative analysis of gene expressions related to inflammation in canine spastic artery after subarachnoid hemorrhage. Stroke 2001;32:212-7.
[4] Anderson DC, Springer TA. Leukocyte adhesion deficiency: an inherited defect in the Mac-1, LFA-1, and p150,95 glycoproteins. Annu Rev Med 1987;38:175-94.
[5] Ascenzi P, Bocedi A, Visca P, et al. Hemoglobin and heme scavenging. IUBMB Life 2005;57:749-59.
[6] Ascer E, Bertolami MC, Venturinelli ML, et al. Atorvastatin reduces proinflammatory markers in hypercholesterolemic patients. Atherosclerosis 2004;177:161-6.
[7] Asleh R, Guetta J, Kalet-Litman S, et al. Haptoglobin genotype- and diabetes-dependent differences in iron-mediated oxidative stress in vitro and in vivo. Circ Res 2005;96:435-41.
[8] Atkinson SH, Mwangi TW, Uyoga SM, et al. The haptoglobin 2-2 genotype is associated with a reduced incidence of Plasmodium falciparum malaria in children on the coast of Kenya. Clin Infect Dis 2007;44:802-9.
[9] Bavbek M, Polin R, Kwan AL, et al. Monoclonal antibodies against ICAM-1 and CD18 attenuate cerebral vasospasm after experimental subarachnoid hemorrhage in rabbits. Stroke 1998;29:1930-5 [discussion 1935-1936].
[10] Bejjani GK, Duong DH, Kalamarides M, et al. Cerebral vasospasm after tumor resection. A case report. Neurochirurgie 1997;43:164-8.
[11] Blum S, Asaf R, Guetta J, et al. Haptoglobin genotype determines myocardial infarct size in diabetic mice. J Am Coll Cardiol 2007;49: 82-7.
[12] Borsody M, Burke A, Coplin W, et al. Haptoglobin and the development of cerebral artery vasospasm after subarachnoid hemorrhage. Neurology 2006;66:634-40.
[13] Bowman BH, Kurosky A. Haptoglobin: the evolutionary product of duplication, unequal crossing over, and point mutation. Adv Hum Genet 1982;12:189-261, 453-184.
[14] Bowman G, Bonneau RH, Chinchilli VM, et al. A novel inhibitor of inflammatory cytokine production (CNI-1493) reduces rodent post-hemorrhagic vasospasm. Neurocrit Care 2006;5:222-9.
[15] Bowman G, Dixit S, Bonneau RH, et al. Neutralizing antibody against interleukin-6 attenuates posthemorrhagic vasospasm in the rat femoral artery model. Neurosurgery 2004;54:719-25 [discussion 725-716].
[16] Bruder N, Cohen B, Pellissier D, et al. The effect of hemodilution on cerebral blood flow velocity in anesthetized patients. Anesth Analg 1998;86:320-4.
[17] Campbell JJ, Qin S, Bacon KB, et al. Biology of chemokine and classical chemoattractant receptors: differential requirements for adhesion-triggering versus chemotactic responses in lymphoid cells. J Cell Biol 1996;134:255-66.
[18] Chaichana K, Riley 3rd LH, Tamargo RJ. Delayed cerebral vasospasm secondary to bacterial meningitis after lumbosacral spinal surgery: case report. Neurosurgery 2007;60:E206-7 discussion E207.
[19] Chaichana KL, Levy AP, Miller-Lotan R, et al. Haptoglobin 2-2 genotype determines chronic vasospasm after experimental subarachnoid hemorrhage. Stroke 2007;38:3266-71.
[20] Chan JR, Hyduk SJ, Cybulsky MI. Chemoattractants induce a rapid and transient up-regulation of monocyte alpha4 integrin affinity for vascular cell adhesion molecule 1 which mediates arrest: an early step in the process of emigration. J Exp Med 2001; 193:1149-58.
[21] Chapelle JP, Albert A, Smeets JP, et al. Effect of the haptoglobin phenotype on the size of a myocardial infarct. N Engl J Med 1982; 307:457-63.
[22] Chello M, Carassiti M, Agro F, et al. Simvastatin blunts the increase of circulating adhesion molecules after coronary artery bypass surgery with cardiopulmonary bypass. J Cardiothorac Vasc Anesth 2004;18:605-9.
[23] Cheong JH, Kim JM, Bak KH, et al. Correlation between cerebral vasospasm after subarachnoid hemorrhage and intracellular adhesion molecule-1 levels in serum and cerebrospinal fluid. J Korean Med Sci 2005;38:1–11.
[24] Chyatte D. Prevention of chronic cerebral vasospasm in dogs with ibuprofen and high-dose methylprednisolone. Stroke 1989;20:1021-6.
[25] Chyatte D, Fode NC, Nichols DA, et al. Preliminary report: effects of high dose methylprednisolone on delayed cerebral ischemia in patients at high risk for vasospasm after aneurysmal subarachnoid hemorrhage. Neurosurgery 1987;21:157-60.
[26] Chyatte D, Rusch N, Sundt Jr TM. Prevention of chronic experimental cerebral vasospasm with ibuprofen and high-dose methylprednisolone. J Neurosurg 1983;59:925-32.
[27] Chyatte D, Sundt Jr TM. Response of chronic experimental cerebral vasospasm to methylprednisolone and dexamethasone. J Neurosurg 1984;60:923-6.
[28] Claassen J, Bernardini GL, Kreiter K, et al. Effect of cisternal and ventricular blood on risk of delayed cerebral ischemia after subarachnoid hemorrhage: the Fisher scale revisited. Stroke 2001; 32:2012-20.
[29] Clatterbuck RE, Gailloud P, Ogata L, et al. Prevention of cerebral vasospasm by a humanized anti-CD11/CD18 monoclonal antibody administered after experimental subarachnoid hemorrhage in nonhuman primates. J Neurosurg 2003;99:376-82.
[30] Clatterbuck RE, Oshiro EM, Hoffman PA, et al. Inhibition of vasospasm with lymphocyte function-associated antigen-1 monoclonal antibody in a femoral artery model in rats. J Neurosurg 2002;97: 676-82.
[31] Clozel M, Breu V, Burri K, et al. Pathophysiological role of endothelin revealed by the first orally active endothelin receptor antagonist. Nature 1993;365:759-61.
[32] Cosby K, Partovi KS, Crawford JH, et al. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. Nat Med 2003;9:1498-505.
[33] Cosentino F, McMahon EG, Carter JS, et al. Effect of endothelinA-receptor antagonist BQ-123 and phosphoramidon on cerebral vasospasm. J Cardiovasc Pharmacol 1993;22(Suppl 8):S332-5.
[34] Costacou T, Ferrell RE, Orchard TJ. Haptoglobin genotype: a determinant of cardiovascular complication risk in type 1 diabetes. Diabetes 2008;57:1702-6.
[35] Crompton MR. The pathogenesis of cerebral infarction following the rupture of cerebral berry aneurysms. Brain 1964;87:491-510.
[36] Cuzzocrea S. Shock, inflammation and PARP. Pharmacol Res 2005; 52:72-82.
[37] Dahlqvist SR, Frohlander N. Haptoglobin groups and rheumatoid arthritis. Hum Hered 1985;35:207-11.
[38] Datta PK, Rappaport J. HIV and complement: hijacking an immune defense. Biomed Pharmacother 2006;60:561-8.
[39] de Fougerolles AR, Stacker SA, Schwarting R, et al. Characterization of ICAM-2 and evidence for a third counter-receptor for LFA-1. J Exp Med 1991;174:253-67.
[40] Diamond MS, Staunton DE, Marlin SD, et al. Binding of the integrin Mac-1 (CD11b/CD18) to the third immunoglobulin-like domain of ICAM-1 (CD54) and its regulation by glycosylation. Cell 1991;65: 961-71.
[41] Dietrich HH, Dacey Jr RG. Molecular keys to the problems of cerebral vasospasm. Neurosurgery 2000;46:517-30.
[42] Doering TJ, Brix J, Schneider B, et al. Cerebral hemodynamics and cerebral metabolism during cold and warm stress. Am J Phys Med Rehabil 1996;75:408-15.

[43] Doo YC, Han SJ, Han SW, et al. Effect of preexisting statin use on expression of C-reactive protein, adhesion molecules, interleukin-6, and antioxidized low-density lipoprotein antibody in patients with unstable angina undergoing coronary stenting. Clin Cardiol 2005;28: 72-6.
[44] Dorsch NW. Cerebral arterial spasm—a clinical review. Br J Neurosurg 1995;9:403-12.
[45] Dulkerian SJ, Kilpatrick L, Costarino Jr AT, et al. Cytokine elevations in infants with bacterial and aseptic meningitis. J Pediatr 1995;126:872-6.
[46] Ecker A, Riemenschneider PA. Arteriographic demonstration of spasm of the intracranial arteries, with special reference to saccular arterial aneurysms. J Neurosurg 1951;8:660-7.
[47] Edwards DH, Byrne JV, Griffith TM. The effect of chronic subarachnoid hemorrhage on basal endothelium-derived relaxing factor activity in intrathecal cerebral arteries. J Neurosurg 1992;76: 830-7.
[48] Edwards DH, Griffith TM, Ryley HC, et al. Haptoglobin-haemoglobin complex in human plasma inhibits endothelium dependent relaxation: evidence that endothelium derived relaxing factor acts as a local autocoid. Cardiovasc Res 1986;20:549-56.
[49] Egemen N, Turker RK, Sanlidilek U, et al. The effect of intrathecal sodium nitroprusside on severe chronic vasospasm. Neurol Res 1993; 15:310-5.
[50] Egge A, Waterloo K, Sjoholm H, et al. Prophylactic hyperdynamic postoperative fluid therapy after aneurysmal subarachnoid hemorrhage: a clinical, prospective, randomized, controlled study. Neurosurgery 2001;49:593-605 [discussion 605-596].
[51] el Hendawy M, Wronski J, Juniewicz H, et al. Cerebral vasospasm detection by TCD after supratentorial brain tumours surgery. Neurol Neurochir Pol 2000;34:114-23.
[52] Elenkov IJ. Glucocorticoids and the Th1/Th2 balance. Ann N Y Acad Sci 2004;1024:138-46.
[53] Endo A. The discovery and development of HMG-CoA reductase inhibitors. J Lipid Res 1992;33:1569-82.
[54] Fahmy Elnoamany M, Abdelraouf Dawood A. Right ventricular myocardial isovolumic relaxation time as novel method for evaluation of pulmonary hypertension: correlation with endothelin-1 levels. J Am Soc Echocardiogr 2007;20:462-9.
[55] Fassbender K, Hodapp B, Rossol S, et al. Inflammatory cytokines in subarachnoid haemorrhage: association with abnormal blood flow velocities in basal cerebral arteries. J Neurol Neurosurg Psychiatry 2001;70:534-7.
[56] Fassbender K, Ries S, Schminke U, et al. Inflammatory cytokines in CSF in bacterial meningitis: association with altered blood flow velocities in basal cerebral arteries. J Neurol Neurosurg Psychiatry 1996;61:57-61.
[57] Fassbender K, Schminke U, Ries S, et al. Endothelial-derived adhesion molecules in bacterial meningitis: association to cytokine release and intrathecal leukocyte-recruitment. J Neuroimmunol 1997.
[58] Feigin VL, Rinkel GJ, Algra A, et al. Calcium antagonists in patients with aneurysmal subarachnoid hemorrhage: a systematic review. Neurology 1998;50:876-83.
[59] Ferris EJ, Rudikoff JC, Shapiro JH. Cerebral angiography of bacterial infection. Radiology 1968;90:727-34.
[60] Filer A, Raza K, Salmon M, et al. Targeting stromal cells in chronic inflammation. Discov Med 2007;7:20-6.
[61] Findlay JM, Kassell NF, Weir BK, et al. A randomized trial of intraoperative, intracisternal tissue plasminogen activator for the prevention of vasospasm. Neurosurgery 1995;37:168-76 [discussion 177-168].
[62] Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1-9.
[63] Fisher CM, Roberson GH, Ojemann RG. Cerebral vasospasm with ruptured saccular aneurysm–—the clinical manifestations. Neurosurgery 1977;1:245-8.
[64] Florey H. Microscopical observations on the circulation of blood in the cerebral cortex. Brain 1925;48:43-64.
[65] Frazier JL, Pradilla G, Wang PP, et al. Inhibition of cerebral vasospasm by intracranial delivery of ibuprofen from a controlled-release polymer in a rabbit model of subarachnoid hemorrhage. J Neurosurg 2004;101:93-8.
[66] Gabikian P, Clatterbuck RE, Eberhart CG, et al. Prevention of experimental cerebral vasospasm by intracranial delivery of a nitric oxide donor from a controlled-release polymer: toxicity and efficacy studies in rabbits and rats. Stroke 2002;33:2681-6.
[67] Gaetani P, Tartara F, Pignatti P, et al. Cisternal CSF levels of cytokines after subarachnoid hemorrhage. Neurol Res 1998;20: 337-42.
[68] Gallia GL, Tamargo RJ. Leukocyte-endothelial cell interactions in chronic vasospasm after subarachnoid hemorrhage. Neurol Res 2006; 28:750-8.
[69] German JW, Gross CE, Giclas P, et al. Systemic complement depletion inhibits experimental cerebral vasospasm. Neurosurgery 1996;39:141-5 [discussion 145-146].
[70] Giblett ER. The haptoglobins of human serum. J Forensic Sci 1963;8: 446-52.
[71] Green GA. Understanding NSAIDs: from aspirin to COX-2. Clin Cornerstone 2001;3:50-60.
[72] Griffith TM, Edwards DH, Lewis MJ, et al. The nature of endothelium-derived vascular relaxant factor. Nature 1984;308: 645-7.
[73] Gull WM. Cases of aneurism of the cerebral vessels. Guys Hosp Rep 1859;5:281-304.
[74] Gutteridge JM. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 1995;41:1819-28.
[75] Haley Jr EC, Kassell NF, Apperson-Hansen C, et al. A randomized, double-blind, vehicle-controlled trial of tirilazad mesylate in patients with aneurysmal subarachnoid hemorrhage: a cooperative study in North America. J Neurosurg 1997;86:467-74.
[76] Haley Jr EC, Kassell NF, Torner JC, et al. A randomized trial of two doses of nicardipine in aneurysmal subarachnoid hemorrhage. A report of the Cooperative Aneurysm Study. J Neurosurg 1994;80: 788-96.
[77] Hamann G, Isenberg E, Strittmatter M, et al. Absence of elevation of big endothelin in subarachnoid hemorrhage. Stroke 1993;24:383-6.
[78] Handa Y, Hayashi M, Takeuchi H, et al. Effect of cyclosporine on the development of cerebral vasospasm in a primate model. Neurosurgery 1991;28:380-5 [discussion 385-386].
[79] Handa Y, Kabuto M, Kobayashi H, et al. The correlation between immunological reaction in the arterial wall and the time course of the development of cerebral vasospasm in a primate model. Neurosurgery 1991;28:542-9.
[80] Handa Y, Kubota T, Kaneko M, et al. Expression of intercellular adhesion molecule 1 (ICAM-1) on the cerebral artery following subarachnoid haemorrhage in rats. Acta Neurochir (Wien) 1995;132: 92-7.
[81] Hashi K, Takakura K, Sano K, et al. Intravenous hydrocortisone in large doses in the treatment of delayed ischemic neurological deficits following subarachnoid hemorrhage–results of a multi-center controlled double-blind clinical study. No To Shinkei 1988;40: 373-82.
[82] Hendryk S, Jarzab B, Josko J. Increase of the IL-1 beta and IL-6 levels in CSF in patients with vasospasm following aneurysmal SAH. Neuro Endocrinol Lett 2004;25:141-7.
[83] Henker R, Carlson KK. Fever: applying research to bedside practice. AACN Adv Crit Care 2007;18:76-87.
[84] Hijdra A, van Gijn J, Nagelkerke NJ, et al. Prediction of delayed cerebral ischemia, rebleeding, and outcome after aneurysmal subarachnoid hemorrhage. Stroke 1988;19:1250-6.
[85] Hino A, Tokuyama Y, Kobayashi M, et al. Increased expression of endothelin B receptor mRNA following subarachnoid hemorrhage in monkeys. J Cereb Blood Flow Metab 1996;16:688-97.

[86] Hofbauer R, Frass M, Gmeiner B, et al. Rapid, fluorescence-based assay for microtiter plates to test drug influences on neutrophil transmigration through endothelial cell monolayers. Life Sci 1999;65: 2453-61.
[87] Hoh BL, Ogilvy CS. Endovascular treatment of cerebral vasospasm: transluminal balloon angioplasty, intra-arterial papaverine, and intra-arterial nicardipine. Neurosurg Clin N Am 2005;16:501-516, vi.
[88] Hooper DC, Steer CJ, Dinarello CA, et al. Haptoglobin and albumin synthesis in isolated rat hepatocytes. Response to potential mediators of the acute-phase reaction. Biochim Biophys Acta 1981; 653:118-29.
[89] Hoshi T, Shimizu T, Kito K, et al. Immunological study of late cerebral vasospasm in subarachnoid hemorrhage. Detection of immunoglobulins, C3, and fibrinogen in cerebral arterial walls by immunofluorescence method. Neurol Med Chir (Tokyo) 1984;24: 647-54.
[90] Huang J, Choudhri TF, Winfree CJ, et al. Postischemic cerebrovascular E-selectin expression mediates tissue injury in murine stroke. Stroke 2000;31:3047-53.
[91] Huang J, Upadhyay UM, Tamargo RJ. Inflammation in stroke and focal cerebral ischemia. Surg Neurol 2006;66:232-45.
[92] Hughes JT, Schianchi PM. Cerebral artery spasm. A histological study at necropsy of the blood vessels in cases of subarachnoid hemorrhage. J Neurosurg 1978;48:515-25.
[93] Inoue A, Yanagisawa M, Kimura S, et al. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc Natl Acad Sci U S A 1989;86: 2863-7.
[94] Jackson IJ. Aseptic hemogenic meningitis. An experimental study of aseptic meningeal reactions due to blood and its breakdown products. Arch Neurol Psychiatry 1949;62:572-89.
[95] Jaffar Z, Ferrini ME, Buford MC, et al. Prostaglandin I2-IP signaling blocks allergic pulmonary inflammation by preventing recruitment of $CD4^+$ Th2 cells into the airways in a mouse model of asthma. J Immunol 2007;179:6193-203.
[96] Javid J. The effect of haptoglobin polymer size on hemoglobin binding capacity. Vox Sang 1965;10:320-5.
[97] Jost SC, Diringer MN, Zazulia AR, et al. Effect of normal saline bolus on cerebral blood flow in regions with low baseline flow in patients with vasospasm following subarachnoid hemorrhage. J Neurosurg 2005;103:25-30.
[98] Kallen J, Welzenbach K, Ramage P, et al. Structural basis for LFA-1 inhibition upon lovastatin binding to the CD11a I-domain. J Mol Biol 1999;292:1-9.
[99] Kaminogo M, Yonekura M, Onizuka M, et al. Combination of serine protease inhibitor FUT-175 and thromboxane synthetase inhibitor OKY-046 decreases cerebral vasospasm in patients with subarachnoid hemorrhage. Neurol Med Chir (Tokyo) 1998;38:704-8 [discussion 708-709].
[100] Kansas GS. Selectins and their ligands: current concepts and controversies. Blood 1996;88:3259-87.
[101] Kapiotis S, Sengoelge G, Sperr WR, et al. Ibuprofen inhibits pyrogen-dependent expression of VCAM-1 and ICAM-1 on human endothelial cells. Life Sci 1996;58:2167-81.
[102] Kassell NF, Haley Jr EC, Apperson-Hansen C, et al. Randomized, double-blind, vehicle-controlled trial of tirilazad mesylate in patients with aneurysmal subarachnoid hemorrhage: a cooperative study in Europe, Australia, and New Zealand. J Neurosurg 1996;84:221-8.
[103] Kassell NF, Helm G, Simmons N, et al. Treatment of cerebral vasospasm with intra-arterial papaverine. J Neurosurg 1992;77: 848-52.
[104] Kassell NF, Torner JC, Haley Jr EC, et al. The International Cooperative Study on the Timing of Aneurysm Surgery. Part 1: overall management results. J Neurosurg 1990;73:18-36.
[105] Kassell NF, Torner JC, Jane JA, et al. The International Cooperative Study on the Timing of Aneurysm Surgery. Part 2: surgical results. J Neurosurg 1990;73:37-47.
[106] Kasuya H, Onda H, Sasahara A, et al. Application of nicardipine prolonged-release implants: analysis of 97 consecutive patients with acute subarachnoid hemorrhage. Neurosurgery 2005;56:895-902 [discussion 895-902].
[107] Kasuya H, Shimizu T. Activated complement components C3a and C4a in cerebrospinal fluid and plasma following subarachnoid hemorrhage. J Neurosurg 1989;71:741-6.
[108] Kaynar MY, Tanriverdi T, Kafadar AM, et al. Detection of soluble intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in both cerebrospinal fluid and serum of patients after aneurysmal subarachnoid hemorrhage. J Neurosurg 2004;101: 1030-6.
[109] Keyrouz SG, Diringer MN. Clinical review: prevention and therapy of vasospasm in subarachnoid hemorrhage. Crit Care 2007;11:220.
[110] Kino K, Mizumoto K, Watanabe J, et al. Immunohistochemical studies on hemoglobin-haptoglobin and hemoglobin catabolism sites. J Histochem Cytochem 1987;35:381-6.
[111] Kino K, Tsunoo H, Higa Y, et al. Hemoglobin-haptoglobin receptor in rat liver plasma membrane. J Biol Chem 1980;255:9616-20.
[112] Kino T, Hatanaka H, Hashimoto M, et al. FK-506, a novel immunosuppressant isolated from a Streptomyces. I. Fermentation, isolation, and physico-chemical and biological characteristics. J Antibiot (Tokyo) 1987;40:1249-55.
[113] Kinouchi H, Ogasawara K, Shimizu H, et al. Prevention of symptomatic vasospasm after aneurysmal subarachnoid hemorrhage by intraoperative cisternal fibrinolysis using tissue-type plasminogen activator combined with continuous cisternal drainage. Neurol Med Chir (Tokyo) 2004;44:569-75 [discussion 576-567].
[114] Klimo Jr P, Kestle JR, MacDonald JD, et al. Marked reduction of cerebral vasospasm with lumbar drainage of cerebrospinal fluid after subarachnoid hemorrhage. J Neurosurg 2004;100:215-24.
[115] Koh KK, Son JW, Ahn JY, et al. Vascular effects of diet and statin in hypercholesterolemic patients. Int J Cardiol 2004;95:185-91.
[116] Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975;256:495-7.
[117] Kohler W, Prokop O. Relationship between haptoglobin and Streptococcus pyogenes T4 antigens. Nature 1978;271:373.
[118] Kordestani RK, Counelis GJ, McBride DQ, et al. Cerebral arterial spasm after penetrating craniocerebral gunshot wounds: transcranial Doppler and cerebral blood flow findings. Neurosurgery 1997;41: 351-9 [discussion 359-360].
[119] Kramer AH, Gurka MJ, Nathan B, et al. Statin use was not associated with less vasospasm or improved outcome after subarachnoid hemorrhage. Neurosurgery 2008;62:422-7 [discussion 427-430].
[120] Kristiansen M, Graversen JH, Jacobsen C, et al. Identification of the haemoglobin scavenger receptor. Nature 2001;409:198-201.
[121] Lange V. Haptoglobin polymorphism—not only a genetic marker. Anthropol Anz 1992;50:281-302.
[122] Langlois MR, Delanghe JR. Biological and clinical significance of haptoglobin polymorphism in humans. Clin Chem 1996;42: 1589-600.
[123] Larson RS, Springer TA. Structure and function of leukocyte integrins. Immunol Rev 1990;114:181-217.
[124] Lasky LA. Selectins: interpreters of cell-specific carbohydrate information during inflammation. Science 1992;258:964-9.
[125] Lawrence MB, Springer TA. Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. Cell 1991;65:859-73.
[126] Lemanske Jr RF, Busse WW. Asthma. JAMA 1997;278:1855-73.
[127] Lennihan L, Mayer SA, Fink ME, et al. Effect of hypervolemic therapy on cerebral blood flow after subarachnoid hemorrhage: a randomized controlled trial. Stroke 2000;31:383-91.
[128] Levy AP. Genetics of diabetic cardiovascular disease: identification of a major susceptibility gene. Acta Diabetol 2003;40(Suppl 2): S330-3.
[129] Levy AP, Levy JE, Kalet-Litman S, et al. Haptoglobin genotype is a determinant of iron, lipid peroxidation, and macrophage accumulation

in the atherosclerotic plaque. Arterioscler Thromb Vasc Biol 2007;27: 134-40.
[130] Ley K, Gaehtgens P, Fennie C, et al. Lectin-like cell adhesion molecule 1 mediates leukocyte rolling in mesenteric venules in vivo. Blood 1991;77:2553-5.
[131] Lin CL, Dumont AS, Calisaneller T, et al. Monoclonal antibody against E selectin attenuates subarachnoid hemorrhage-induced cerebral vasospasm. Surg Neurol 2005;64:201-5 [discussion 205-206].
[132] Lollo BA, Chan KW, Hanson EM, et al. Direct evidence for two affinity states for lymphocyte function-associated antigen 1 on activated T cells. J Biol Chem 1993;268:21693-700.
[133] Lynch JR, Wang H, McGirt MJ, et al. Simvastatin reduces vasospasm after aneurysmal subarachnoid hemorrhage: results of a pilot randomized clinical trial. Stroke 2005;36:2024-6.
[134] Mack WJ, Ducruet AF, Hickman ZL, et al. Early plasma complement C3a levels correlate with functional outcome after aneurysmal subarachnoid hemorrhage. Neurosurgery 2007;61:255-60 [discussion 260-251].
[135] Mack WJ, Mocco J, Hoh DJ, Huang J, Choudhri TF, Kreiter KT, Lozier A, Opperman M, Poiksik A, Yorgason J, Solomon RA, MAyer SA, Connolly ES. Outcome prediction with serum intercellular adhesion molecule-1 levels after aneurysmal subarachnoid hemorrhage. J Neurosurg 2002;96:71-5.
[136] Maiuri F, Gallicchio B, Donati P, et al. The blood leukocyte count and its prognostic significance in subarachnoid hemorrhage. J Neurosurg Sci 1987;31:45-8.
[137] Manning AM, Davis RJ. Targeting JNK for therapeutic benefit: from junk to gold? Nat Rev Drug Discov 2003;2:554-65.
[138] Manno EM, Gress DR, Ogilvy CS, et al. The safety and efficacy of cyclosporine A in the prevention of vasospasm in patients with Fisher grade 3 subarachnoid hemorrhages: a pilot study. Neurosurgery 1997; 40:289-93.
[139] Martin NA, Doberstein C, Alexander M, et al. Posttraumatic cerebral arterial spasm. J Neurotrauma 1995;12:897-901.
[140] Martin NA, Doberstein C, Zane C, et al. Posttraumatic cerebral arterial spasm: transcranial Doppler ultrasound, cerebral blood flow, and angiographic findings. J Neurosurg 1992;77:575-83.
[141] Mascia L, Fedorko L, Stewart DJ, et al. Temporal relationship between endothelin-1 concentrations and cerebral vasospasm in patients with aneurysmal subarachnoid hemorrhage. Stroke 2001;32: 1185-90.
[142] Matsumura Y, Ikegawa R, Suzuki Y, et al. Phosphoramidon prevents cerebral vasospasm following subarachnoid hemorrhage in dogs: the relationship to endothelin-1 levels in the cerebrospinal fluid. Life Sci 1991;49:841-8.
[143] McCormick DJ, Atassi MZ. Hemoglobin binding with haptoglobin: delineation of the haptoglobin binding site on the alpha-chain of human hemoglobin. J Protein Chem 1990;9:735-42.
[144] McGirt MJ, Lynch JR, Parra A, et al. Simvastatin increases endothelial nitric oxide synthase and ameliorates cerebral vasospasm resulting from subarachnoid hemorrhage. Stroke 2002;33:2950-6.
[145] McGirt MJ, Mavropoulos JC, McGirt LY, et al. Leukocytosis as an independent risk factor for cerebral vasospasm following aneurysmal subarachnoid hemorrhage. J Neurosurg 2003;98:1222-6.
[146] Melamed-Frank M, Lache O, Enav BI, et al. Structure-function analysis of the antioxidant properties of haptoglobin. Blood 2001;98: 3693-8.
[147] Mocco J, Mack WJ, Kim GH, et al. Rise in serum soluble intercellular adhesion molecule-1 levels with vasospasm following aneurysmal subarachnoid hemorrhage. J Neurosurg 2002;97:537-41.
[148] Moestrup SK, Moller HJ. CD163: a regulated hemoglobin scavenger receptor with a role in the anti-inflammatory response. Ann Med 2004;36:347-54.
[149] Mori T, Nagata K, Ishida T, et al. Sequential morphological changes of the constrictive basilar artery in a canine model of experimental cerebral vasospasm by talc injection. J Vet Med Sci 1994;56:535-40.
[150] Mori T, Nagata K, Ishida T, et al. FK-506: a new immunosuppressive agent, failed to reduce cerebral vasospasm after experimental subarachnoid hemorrhage. J Vet Med Sci 1993;55:581-6.
[151] Mori T, Nagata K, Matsui T, et al. Superoxide anions in the pathogenesis of talc-induced cerebral vasocontraction. Neuropathol Appl Neurobiol 1995;21:378-85.
[152] Muizelaar JP, Zwienenberg M, Rudisill NA, et al. The prophylactic use of transluminal balloon angioplasty in patients with Fisher grade 3 subarachnoid hemorrhage: a pilot study. J Neurosurg 1999;91: 51-8.
[153] Mukai AO, Krebs VL, Bertoli CJ, et al. TNF-alpha and IL-6 in the diagnosis of bacterial and aseptic meningitis in children. Pediatr Neurol 2006;34:25-9.
[154] Nagata K, Sasaki T, Iwama J, et al. Failure of FK-506, a new immunosuppressant, to prevent cerebral vasospasm in a canine two-hemorrhage model. J Neurosurg 1993;79:710-5.
[155] Nagata K, Sasaki T, Mori T, et al. Cisternal talc injection in dog can induce delayed and prolonged arterial constriction resembling cerebral vasospasm morphologically and pharmacologically. Surg Neurol 1996;45:442-7.
[156] Nam DH, Kim JS, Hong SC, et al. Expression of interleukin-1 beta in lipopolysaccharide stimulated monocytes derived from patients with aneurysmal subarachnoid hemorrhage is correlated with cerebral vasospasm. Neurosci Lett 2001;312:41-4.
[157] Neil-Dwyer G, Cruickshank J. The blood leucocyte count and its prognostic significance in subarachnoid haemorrhage. Brain 1974;97: 79-86.
[158] Niikawa S, Hara S, Ohe N, et al. Correlation between blood parameters and symptomatic vasospasm in subarachnoid hemorrhage patients. Neurol Med Chir (Tokyo) 1997;37:881-4 [discussion 884-885].
[159] Nishizawa S, Chen D, Yokoyama T, et al. Endothelin-1 initiates the development of vasospasm after subarachnoid haemorrhage through protein kinase C activation, but does not contribute to prolonged vasospasm. Acta Neurochir (Wien) 2000;142:1409-15.
[160] Nishizawa S, Laher I. Signaling mechanisms in cerebral vasospasm. Trends Cardiovasc Med 2005;15:24-34.
[161] Nishizawa S, Peterson JW, Shimoyama I, et al. Therapeutic effect of a new immunosuppressant, FK-506, on vasospasm after subarachnoid hemorrhage. Neurosurgery 1993;32:986-91 [discussion 991-982].
[162] Nissen JJ, Mantle D, Blackburn A, et al. The selectin superfamily: the role of selectin adhesion molecules in delayed cerebral ischaemia after aneurysmal subarachnoid haemorrhage. Acta Neurochir Suppl 2000;76:55-60.
[163] Nissen JJ, Mantle D, Gregson B, et al. Serum concentration of adhesion molecules in patients with delayed ischaemic neurological deficit after aneurysmal subarachnoid haemorrhage: the immunoglobulin and selectin superfamilies. J Neurol Neurosurg Psychiatry 2001;71:329-33.
[164] Nissim A, Chernajovsky Y. Historical development of monoclonal antibody therapeutics. Handb Exp Pharmacol 2008:3–18.
[165] O'Shea JJ, Murray PJ. Cytokine signaling modules in inflammatory responses. Immunity 2008;28:477-87.
[166] Oertel M, Boscardin WJ, Obrist WD, et al. Posttraumatic vasospasm: the epidemiology, severity, and time course of an underestimated phenomenon: a prospective study performed in 299 patients. J Neurosurg 2005;103:812-24.
[167] Oliveira-Filho J, Ezzeddine MA, Segal AZ, et al. Fever in subarachnoid hemorrhage: relationship to vasospasm and outcome. Neurology 2001;56:1299-304.
[168] Oshiro EM, Hoffman PA, Dietsch GN, et al. Inhibition of experimental vasospasm with anti-intercellular adhesion molecule-1 monoclonal antibody in rats. Stroke 1997;28:2031-7 [discussion 2037-2038].
[169] Ostergaard JR, Kristensen BO, Svehag SE, et al. Immune complexes and complement activation following rupture of intracranial saccular aneurysms. J Neurosurg 1987;66:891-7.

[170] Otterbein LE, Soares MP, Yamashita K, et al. Heme oxygenase-1: unleashing the protective properties of heme. Trends Immunol 2003; 24:449-55.
[171] Parra A, Kreiter KT, Williams S, et al. Effect of prior statin use on functional outcome and delayed vasospasm after acute aneurysmal subarachnoid hemorrhage: a matched controlled cohort study. Neurosurgery 2005;56:476-84 [discussion 476-484].
[172] Pellettieri L, Carlson CA, Lindholm L. Is the vasospasm following subarachnoidal hemorrhage an immunoreactive disease. Experientia 1981;37:1170-1.
[173] Pellettieri L, Nilsson B, Carlsson CA, et al. Serum immunocomplexes in patients with subarachnoid hemorrhage. Neurosurgery 1986;19: 767-71.
[174] Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest 2003;111:1805-12.
[175] Peterson JW, Kwun BD, Hackett JD, et al. The role of inflammation in experimental cerebral vasospasm. J Neurosurg 1990;72:767-74.
[176] Peterson JW, Nishizawa S, Hackett JD, et al. Cyclosporine A reduces cerebral vasospasm after subarachnoid hemorrhage in dogs. Stroke 1990;21:133-7.
[177] Pfister HW, Borasio GD, Dirnagl U, et al. Cerebrovascular complications of bacterial meningitis in adults. Neurology 1992;42: 1497-504.
[178] Pickard JD, Murray GD, Illingworth R, et al. Effect of oral nimodipine on cerebral infarction and outcome after subarachnoid haemorrhage: British aneurysm nimodipine trial. BMJ 1989;298: 636-42.
[179] Pigott R, Dillon LP, Hemingway IH, et al. Soluble forms of E-selectin, ICAM-1 and VCAM-1 are present in the supernatants of cytokine activated cultured endothelial cells. Biochem Biophys Res Commun 1992;187:584-9.
[180] Pimstone NR, Tenhunen R, Seitz PT, et al. The enzymatic degradation of hemoglobin to bile pigments by macrophages. J Exp Med 1971;133:1264-81.
[181] Pluta RM. Delayed cerebral vasospasm and nitric oxide: review, new hypothesis, and proposed treatment. Pharmacol Ther 2005;105: 23-56.
[182] Pluta RM. Dysfunction of nitric oxide synthases as a cause and therapeutic target in delayed cerebral vasospasm after SAH. Neurol Res 2006;28:730-7.
[183] Pluta RM, Boock RJ, Afshar JK, et al. Source and cause of endothelin-1 release into cerebrospinal fluid after subarachnoid hemorrhage. J Neurosurg 1997;87:287-93.
[184] Pluta RM, Dejam A, Grimes G, et al. Nitrite infusions to prevent delayed cerebral vasospasm in a primate model of subarachnoid hemorrhage. JAMA 2005;293:1477-84.
[185] Pluta RM, Oldfield EH, Boock RJ. Reversal and prevention of cerebral vasospasm by intracarotid infusions of nitric oxide donors in a primate model of subarachnoid hemorrhage. J Neurosurg 1997;87: 746-51.
[186] Polin RS, Bavbek M, Shaffrey ME, et al. Detection of soluble E-selectin, ICAM-1, VCAM-1, and L-selectin in the cerebrospinal fluid of patients after subarachnoid hemorrhage. J Neurosurg 1998;89: 559-67.
[187] Polin RS, Hansen CA, German P, et al. Intra-arterially administered papaverine for the treatment of symptomatic cerebral vasospasm. Neurosurgery 1998;42:1256-64 [discussion 1264-1257].
[188] Pozzesi N, Gizzi S, Gori F, et al. IL-2 induces and altered CD4/CD8 ratio of splenic T lymphocytes from transgenic mice overexpressing the glucocorticoid-induced protein GILZ. J Chemother 2007;19: 562-9.
[189] Pradilla G, Thai QA, Legnani FG, et al. Local delivery of ibuprofen via controlled-release polymers prevents angiographic vasospasm in a monkey model of subarachnoid hemorrhage. Neurosurgery 2005; 57:184-90 [discussion 184-190].
[190] Pradilla G, Thai QA, Legnani FG, et al. Delayed intracranial delivery of a nitric oxide donor from a controlled-release polymer prevents experimental cerebral vasospasm in rabbits. Neurosurgery 2004;55: 1393-9 [discussion 1399-1400].
[191] Pradilla G, Wang PP, Legnani FG, et al. Prevention of vasospasm by anti-CD11/CD18 monoclonal antibody therapy following subarachnoid hemorrhage in rabbits. J Neurosurg 2004;101:88-92.
[192] Pritchard DI. Sourcing a chemical succession for cyclosporin from parasites and human pathogens. Drug Discov Today 2005; 10:688-91.
[193] Qureshi AI, Sung GY, Razumovsky AY, et al. Early identification of patients at risk for symptomatic vasospasm after aneurysmal subarachnoid hemorrhage. Crit Care Med 2000;28:984-90.
[194] Rantapaa Dahlqvist S, Beckman G, Beckman L. Serum protein markers in systemic lupus erythematosus. Hum Hered 1988;38:44-7.
[195] Recinos PF, Pradilla G, Thai QA, et al. Controlled release of lipopolysaccharide in the subarachnoid space of rabbits induces chronic vasospasm in the absence of blood. Surg Neurol 2006;66: 463-9 [discussion 469].
[196] Rezaie-Majd A, Prager GW, Bucek RA, et al. Simvastatin reduces the expression of adhesion molecules in circulating monocytes from hypercholesterolemic patients. Arterioscler Thromb Vasc Biol 2003; 23:397-403.
[197] Ries S, Schminke U, Fassbender K, et al. Cerebrovascular involvement in the acute phase of bacterial meningitis. J Neurol 1997;244:51-5.
[198] Rinkel GJ, Feigin VL, Algra A, et al. Circulatory volume expansion therapy for aneurysmal subarachnoid haemorrhage. Cochrane Database Syst Rev 2004:CD000483.
[199] Rittig MG, Kaufmann A, Robins A, et al. Smooth and rough lipopolysaccharide phenotypes of Brucella induce different intracellular trafficking and cytokine/chemokine release in human monocytes. J Leukoc Biol 2003;74:1045-55.
[200] Rittner HL, Brack A. Leukocytes as mediators of pain and analgesia. Curr Rheumatol Rep 2007;9:503-10.
[201] Robertson EG. Cerebral lesions due to intracranial aneurysms. Brain 1949;72:150-85.
[202] Rosen SD. Cell surface lectins in the immune system. Semin Immunol 1993;5:237-47.
[203] Rother RP, Bell L, Hillmen P, et al. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. JAMA 2005;293:1653-62.
[204] Rothoerl RD, Axmann C, Pina AL, et al. Possible role of the C-reactive protein and white blood cell count in the pathogenesis of cerebral vasospasm following aneurysmal subarachnoid hemorrhage. J Neurosurg Anesthesiol 2006;18:68-72.
[205] Rousseaux P, Scherpereel B, Bernard MH, et al. Fever and cerebral vasospasm in ruptured intracranial aneurysms. Surg Neurol 1980;14: 459-65.
[206] Rowe BH, Chahal AM, Spooner CH, et al. Increasing the use of anti-inflammatory agents for acute asthma in the emergency department: experience with an asthma care map. Can Respir J 2008;15:20-6.
[207] Ryba M, Grieb P, Bidzinski J, et al. Cyclosporine A for the prevention of neurological deficit following subarachnoid hemorrhage. Stroke 1991;22:531.
[208] Ryba M, Jarzabek-Chorzelska M, Chorzelski T, et al. Is vascular angiopathy following intracranial aneurysm rupture immunologically mediated. Acta Neurochir (Wien) 1992;117:34-7.
[209] Ryba M, Pastuszko M, Dziewiecki C, et al. A strategy for analyzing multiple parameters with application to aneurysmal SAH patients all of them clipped but treated with and without cyclosporine. Acta Neurochir (Wien) 1993;122:194-9.
[210] Ryba M, Pastuszko M, Iwanska K, et al. Cyclosporine A prevents neurological deterioration of patients with SAH—a preliminary report. Acta Neurochir (Wien) 1991;112:25-7.
[211] Sadrzadeh SM, Graf E, Panter SS, et al. Hemoglobin. A biologic fenton reagent. J Biol Chem 1984;259:14354-6.
[212] Saeed SA, Ahmad N, Ahmed S. Dual inhibition of cyclooxygenase and lipoxygenase by human haptoglobin: its polymorphism and

relation to hemoglobin binding. Biochem Biophys Res Commun 2007;353:915-20.
[213] Sakurai T, Yanagisawa M, Inoue A, et al. cDNA cloning, sequence analysis and tissue distribution of rat preproendothelin-1 mRNA. Biochem Biophys Res Commun 1991;175:44-7.
[214] Sander D, Klingelhofer J. Cerebral vasospasm following post-traumatic subarachnoid hemorrhage evaluated by transcranial Doppler ultrasonography. J Neurol Sci 1993;119:1-7.
[215] Satoh M, Date I, Nakajima M, et al. Inhibition of poly(ADP-ribose) polymerase attenuates cerebral vasospasm after subarachnoid hemorrhage in rabbits. Stroke 2001;32:225-31.
[216] Schenkel AR, Mamdouh Z, Muller WA. Locomotion of monocytes on endothelium is a critical step during extravasation. Nat Immunol 2004;5:393-400.
[217] Schoch B, Regel JP, Wichert M, et al. Analysis of intrathecal interleukin-6 as a potential predictive factor for vasospasm in subarachnoid hemorrhage. Neurosurgery 2007;60:828-36 [discussion 828-836].
[218] Seifert V, Loffler BM, Zimmermann M, et al. Endothelin concentrations in patients with aneurysmal subarachnoid hemorrhage. Correlation with cerebral vasospasm, delayed ischemic neurological deficits, and volume of hematoma. J Neurosurg 1995;82:55-62.
[219] Serhan CN. Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. Annu Rev Immunol 2007;25:101-37.
[220] Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. Nat Immunol 2005;6:1191-7.
[221] Sethi S, Sharma P, Dikshit M. Nitric oxide- and oxygen-derived free radical generation from control and lipopolysaccharide-treated rat polymorphonuclear leukocyte. Nitric Oxide 2001;5:482-93.
[222] Shimizu T, Kito K, Hoshi T, et al. Immunological study of late cerebral vasospasm in subarachnoid hemorrhage. Neurol Med Chir (Tokyo) 1982;22:613-9.
[223] Shinyama H, Uchida T, Kido H, et al. Phosphoramidon inhibits the conversion of intracisternally administered big endothelin-1 to endothelin-1. Biochem Biophys Res Commun 1991;178:24-30.
[224] Shor M, Boaz M, Gavish D, et al. Relation of haptoglobin phenotype to early vascular changes in patients with diabetes mellitus. Am J Cardiol 2007;100:1767-70.
[225] Shreenivas S, Oparil S. The role of endothelin-1 in human hypertension. Clin Hemorheol Microcirc 2007;37:157-78.
[226] Sills Jr AK, Clatterbuck RE, Thompson RC, et al. Endothelial cell expression of intercellular adhesion molecule 1 in experimental posthemorrhagic vasospasm. Neurosurgery 1997;41:453-60 [discussion 460-451].
[227] Smithies O. Zone electrophoresis in starch gels: group variations in the serum proteins of normal human adults. Biochem J 1955;61: 629-41.
[228] Solenski NJ, Haley Jr EC, Kassell NF, et al. Medical complications of aneurysmal subarachnoid hemorrhage: a report of the multicenter, cooperative aneurysm study. Participants of the Multicenter Cooperative Aneurysm Study. Crit Care Med 1995;23:1007-17.
[229] Spallone A, Acqui M, Pastore FS, et al. Relationship between leukocytosis and ischemic complications following aneurysmal subarachnoid hemorrhage. Surg Neurol 1987;27:253-8.
[230] Springer TA. Adhesion receptors of the immune system. Nature 1990;346:425-34.
[231] Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 1994;76:301-14.
[232] Springer TA. Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration. Annu Rev Physiol 1995;57: 827-72.
[233] Suarez JI, Shannon L, Zaidat OO, et al. Effect of human albumin administration on clinical outcome and hospital cost in patients with subarachnoid hemorrhage. J Neurosurg 2004;100:585-90.
[234] Suarez JI, Tarr RW, Selman WR. Aneurysmal subarachnoid hemorrhage. N Engl J Med 2006;354:387-96.
[235] Suzuki SC, Takeichi M. Cadherins in neuronal morphogenesis and function. Dev Growth Differ 2008.
[236] Takemae T, Mizukami M, Kin H, et al. Computed tomography of ruptured intracranial aneurysms in acute stage-relationship between vasospasm and high density on CT scan. (author's transl)No To Shinkei 1978;30:861-6.
[237] Taneda M, Kataoka K, Akai F, et al. Traumatic subarachnoid hemorrhage as a predictable indicator of delayed ischemic symptoms. J Neurosurg 1996;84:762-8.
[238] Thai QA, Oshiro EM, Tamargo RJ. Inhibition of experimental vasospasm in rats with the periadventitial administration of ibuprofen using controlled-release polymers. Stroke 1999;30:140-7.
[239] Tseng MY, Czosnyka M, Richards H, et al. Effects of acute treatment with pravastatin on cerebral vasospasm, autoregulation, and delayed ischemic deficits after aneurysmal subarachnoid hemorrhage: a phase II randomized placebo-controlled trial. Stroke 2005;36:1627-32.
[240] Tseng MY, Hutchinson PJ, Czosnyka M, et al. Effects of acute pravastatin treatment on intensity of rescue therapy, length of inpatient stay, and 6-month outcome in patients after aneurysmal subarachnoid hemorrhage. Stroke 2007;38:1545-50.
[241] Ursino M, Giulioni M, Lodi CA. Relationships among cerebral perfusion pressure, autoregulation, and transcranial Doppler waveform: a modeling study. J Neurosurg 1998;89:255-66.
[242] Vajkoczy P, Horn P, Bauhuf C, et al. Effect of intra-arterial papaverine on regional cerebral blood flow in hemodynamically relevant cerebral vasospasm. Stroke 2001;32:498-505.
[243] Vajkoczy P, Meyer B, Weidauer S, et al. Clazosentan (AXV-034343), a selective endothelin A receptor antagonist, in the prevention of cerebral vasospasm following severe aneurysmal subarachnoid hemorrhage: results of a randomized, double-blind, placebo-controlled, multicenter phase IIa study. J Neurosurg 2005; 103:9–17.
[244] van Deuren M, van der Ven-Jongekrijg J, Bartelink AK, et al. Correlation between proinflammatory cytokines and antiinflammatory mediators and the severity of disease in meningococcal infections. J Infect Dis 1995;172:433-9.
[245] Van Vlierberghe H, Langlois M, Delanghe J. Haptoglobin polymorphisms and iron homeostasis in health and in disease. Clin Chim Acta 2004;345:35-42.
[246] Walton JN. The prognosis and management of subarachnoid haemorrhage. Can Med Assoc J 1955;72:165-75.
[247] Wang Y, Zhong M, Tan XX, et al. Expression change of interleukin-8 gene in rabbit basilar artery after subarachnoid hemorrhage. Neurosci Bull 2007;23:151-5.
[248] Watanabe M, Sangawa A, Sasaki Y, et al. Distribution of inflammatory cells in adventitia changed with advancing atherosclerosis of human coronary artery. J Atheroscler Thromb 2007;14: 325-31.
[249] Weber C, Erl W, Weber KS, et al. HMG-CoA reductase inhibitors decrease CD11b expression and CD11b-dependent adhesion of monocytes to endothelium and reduce increased adhesiveness of monocytes isolated from patients with hypercholesterolemia. J Am Coll Cardiol 1997;30:1212-7.
[250] Weir B, Disney L, Grace M, et al. Daily trends in white blood cell count and temperature after subarachnoid hemorrhage from aneurysm. Neurosurgery 1989;25:161-5.
[251] Weir B, Grace M, Hansen J, et al. Time course of vasospasm in man. J Neurosurg 1978;48:173-8.
[252] Wejman JC, Hovsepian D, Wall JS, et al. Structure and assembly of haptoglobin polymers by electron microscopy. J Mol Biol 1984;174: 343-68.
[253] Wejman JC, Hovsepian D, Wall JS, et al. Structure of haptoglobin and the haptoglobin-hemoglobin complex by electron microscopy. J Mol Biol 1984;174:319-41.
[254] Weyerbrock A, Walbridge S, Pluta RM, et al. Selective opening of the blood-tumor barrier by a nitric oxide donor and long-term survival in rats with C6 gliomas. J Neurosurg 2003;99:728-37.

[255] White RP, Robertson JT. Comparison of piroxicam, meclofenamate, ibuprofen, aspirin, and prostacyclin efficacy in a chronic model of cerebral vasospasm. Neurosurgery 1983;12:40-6.
[256] Woodside DG, Vanderslice P. Cell adhesion antagonists: therapeutic potential in asthma and chronic obstructive pulmonary disease. BioDrugs 2008;22:85–100.
[257] Yamashima T, Kashihara K, Ikeda K, et al. Three phases of cerebral arteriopathy in meningitis: vasospasm and vasodilatation followed by organic stenosis. Neurosurgery 1985;16:546-53.
[258] Yamashima T, Kida S, Yamamoto S. An electron microscopic study of cerebral vasospasm with resultant myonecrosis in cases of subarachnoid haemorrhage, meningitis and trans-sylvian surgery. J Neurol 1986;233:348-57.
[259] Yamaura I, Tani E, Maeda Y, et al. Endothelin-1 of canine basilar artery in vasospasm. J Neurosurg 1992;76:99–105.
[260] Yanamoto H, Kikuchi H, Okamoto S, et al. Cerebral vasospasm caused by cisternal injection of polystyrene latex beads in rabbits is inhibited by a serine protease inhibitor. Surg Neurol 1994;42:374-81.
[261] Yanamoto H, Kikuchi H, Okamoto S, et al. Preventive effect of synthetic serine protease inhibitor, FUT-175, on cerebral vasospasm in rabbits. Neurosurgery 1992;30:351-6 [discussion 356-357].
[262] Yanamoto H, Kikuchi H, Sato M, et al. Therapeutic trial of cerebral vasospasm with the serine protease inhibitor, FUT-175, administered in the acute stage after subarachnoid hemorrhage. Neurosurgery 1992;30:358-63.
[263] Yatsushige H, Yamaguchi M, Zhou C, et al. Role of c-Jun N-terminal kinase in cerebral vasospasm after experimental subarachnoid hemorrhage. Stroke 2005;36:1538-43.
[264] Zhou C, Yamaguchi M, Kusaka G, et al. Caspase inhibitors prevent endothelial apoptosis and cerebral vasospasm in dog model of experimental subarachnoid hemorrhage. J Cereb Blood Flow Metab 2004;24:419-31.
[265] Zubkov AY, Lewis AI, Raila FA, et al. Risk factors for the development of post-traumatic cerebral vasospasm. Surg Neurol 2000;53:126-30.
[266] Zubkov AY, Rollins KS, Parent AD, et al. Mechanism of endothelin-1-induced contraction in rabbit basilar artery. Stroke 2000;31:526-33.
[267] Zuccarello M, Boccaletti R, Romano A, et al. Endothelin B receptor antagonists attenuate subarachnoid hemorrhage-induced cerebral vasospasm. Stroke 1998;29:1924-9.
[268] Zucker MB. A study of substances in blood serum and platelets which stimulate smooth muscle. Am J Physiol 1944;142:12-26.