Cerebrovascular Inflammation Following Subarachnoid Hemorrhage

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ABSTRACT—Aneurysmal subarachnoid hemorrhage frequently results in complications including intracranial hypertension, rebleeding and vasospasm. The extravasated blood is responsible for a cascade of reactions involving release of various vasoactive and pro-inflammatory factors (several of which are purported to induce vasospasm) from blood and vascular components in the subarachnoid space. The authors review the available evidence linking these factors to the development of inflammatory lesions of the cerebral vasculature, emphasizing: 1) neurogenic inflammation due to massive release of sensory nerve neuropeptides; 2) hemoglobin from lysed erythrocytes, which creates functional lesions of endothelial and smooth muscle cells; 3) activity, expression and metabolites of lipoxygenases cyclooxygenases and nitric oxide synthases; 4) the possible role of endothelin-1 as a pro-inflammatory agent; 5) serotonin, histamine and bradykinin which are especially involved in blood-brain barrier disruption; 6) the prothrombotic and pro-inflammatory action of complement and thrombin towards endothelium; 7) the multiple actions of activated platelets, including platelet-derived growth factor production; 8) the presence of perivascular and intramural macrophages and granulocytes and their interaction with adhesion molecules; 9) the evolution, origins, and effects of pro-inflammatory cytokines, especially IL-1, TNF-α and IL-6. Human and animal studies on the use of anti-inflammatory agents in subarachnoid hemorrhage include superoxide and other radical scavengers, lipid peroxidation inhibitors, iron chelators, NSAIDs, glucocorticoids, and serine protease inhibitors. Many animal studies claim reduced vasospasm, but these effects are not always confirmed in human trials, where symptomatic vasospasm and outcome are the major endpoints. Despite recent work on penetrating vessel constriction, there is a paucity of studies on inflammatory markers in the microcirculation.

Keywords: Subarachnoid hemorrhage, Vascular inflammation, Neurogenic inflammation, Symptomatic vasospasm, Anti-inflammatory agent

1. Introduction .................................................. 228
2. General effects of subarachnoid hemorrhage on cerebral arteries .................................................. 228
   2-1. Structural changes
   2-2. Functional effects
3. Evidence in favor of posthemorrhagic inflammation .................................................. 229
   3-1. Neurogenic inflammation in subarachnoid hemorrhage
   3-2. Hemoglobin and derived compounds
   3-3. Expression, activity and metabolites of lipoxygenases, cyclooxygenases and nitric oxide synthases
4. Studies on anti-inflammatory agents ..................... 238
   4-1. Superoxide radical scavengers
   4-2. Lipid peroxidation and lazaroids
   4-3. Iron chelators and other anti-oxidants
   4-4. NSAIDs and glucocorticoids
   4-5. Other treatments
5. Conclusions .................................................. 240

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1. Introduction

A SAH occurs when arterial blood brutally floods the subarachnoid space, most frequently following the rupture of an aneurysm. The blood mixes with the CSF that normally occupies this space under low pressure and can thus come into contact with the adventitial surface of the large arteries and the circle of Willis at the base of the brain, the pial arteries and a short initial portion of the cortical penetrating arteries (the Virchow-Robin space). If the SAH is not immediately fatal, it may be subject to complications, in particular acute intracranial hypertension and, later, cerebral VS and rebleeding. In the longer term, normal-pressure hydrocephalus may develop. Until recently, the classical treatment of the arterial aneurysm was aneurysm clipping and clot removal by neurosurgery, conditioned by the presence of the cerebral VS which contraindicates any operation before the third week by which time the VS resolves spontaneously. The VS is maximal in the week following the bleed. It consists of a significant durable narrowing of the artery caliber which, if severe, is accompanied by cerebral ischemia responsible for secondary mortality or morbidity.

2. General effects of SAH on cerebral arteries

SAH leads to abnormal brutal contact of the extraluminal wall of the arteries with all components of blood: the cells, i.e., erythrocytes, leukocytes, and platelets, and plasma components. This blood does not simply stagnate in the subarachnoid space: oxyHb is slowly released from erythrocytes which are lysed and diffuses right through the artery wall to the endothelium (1). A cascade of complex reactions begins that generates new diffusible agents and enzymes which initiate morphological and functional modifications of the cerebral arteries. Among these changes, inflammatory reactions seem to play an important role that is probably underestimated. Although, in our opinion, inflammatory reactions in the subarachnoid compartment are more reactive to many vasoconstrictors (e.g., ref. 19), have shown that after induced SAH the cerebral arteries are more reactive to many vasoconstrictors (e.g., ref. 19), including small pial arteries (20). They also show lesser capacity to dilate to recognized vasodilators (21–24). This decrease in induced vasodilation seems related to inflammatory secondary to the brain ischemic processes initiated by SAH-induced VS.

2-1. Structural changes

Cerebral arteries are composed of three tunics: the external layer, the adventitia, contains axons of the perivascular nerves in a collagen sheath; the media is the smooth muscle layer, and the intima, the internal layer, is formed by the internal elastic laminal on which lie the endothelial cells. Blood is pathogenic for all three layers but the consequences of the hemorrhage vary according to the tunic considered.

Denervation phenomena in the adventitia

The direct contact of the blood causes the disappearance of nerve fiber labelling. This “denervation” is clearly demonstrated towards the third day post-SAH when the immunolabelling of SP- and CGRP-containing fibers disappears (2–5). This lack of labelling coincides with the fall in the concentration of these neuropeptides in the CSF (6). In fact, the action of blood on the adventitia is global because nerve fibers containing noradrenaline, acetylcholine or VIP (vasoactive intestinal peptide) (7, 8), and NOS (9) are also affected. This phenomenon implies the loss of neurogenic control of cerebral arteries in this period.

Myonecrosis of the media

A certain degree of myonecrosis can be observed in the media (10–14). There is also a certain loss of contractile protein in the smooth muscle (15, 16), whereas the amount of interstitial collagen increases (15). The proliferation and the rearrangement of the collagen lattice is a noncontractile component of the artery narrowing after SAH (17).

Intima

The structural modifications here involve the endothelial tight junctions with increased permeability of the BBB (18).

2-2. Functional effects

The structural changes are accompanied by functional modifications of the smooth muscle and the endothelium that affect the cerebral artery motricity. In vitro studies have shown that after induced SAH the cerebral arteries are more reactive to many vasoconstrictors (e.g., ref. 19), including small pial arteries (20). They also show lesser capacity to dilate to recognized vasodilators (21–24). This decrease in induced vasodilation seems related to
a functional lesion of the contractile elements in the artery (25) and also to the endothelium (26, 27). Endothelium-dependent dilation seems to be reduced, partly because of reduced expression of guanylate cyclase (28). Experimentally, neurogenic and endothelium-dependent vasodilation have been shown to be altered by the presence of Hb in the bath (29). A more complete review can be found elsewhere (30). Cerebral vasomotor disturbances have been demonstrated in humans after SAH in measurements of cerebral blood flow by 133-xenon tomography. One of the most important functional consequences of SAH is the relative loss of cerebrovascular dilatory capacity after acetazolamide injection (31).

3. Evidence in favor of posthemorrhagic inflammation

Two types of inflammation have been described in the central nervous system. The first consists of classical inflammatory phenomena due to infection, trauma or immunitary disease; the second consists of neurogenic inflammation caused by excessive release from the terminals of trigeminal sensory nerves of peptides such as SP and CGRP. In SAH, the two phenomena are intimately tied up together. The signs of inflammation after SAH are intense headache (pain due to activation of trigeminal sensory fibers), early cerebral vasodilation, vascular permeability problems (BBB opening), PG production and the presence of inflammatory cells.

3-1. Neurogenic inflammation in SAH

Anatomical basis

Although the concept of neurogenic inflammation was known well before the discovery of SP, work since performed has revealed the primordial role of this peptide and the involvement of the sensory nervous system in this phenomenon. In the brain, most of the sensory innervation is comprised in the trigeminal nerve. It covers the internal carotid system (anterior and middle cerebral arteries), the rostral two-thirds of the basilar artery and the superior cerebellar artery, the rest of the vertebrobasilar system being innervated via the first cervical roots (32, 33). The trigeminal projections on these arteries are ipsilateral, except for those to the anterior cerebral arteries which are bilateral (34). Only relatively recently has it been shown that stimulation of Gasser’s ganglion (trigeminal ganglion), which contains the cell bodies of neurons projecting to cerebral arteries, leads to cerebral vasodilation in animals (35, 36) and humans (37, 38). This dilation is due to antidromic release of the neuropeptides SP and CGRP by the trigeminovascular fibers.

Post-SAH vasodilatation and neuropeptide release

The antidromic release of SP and CGRP has been demonstrated by measurements showing rapidly increased concentrations in the CSF after SAH (6). This is probably the cause of the early cerebral vasodilation observed after SAH which precedes a possible VS (31). However, it is difficult to determine if this transient phase is consistently present in humans.

Disturbances of vascular permeability and the BBB

Endothelial cells of cerebral arteries and microvessels are connected to each other by specific proteins forming the BBB, a mechanical barrier to the movement of most molecules circulating in the blood. Many pathological processes (ischemia, infections, hypertensive insult, etc.) alter the integrity of this barrier, including SAH. One of the characteristics of the inflammation caused by SAH, whether or not of neurogenic origin, is the increase in vascular permeability produced by the dislocation of these proteins, which in the case of large arteries allows numerous substances to enter the media and modify the function (18, 39 – 42). The rupture is caused at least partially by the presence of SP (43) and potentiated by CGRP (44), but other substances released after SAH probably contribute significantly. These include histamine (45, 46), 5-HT (46), ET (47) and bradykinin. The effect of bradykinin on the BBB is probably indirect, through an increase in the release of SP and CGRP from the sensory fibers (48). PGs and LTs probably also contribute because their precursor, AA, is formed in greatly increased quantity (ref. 49, and see later). The release of SP also degranulates mast cells, the products of which contribute to leukocyte activation, stimulating their adhesion to the endothelium and facilitating BBB rupture (50).

End of neurogenic inflammation

The neurogenic inflammation lasts as long as the neuropeptides and other neuromodulators are released from the perivascular nerve endings. Complete exhaustion of the stocks occurs after 2 – 3 days and the fibers are then no longer visible histochimically. It is noteworthy that this coincides roughly with the occurrence of VS and has even been suggested to contribute to it (51). The end of the neurogenic inflammation does not terminate the other inflammatory phenomena.

3-2. Hb and derived compounds

General effects and timing of Hb release

The extravasation of blood due to aneurysm rupture gives rise to the release into the perivascular space of several compounds derived from erythrocytes which are progressively lysed. Fundamental evidence has been adduced for the participation of Hb, especially oxyHb, in the development of delayed VS (52, 53). A minor role seems possible for deoxyhemoglobin and bilirubin and its oxidative products (54), but methemoglobin alone is without incidence (53). However, there may be some participation of smaller molecules, including ATP, in the overall action of hemo-
lysate (see below).

Although pure oxyHb does display direct vasoconstrictor activity, it seems insufficiently active to explain the extremely high degree of arterial narrowing frequently observed. However, the sources of NO in the cerebral arteries (55) are such that it has been supposed that Hb scavenging of NO could induce VS. The prolonged presence of the perivascular clot seems necessary to obtain VS, since clot removal up to 3 days post-hemorrhage reduces VS, but after 5 days, it has little influence (56). Concentrations of Hb obtained by microdialysis (molecules necessarily in solution) revealed increasing concentrations of oxy/deoxyHb in the CSF of monkeys, peaking at 7 days at 100× concentrations in shams (57). The early penetration of Hb into the artery wall has also been demonstrated (1). Overall, this evidence suggests, therefore, that the products of lysed blood must be in contact with the arteries for several days for prolonged VS to develop. This is in accordance with several lines of evidence of a role for Hb in creating an inflammatory state in the cerebral arteries which contributes to the delayed VS, as we discuss below.

Hb and eicosanoids

Hb appears to possess enzyme-independent catalytic activity, allowing it to convert AA in brain preparations to PGE₂ (58) or to lipoxygenase products (59). PG formation was blocked by scavengers or metal chelators but not blocked by indomethacin. In experiments on rabbits, intracisternal injection of 10 or 100 µg of Hb induced the formation of PGE₂ in CSF, the concentration being correlated with the fever induced (60).

Hb and free radicals

There is considerable evidence that Hb from lysed erythrocytes may contribute to the formation of ROS, including superoxide anion, hydrogen peroxyde and hydroxyl radical. In conjunction with excess NO (if there is induction of NOS-2), peroxynitrite will also be formed. Evidence of lipid peroxidation has been determined (61, 62), and evidence of tyrosine nitration has been presented (63).

When Macdonald and Weir (64) reviewed evidence on the role of free radicals in VS induction, they concluded that the data on free radicals detected after SAH, the damage imputable to free radicals, and the effects of free radicals on smooth muscle were insufficient to attribute with any certainty a role to free radicals. However, Giulivi et al. (65) have shown auto-oxidation of oxyHb to O₂⁻ followed by dismutation to H₂O₂. It has also been shown that oxyHb reduces Fe(III) to Fe(II), which catalyzes the formation of hydroxyl radicals from H₂O₂ (66). The effects of a gel impregnated with oxyHb were tested in vivo in monkeys, which induced artery narrowing of 40% at 7 days. This was reduced to 27% by treatment with SOD/catalase, but was not significantly different from placebo (62). However, significant malondialdehyde formation was noted in the SAH but not in the sham group.

Work on SMCs in vitro has documented the involvement of free radicals in Hb effects. Single rat SMCs stimulated by oxyHb showed contraction, increased K⁺ currents, decreased membrane resistance, bleb formation and death. Catalase or dimethyl sulfoxide (hydroxyl radical scavenger) afforded protection, but SOD only partially, and OH radicals reproduced these effects (67). Work with SMC cultures similarly revealed that contraction and intracellular Ca²⁺ increase by oxyHb were attributable to the action of OH radicals (68, 69).

Hb toxicity towards endothelial cells

Work with cultures has revealed a number of specific lesional effects of Hb. In carotid endothelial cells, oxyHb causes the appearance of detachment vacuoles and decreased cell density in a time- and dose-dependent manner (70). It also stimulated AA release, an effect inhibited by PLA₂ inhibition or Ca²⁺ chelation, which indicates calcium entry. Partial protection was afforded by SOD. Tyrosine phosphorylation was increased in pulmonary artery endothelial cells by hemolysate, in association with mobilization of intracellular calcium (71). Bovine intracranial endothelial cells showed sensitivity to a blood/CSF incubation mixture after 3 days or more. The degree of cytotoxicity was related to the concentrations of Hb over time (72). Other studies have shown similar effects associated with DNA damage and apoptosis (73, 74).

Regulation by Hb of protein synthesis/gene expression

Heme is degraded by HO. It has been shown that an upregulation of the inducible isoform (HO-1) occurs after SAH or injection of oxyHb or lysed blood into the cisterna magna. In one study, many rat brain regions were affected (microglia and some astrocytes) (75), whereas in another the major increase was in the (rat) basilar artery where new mRNA was induced (76). The use of HO-1 antisense oligonucleotide delayed Hb clearance and aggravated the late VS. However, a third study in monkeys found no change in mRNA but increased stocks of HO-1 and ferritin proteins in the adventitia with maximums at 3 and 6 days, respectively (77).

Hemolysate induces fibroblast collagen compaction, as do oxyHb and ATP, but with different time courses (78). Early genes are expressed in cultured SMCs under the influence of hemolysate (79). NOS-2 can be induced in rat SMCs by hemin (80) or by Hb, especially in the presence of IL-1β (81).
3.3. Expression, activity and metabolites of lipoxigenases, COXs and NOS

The eicosanoid pathways

AA is the key molecule from which eicosanoids are formed. Prostanoids or LTs are formed through cascades of reactions that begin with the transformation of AA by either a COX or a lipoxigenase, of which 5-lipoxigenase is the more important here. The metabolites are the PGs and TXs on the one hand, and the LTs on the other hand. The production of these metabolites seems to be proportional to the amount of bleeding (82) or rebleeding (83).

PLA2 and AA

AA is produced by the hydrolysis of membrane phospholipids by PLA2. This reaction is dependent on calcium, so that any pathological phenomenon such as SAH that causes increase in free calcium movement through the cell membrane will activate PLA2, leading to more eicosanoid production. The increase in cytosolic calcium in SAH concerns both SMCs (84, 85) and endothelial cells (71, 86). AA itself possesses noxious activity, since the cytotoxic effects of oxyHb on endothelial cells in culture are partly attributable to increased AA release (87). It also contributes to BBB rupture (88). Other substances present after SAH cause release of AA, for example histamine secreted by platelets and mast cells.

PGs and TXAs

After SAH, there are sizable changes in the concentrations of CSF prostanoids related to the hemorrhage volume. The variations show a time-dependent pattern.

PGE2: This is the eicosanoid molecule present in the highest concentration in the CSF after SAH. The concentration increases by more than 25 times soon after the hemorrhage and remains high for several days (89–91). This rise probably explains the fever usually found in SAH (60). The preferential production of PGE2 may be explained by the fact that SP and CGRP stimulate the COX pathway in platelets and favor particularly the PGE2 pathway (92). A recent study showed that PGE2 could be produced by microvessel endothelial cells in culture, probably through COX2 induction, after exposure to TNF-α (93). There is evidence of early increased TNF-α concentration in the CSF (91, 94). A final point is that in large cerebral arteries PGE2 has been shown to induce contraction (95).

PGI2: Like NO, PGI2 is vasodilator and an inhibitor of platelet and leukocyte activation. Cerebral vasodilation by histamine (released after SAH) is accompanied by increased CSF concentrations of PGE2 and PGI2 (96). This increase is shortlived, however, the PGI2 concentration falling to extremely low levels after 2–3 days (91, 97, 98). This low level has been suggested to favor the appearance of VS, in relation to a disequilibrium of PGI2/TXA (99).

PGF2α: According to certain authors (100, 101), the CSF concentration of PGF2α is significantly augmented after SAH. Moreover, labelling for PGF2α in neurons and intracranial vessels was found to be proportional to the increase in intracranial pressure induced by SAH (102). In vitro data suggest that PGF2α and PGE2 may lesion the BBB (93).

TXA2: TXA2 is a powerful vasoconstrictor and stimulant of platelet aggregation. It is produced mainly by activated platelets themselves from the endoperoxides formed by the action of COX on AA. According to some authors its concentration in CSF is significantly increased after SAH (100), but not according to other authors (101). Although it was suggested to play a role in the development of VS, the inhibition of TXA2 synthesis did not prevent arterial narrowing during the chronic phase of VS (103).

The 5-lipoxigenase pathway and LTs

After SAH the 5-lipoxigenase pathway is stimulated. In the CSF, the LT concentration increases rapidly, within the first half-hour, returning progressively to the baseline level at about 24 h (104). The concentrations are correlated with the development of VS (105–107). Strong LTC4 labelling is present in the media and the adventitia which changes little after SAH, whereas LTC4 concentrations increase significantly after SAH in the cortex (108). However, after SAH the neutrophils and macrophages which infiltrate the adventitia are strongly labelled for LTC4. These cells are thus the major producers of LTC4 (109). Its action is mainly to promote activation and permeabilization of endothelial cells, whereas its vasomotor activity is weak (110). Its participation in VS has been suggested on the basis of experiments with LTC4-receptor antagonists that attenuated the VS (111).

Expression/activity of COX-2 after SAH

COX-2 is the inducible isof orm of the enzyme that converts AA to PGG2 and PGH2, from which all other PGs and TXs are formed. This induction is a well-known phenomenon in peripheral arteries and veins (112). In the cerebral arteries COX-2 is expressed constitutionally (113), but its overexpression after SAH has been little studied. In an immunohistochemical study, it was found only in the endothelial layer 3 days after SAH (91), although a previous immunoblot study showed its overexpression in the arteries after 2 days (114). In our study (91), the expression of COX-2 was preceded during day 1 by a rise in the CSF concentration of TNF-α. The role of TNF-α in inducing COX-2 in endothelial cells has been recently demonstrated (93, 115), although the mechanisms at work in SAH are probably more elaborate and varied. Experimentally, it has also been found that ET-1 (see later discussion) may stimulate the expression of COX-2 (116). IL-1β also induces COX-2 expression (117) and this effect is antagonised by a PAF antagonist.

The exact consequences of this overexpression are not understood at the present time, but Mark et al. (93) showed...
increased release of PGs by cultured cerebral endothelial cells. Excessive COX activity may also lead to oxygen radical production. It is also suspected that COX-2 may influence gene transcription (118, 119), so that structural /biochemical changes may result from such overexpression. According to a recent study, in physiological conditions bradykinin may dilate cerebral arterioles by causing the release of COX-2 metabolites (120).

NOS expression/activity after SAH

NOS exists in three different isoforms: NOS-1 or “neuronal” NOS; NOS-2, the inducible form; and NOS-3, the “endothelial” enzyme. NOS-2 can be induced in a wide variety of cells, and its presence is associated with inflammation. The quantities of NO produced are usually far greater than for the other isoforms. There is a basal or stimulus-induced release of NO from endothelial cells that is vasodilatory. In the brain, this tonic level of NO includes the release of NO due to neuronal activity, some of which also affects the vascular tone. SAH was recently shown to affect the short-term levels of NO in the rat brain as measured by assaying the metabolites nitrite and nitrate (121). Between 10 and 120 or 180 min after SAH, there were significantly decreased levels of these metabolites in the 5 regions studied, although recovery towards baseline levels had commenced by 180 min. It is probable that this reflects rapid scavenging of NO by the Hb and that it contributes to the acute ischemic injury caused by SAH through acute vasoconstriction and other (neuronal) mechanisms. This fall in brain NO may also significantly potentiate the procoagulant actions of platelets and complement in the subarachnoid space.

In the longer term, there are changes in NOS-3 expression. At 7 days post-SAH in the rat, a significant decrease in NOS-3 mRNA (−56%) was found (122), although another group found a non-significant decrease (−22%) in NOS-3 protein, but a substantial fall in soluble cyclic guanylate cyclase (123). In the rat femoral artery model, perivascular blood clot induced biphasic changes in NOS-3 protein levels; i.e., increase at 3 days and decrease (−39%) at 7 days (124).

Exploration of NOS-2 expression suggests a link between the “inflammatory” form of NOS and VS. An immunohistochemical study of NOS-2 expression determined significant staining in endothelial, muscular, and especially adventitial cells at 7 days post-SAH in the rat (125). The intensity was greatest in animals with angiographic VS. mRNA for NOS-2 was studied in a different rat SAH model (63) and found to be increased mostly in vascular tissue at days 1–7. Treatment with aminoguanidine (NOS-2 inhibitor) improved the VS status of cerebral arteries. Interestingly, the immunohistochemically explored presence of nitrotyrosine was similar to that of NOS-2 mRNA, suggesting an important local activity leading to peroxynitrite formation. A study in humans after SAH found an increase in the CSF concentration of NO metabolites (nitrite and nitrate) beginning in the acute stage, which persisted 14 days (126). There was some correlation with the patients’ condition since the levels of metabolite were higher for those graded in Fisher group 3 than for group 2. However, high-dose methylprednisolone treatment (versus low-dose treatment) did not significantly reduce the levels of NO metabolites. Overall, these studies suggest that inflammatory levels of NO may be attained locally in the first days following SAH, leading to cellular lesions and favoring perturbed vascular function such as VS. Stimulating endogenous NO production by 1-arginine infusion did not prevent VS in a primate model of SAH (127), nor did adenovirus-mediated NOS gene transfer in dogs (128).

NO/COX interactions

The activity of NO is probably strongly linked to that of the COX enzymes because of the interactions between the two systems. The NO produced in endothelial cells activates COX to produce more eicosanoids (129, 130). In many models of inflammation, there is a concomitant induction of both NOS-2 and COX-2, such as after intracerebroventricular injections of lipopolysaccharide (131) or cerebral ischemia (132), and the NO produced influences the COX-2 activity. NO also facilitates the induction of COX-2 by IL-1 (133). Also, Hb can modify the NO production induced by IL-1 in SMC cultures by increasing the formation of NOS-2 (81).

3.4. ETs

The general origins and effects of ETs in the cerebral vascular territory were reviewed by Salom et al. (134). The evidence of a responsibility of ETs in inducing the delayed vasospasm frequently following SAH was also reviewed by these authors and more recently by Zimmermann and Seifert (135) and Faraci and Heistad (136). However, there has been no systematic survey of the possible inflammatory activity of ETs in this pathology, despite ample indications of their implications in pro-inflammatory mechanisms.

Presence of ETs in CSF after SAH

The presence of ETs in CSF has been documented by several authors in humans following SAH (137 – 144), but in primate models, some authors did not find evidence of increased ET-1 levels (145, 146). This discrepancy is all the more difficult to understand since it has been reported that ET antagonists inhibit vasospasm in primates (147, 148) as well as in other species. By far the most significant increases of ET concern ET-1, only sporadic amounts of ET-3 being noted in one report (141). However, the pre-cursor, big ET-1, was observed in one study to be the pre-
Origin of ETs in CSF

Endothelial cells

ET-1 transcription results in the formation of preproET-1, which is converted to big ET-1 by endopeptidases. Big ET-1 is cleaved to ET-1, the active molecule, by ECE-1. The endothelia of diverse vessels are a recognized source of ETs. In the cerebral vascular bed, these include cerebral microvascular endothelial cells (150, 151), dog basilar artery endothelial cells (152) and human microvascular endothelial cells (153). It is a reasonable assumption, based on data on many non-cerebral large arteries, that the endothelia of all arteries in the subarachnoid space are capable of producing big ET, and the presence of ECE has been reported in endothelial cells in vivo (154). There is evidence that SMC membranes in rabbit basilar artery carry ECE-1 enabling these cells to form ET-1 from big ET-1 (155).

Various stimuli have been shown to be capable of increasing ET-1 release, whether by induction of big ET-1 expression or by stimulation of cleavage and release mechanisms. Thus AA via its conversion to lipoxigenase metabolites seems to induce ET-1 gene expression (156) in aortic endothelial cells in culture. Substances that appear to stimulate release of ET-1 include Hb (157, 158), inflammatory cytokines, in particular IL-1β, TNF-α, IFN-γ, MCP-1 (159, 160). Two different cultures of endothelial cells have shown substantially increased ET-1 release on stimulation by oxyHb (158), although this was denied by Pluta et al. (146), or by erythrocyte lysate (mostly Hb) (157). Release was long-lasting (157) and showed potentiation if platelet-induced production was initiated (158).

Monocytes and macrophages

Recent evidence demonstrated the presence of ET-1 mRNA in monocytes from the CSF of SAH patients 5 days after the initial hemorrhage, at the same time as high levels of ET-1 (137). It has also been demonstrated that macrophages are capable of synthesizing ET-1 (161) and that they react via ET-A receptors to produce TNF-α (162).

Effects of ET-1

Direct vasomotor effects

ET-1 has extremely potent and long-lasting contractile effects on cerebral arteries (136). It acts via two receptors (ET-A and ET-B) on the smooth muscle and requires extracellular calcium for its action. It can also act on endothelial cells to release NO via another subtype of ET-B receptor. Pro-inflammatory effects of ETs

Little direct evidence of pro-inflammatory activity of ETs in cerebral arteries after SAH has so far been obtained, probably because interest has centered essentially on vasomotor activity. The numerous reports concerning such effects in other cell types attest to the possibility of such activity in subarachnoid vessels. We shall therefore enumerate some of the proinflammatory properties of ET reported in other cells and which have been noted in SAH.

Significant BBB opening was found by Narushima et al. (47) after intracisternal ET-1 injection, considerably potentiated by a second injection. Several papers report that ET-1 stimulates COX-2 induction. Several different cell types show this characteristic, including peritoneal macrophages (163), mesangial cells (116) and osteoblastic cells (164), although to date, none are vascular cells. It is noteworthy, however, that eicosanoids, including PGs, are released in greater quantities from human endothelial cells under ET-1 stimulation (165). Cytokine release (IL-6) has been demonstrated by stimulation with ET-1 in human vascular SMCs (166), human umbilical vein endothelial cells (167, 168) and monocytes (169).

A recent study on humans (144) is suggestive of pro-inflammatory behavior of ET-1 after SAH. Patients with a poor neurological condition but no significant VS (at 7 days) had the highest ET-1 levels at admission and a high level at 7 days. Patients with clinical VS also had high ET-1 levels at 7 days, but those with only angiographic VS did not. The greater gravity of the state of patients of the first two groups might be related to the early release of ET-1, causing aggravation of the general inflammatory state.

3-5. Other autacoids

5-HT

Most of the 5-HT is derived from platelets introduced into the subarachnoid space at the time of the aneurysm rupture. The aggregation thus stimulated causes release of 5-HT and other mediators (adenosine diphosphate, TXA₂, histamine) (170). Mast cell degranulation also results in 5-HT release. The CSF 5-HT concentration increases soon after the bleeding, especially in patients who develop VS (101). The actions of this amine are varied, including vasoconstriction in large cerebral arteries versus vasodilation in small cerebral arteries (171, 172), and increased vascular permeability (173–175). It is also algogenic, stimulating sensory fibers of the trigeminovascular system and thus releasing SP antidromically, contributing further to the effects of neurogenic inflammation already mentioned. Confirmation of this interaction in the proinflammatory activity of 5-HT has been provided by experiments in which sensory fibers were destroyed by capsaicin (176).

Histamine

This amine is released from mast cells, platelets and basophils. It has been demonstrated that mast cells migrate to cerebral arteries from the blood (177). After SAH, their number in the artery wall increases significantly (178). The
release of histamine by mast cells is stimulated by bradykinin (179). Activation of platelets by the collagen in the subarachnoid space not only releases stored histamine but also stimulates de novo synthesis (180). Like 5-HT, histamine stimulates trigeminovascular sensory fibers to release SP and potentiate the neurogenic inflammation. It also induces increased vascular permeability and BBB rupture (175). Finally, its pro-inflammatory properties includes induction of increased vascular permeability and BBB rupture by activation of platelets. In a physiological in vivo model, histamine induces vasodilation of cerebral arteries accompanied by induction of increased vascular permeability and BBB rupture (175). Bradykinin aggravates the inflammatory phenomena: it induces vasodilation by releasing PGI \(_2\) and PGI \(_3\) in the CSF, (but not TXB\(_2\), PGD\(_2\) or PGF\(_{2\alpha}\)) (96).

**Bradykinin**

The concentration of bradykinin in CSF is substantially increased immediately after SAH, after which it returns progressively to normal levels during one week at least (181). This peptide is synthesized in the subarachnoid space by activation of the Hageman factor in the blood through its contact with the collagen of the trabeculae. Bradykinin aggravates the inflammatory phenomena: it induces vasodilation by releasing PGI\(_2\) from endothelial cells (182) and alters the integrity of the BBB also through the action of PGIs (175). It also favors the release of SP and CGRP from the sensory fibers (48) and that of histamine (179) and AA from mast cells (49) and platelets (183). It was recently shown also to stimulate the induction of COX-2 in cerebral arterioles (120).

3.6. Complement and thrombin

**General pro-inflammatory properties of complement**

The complement system influences the activity of numerous cells, tissues, and physiological mechanisms of the body. Activation of the complement cascade, with the formation of the membrane-attack complex, results in cytotoxic and cytolytic reactions. The system is in fact a potent mechanism for initiating and amplifying inflammation. Target cells for membrane attack may include erythrocytes and nucleated cells (autologous or foreign). This activity is mediated through the action of fragments of complement components, in particular the anaphylatoxins. Anaphylatoxins are proteolytic products of the serine proteases of the complement system: C3a, C4a and C5a. They are polypeptides containing approximately 75 amino acid residues and meet all the criteria that characterize local hormones. The production of anaphylatoxins follows not only from complement activation, but also from activation of other enzyme systems that may directly cleave C3, C4 and C5. Such enzymes include plasmin, kallikrein, tissue and leukocyte lysosomal enzymes, and bacterial proteases. The anaphylatoxins have powerful effects on blood vessel walls, causing contraction of smooth muscle and an increase in vascular permeability, probably mediated indirectly via release of histamine from mast cells and basophils. C5a is extremely potent at stimulating neutrophil chemotaxis, adherence, respiratory burst generation, and degranulation. C5a also stimulates neutrophils and endothelial cells to express more adhesion molecules, and ligation of the neutrophil C5a receptor is followed by mobilization of membrane AA which is metabolized to PGs and LTs. Also, following ligation of monocyte C5a receptors, IL-1 is released.

**Involvement of complement in SAH**

Several reports have suggested that activated complement is likely to be present and to act on the subarachnoid tissues after SAH. Serial measurements of the lumbar CSF levels in humans revealed that the C3a and C4a levels were significantly elevated in the initial stage of SAH, within 48 h, but decreased rapidly (184). Levels in patients that showed delayed ischemic neurological deficits were significantly higher than in those which did not. Fibropeptide A levels were correlated with activated complement components, suggesting that activation of the coagulation system was responsible for the complement activation. This early increase in complement was confirmed in another study in which terminal complement complex was measured (185); it was also found to disappear by 7 – 10 days post-SAH. One pertinent action of complement is likely to be the accelerated aging of erythrocytes: the addition of plasma proteins to erythrocytes incubated in artificial CSF dramatically increased the hemolysis due to the activity of activated complement (186). Inhibition of the complement system by FUT-175 significantly decreased the incidence of symptomatic VS in patients with severe neurological grade (Hunt and Hess 3 and 4) (187).

**Thrombin**

Thrombin is a serine protease participating in the coagulation cascade which when activated cleaves fibrinogen and other factors of coagulation. The increase in its concentration in CSF after SAH is indicated by increased concentrations of fibropeptide A and thrombin – antithrombin complex (181, 188, 189). However, its intrinsic influence on cerebral arteries after SAH remains unclear (190, 191), despite some correlation of its presence with VS (189) or infarction (188) or outcome (191). Thrombin inhibitors seem to reduce VS (192, 193). At least part of the action of thrombin may result from the induction of PDGF (ref. 193, see next section).

3.7. Platelets

Platelet aggregation and adherence to endothelium is one of the known consequences of SAH (194). It certainly
stems from the endothelial modifications which occur, but a pattern of specific platelet-derived actions due to the extravasation deserves consideration.

**Effects of extravasation: platelet activation and coagulation**

An essential role of platelets is to rapidly plug small tears in the vascular wall by reacting to the stimulus of the collagen exposed by endothelial lesions. The activation process causes them to adopt a pattern of responses including degradation, formation of adhesion molecules, and in association with granulocytes, if present, the production of large quantities of eicosanoids and free radicals (195). In the particular case of SAH, they are exposed to collagens in the blood vessel adventitia and in the arachnoid structures (membrane and trabeculae), and their activation will be hastened by the proximity of leukocytes themselves undergoing activation through the immobilization and contact with non-vascular elements (195). The cascade of extravascular coagulation is triggered by the presence of tissue factor and its interaction with factor VIIa.

**Interactions with leukocytes**

Recent evidence suggests that tissue factor antigen may form on the surface of platelets adhering to neutrophils or monocytes (196). The authors suggest that the release of elastase by the leukocytes may inactivate the tissue factor pathway inhibitor. Elastase release seems to require the autocrine stimulation of leukocytes by LTs and the necessary adhesion to platelets (197). Transcellular metabolism of AA from activated platelets by lipoxygenase in granulocytes is likely to be an important source of LTB4 and 5,12-dihydroeicosatetraenoic acid (198). AA is in turn formed by the stimulation of platelet PLA2 by various factors (e.g., ET, Hb).

Activated PMNLs also interact with platelets via release of the granule protease cathepsin G. This protease acts on the protease-activated receptor 4 expressed by platelets, which mediates calcium signalling when activated (199). Another form of cooperation between platelets and leukocytes revolves around free radical formation which is stimulated in neutrophils by activated platelets. Not only do platelets produce NO from constitutive NOS, but when activated, they also store NO in the shape of nitrosothiols (200) and this interacts with superoxide (from PMNLs or Hb) to form peroxynitrite, augmenting the peroxidative tendency of the CSF/blood magma.

**Platelets and perivascular nerve fibers**

Two forms of interaction between activated platelets and cerebrovascular perivascular sensory nerves are likely to occur. Release of the sensory fiber transmitters SP and CGRP has been shown to occur after experimental SAH (6). In vitro, these peptides stimulate the AA cascade in platelets via both the lipoxygenase (SP only) and the COX (SP and CGRP) enzymes (92). Inversely, activated platelets have been shown to excite nociceptive C-fibers in a skin-nerve preparation (201). We speculate that such a phenomenon may occur in cerebrovascular sensory fibers (C-fibers), inducing both local SP and CGRP release and transmission of a pain message through the trigeminal ganglion to the various central structures. The chemical mediators could be serotonin and PGs, both known to act on C-fiber pain receptors.

**PDGF and TGF-β release**

**PDGF**

An important element released from activated platelets is PDGF. Apart from its marked contractile activity on vascular SMCs (193), PDGF stimulates SMCs from aorta to express and secrete the MCP-1 (202, 203) with a certain specificity, acting via the JE promotor, an immediate early gene. This induction was blocked by dexamethasone, suggesting it to be a regulatable and specific event involved in inflammation. The presence of PDGF in CSF was studied in humans by Gaetani et al. (204). They determined that there was a significant release of PDGF-BB early after SAH (days 1–3), and that higher levels of PDGF-BB found in patients within 72 h of SAH may be predictive of symptomatic VS. In contrast to these results, a study in dogs (205) subjected to a double hemorrhage bore no increase in CSF PDGF-AB immunoreactivity at 1, 3 and 8 days after SAH, but significant increases in serum levels at 3 and 8 days. Such a discrepancy seems likely to be related to the type of PDGF measured. Zhang et al. (193) demonstrated that PDGF-BB was induced two days after SAH in all layers of the rabbit basilar artery and that this induction could be sharply attenuated by inhibitors of serine proteases, including aragatroban which is specific to thrombin. The reduction in PDGF labelling was paralleled by substantial reduction in the degree of VS, and the authors hypothesize that PDGF causes VS via autocrine and paracrine activity.

**TGF-β**

A recent study in humans (206) determined highly significant increases in TGF-β1 at 1–2 days post-hemorrhage (mean increase ×44), which were presumed to be due to extravasated blood (platelets). After falling to a low level at 5 days, a second peak arose at 9–10 days which was attributed to a tissue-specific reaction. TGF-β1, by acting on specific receptors, can modulate extracellular matrix formation and the actions of other cytokines.

**3-8. Inflammatory cells and adhesion molecules**

Many results strongly suggest that SAH elicits an immunological process that includes both cellular and humoral
immunity. There is evidence of involvement of humoral immunity such as an increase in immunocomplexes in CSF (207) and deposition of immunoglobulin G in spastic arterial wall in primates (14). The involvement of immunocompetent cells is suggested by work with cyclosporine A, which is known to suppress cell-mediated immunity and has been reported to reduce the severity of VS in animals (208, 209) and humans (210). However, studies with another cell-mediated immunosuppressive agent, FK-506, failed to prevent vasoconstriction or pathological lesions (211, 212).

**Studies on immunocompetent cells**

It was reported in an early study that inflammatory cells appeared in the subarachnoid space and in large spastic arteries. There were relevant changes in all the layers, the most significant being evidence of necrosis in spastic arteries. There were relevant changes in all the cells appeared in the subarachnoid space and in large arteries. Studies on immunocompetent cells (211, 212).

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**Adhesion molecule expression**

The accumulation of leukocytes in inflamed tissue results from adhesive interactions between leukocytes and endothelial cells, generally in the microcirculation. Intracisternal injection of antibodies to ICAM-1 or CD18, which are two of the key adhesion molecules responsible for the attachment of leukocytes to endothelial cells, inhibit VS in a rabbit model of SAH (216). In addition, T-lymphocytes and macrophages were observed in the subarachnoid space with a peak appearance in brain tissue at 2 days post-SAH (217).

**The aneurysmal wall**

Immunohistochemical methods and scanning electron microscopy show from human ruptured and unruptured aneurysms obtained at surgery that extensive inflammatory and immunological reactions are common in unruptured intracranial aneurysms and may be related to aneurysm formation and rupture. Ruptured aneurysms manifested significant inflammatory cell invasion. Macrophages and monocytes were all frequently present in the wall of aneurysm tissue. The wall of ruptured aneurysms was found to be fragile, possibly because macrophage infiltration into the aneurysmal wall resulted in loss of SMCs and in degradation of matrix proteins (223, 224). Additionally, many T-lymphocytes (CD3) and a few B-lymphocytes (CD20) are present in the wall of aneurysm tissue (223).

**3-9. Cytokines in arteries and CSF**

Cytokines are low molecular weight proteins, divided into several classes: ILs (originally described as messengers between leukocytes), IFNs, growth factors, colony-stimulating factors and chemokines. However, most cytokines are pleiotropic and have multiple, diverse biological activities. Although release of inflammatory cytokines is essential for immunological and physiological homeostasis, an acute exaggerated production or extended release can have harmful effects on the host. Evidence for the involvement of cytokines in the pathology of SAH comes from the detection of high levels of cytokines in
CSF of patients with SAH and also from experimental data obtained after intracisternal injection of cytokines or monoclonal antibodies against cytokines.

### IL-1

Two forms have been identified (α, β) that have similar biological effects and the same receptors on target cells. Together with the antagonist molecule (IL-1ra) and IL-8, they play an important role in regulating inflammation. Both are expressed by astrocytes, fibroblasts, T-cells, macrophages, and monocytes; and IL-1β is also expressed by endothelial cells, platelets, neurons, neutrophils and natural killer cells. IL-1, when released into a local environment impacts a number of cells. Endothelial cells are induced to secrete chemokines such as MCP-1 (see below) and upregulate the expression of vascular adhesion molecules such as E-selectin, ICAM-1 and VCAM-1.

Conflicting results on CSF cytokine levels have been obtained in patients. One study reported no detection of IL-1α and IL-1β in the cerebrospinal fluid of patients with SAH (225), and another study found no IL-1β gradient between jugular venous and arterial serum in the first 96 h after onset of SAH (226). However, although no IL-1β was detectable in the peripheral serum of patients with SAH, in the blood of the internal jugular vein, IL-1β showed a transient increase at 5–9 days after SAH (220, 227). This suggests that only a very slight increase in IL-1β in CSF is sufficient to promote the pathological processes.

Recently, increased synthesis of IL-1α (with other cytokines, IL-6, IL-8) was detected in spastic basilar artery in the dog following experimental SAH. The increased expression of the IL-1α gene was 17-fold at D7 compared with that at D0 and was associated with sustained contraction (222). Osaka et al. (220) found that IL-1β increased only slightly in CSF with a maximum concentration occurring on day 14 and that high doses of methylprednisolone suppressed the changes of IL-1β in CSF. These findings suggest that IL-1β is a powerful molecule acting at very low concentrations. Intracisternal injection of cytokines (0.03 mg IL-1β, 3 mg IL-6 or 10 mg IL-8) induce COX-2-immunoreactive protein in the basilar artery (114).

### IL-1ra

Known alternatively as IL-1-receptor antagonist (IL-1ra) or soluble IL-1ra (sIL-1ra), this molecule is a particularly powerful inflammatory inhibitor of the two forms of IL-1. Because of its role as a natural inhibitor of the IL-1 activity, IL-1ra has been the subject of numerous studies investigating its possible use as an anti-inflammatory agent. In human studies, it was found that on admission, the IL-1ra level was higher (318 ± 302 pg/ml) in patients who were in poor clinical condition (Hunt and Hess Grades III – IV), and in patients with an unfavorable outcome or who experienced an episode of delayed ischemic deficit, there were marked increases in CSF IL-1ra levels between days 3 and 12 (>1000 pg/ml). On the contrary, patients with favorable outcome and good clinical condition had levels of IL-1ra only slightly higher than control patients (228, 229).

### IL-6

Cells known to express IL-6 include CD8 T cells, fibroblasts, endothelial cells (under the influence of ETs), neurons, astrocytes, neutrophils, monocytes and B cells. IL-6 production is generally correlated with cell activation.

In the context of SAH, IL-6 is synthesized with IL-1β and TNF-α as an acute phase reactant. Mononuclear leukocytes from the SAH clot are the major source of IL-6, IL-1β, TNF-α and ET-1 in CSF and expressed increased amounts of mRNA of these molecules (137). In patients with SAH, IL-6 is released into the subarachnoid space (7 ± 2 ng/ml on day 5) and its levels were correlated with those of ET-1 (137). In in vitro experiments, aging of blood or co-incubation of blood with CSF (absence of endothelial cells, neurons, astrocytes or other cellular sources) was sufficient to trigger leukocyte synthesis of IL-6, IL-1β, TNF-α and ET-1. This result demonstrates again that blood cells and serum protein components of the subarachnoid space blood clot following aneurysm rupture play a major role in triggering inflammatory reactions.

Expression of genes related to inflammation was increased in canine spastic artery after SAH. The mRNA expression for IL-6 increased between days 2 and 14 with a maximum on day 7 (222). At day 7, the average level of mRNA was 16-fold higher than at day 0. The increased expression of IL-6 was associated with increased expression of other genes related to inflammation such as IL-1α, IL-8 and ICAM-1. Levels in the CSF were not paralleled by increased values in the serum, reflecting intrathecal synthesis instead of passive transfer across the BBB (220, 230). The CSF concentration of IL-6 is 3000 – 3200 pg/ml in the middle phase (day 5) and less than 1000 – 1200 pg/ml in the late phase of SAH (days 13 – 14). Serum concentration of IL-6 remains low compared to those obtained in the CSF (<50 – 60 pg/ml) (225, 229, 231). Mean values of IL-6 are 11 ± 7 pg/ml in normal CSF and 3.5 ± 2 pg/ml in normal serum. Levels of IL-6 in CSF significantly increase from days 3 – 6 and are significantly higher on days 5 – 7 in patients with symptomatic VS or with delayed ischemic deficit compared with patients with no symptom of VS (220, 230). High-dose methylprednisolone treatment suppresses the high concentration of IL-6 (220). These authors also showed that intracisternal injection of IL-6 (3 μg) in dogs induced long-lasting vasoconstriction of basilar artery after transient vasodilation. Changes in diameter were still present on day 14. This intracisternal injection of IL-6 activated the PG cascade and especially increased both 6-keto
TNF-α

Human TNF-α exists as either a transmembrane or soluble protein. While both membrane-bound and soluble TNF-α are biologically active, soluble TNF-α is reported to be more potent. The variety of cell types known to express TNF-α is enormous. Astrocytes, endothelial cells, and neurons can synthesize cytokines, but microglia and macrophages, which are activated and proliferate in CNS after SAH, appear to be the dominant sources of TNF-α and IL-1 in this context (137). CSF monocytes express increased amounts of mRNA of TNF-α (along with IL-1β, IL-6 and ET-1) in SAH patients: at day 5 following the onset of SAH, the increase in synthesis reached 62–69% of the baseline values (137). The synthesis of TNF-α is induced by many different stimuli including IFN-γ, IL-2, SP, bradykinin, immune complexes, inhibitors of COX and PAF. The production of TNF-α is inhibited by IL-6, TGβ, PGE₂, dexamethasone, cyclosporin A and PAF antagonists.

TNF-α shows a wide spectrum of biological activities. Among them, in vivo, TNF-α in combination with IL-1 is responsible for many alterations of the endothelium. It inhibits anticoagulatory mechanisms and promotes thrombotic processes and therefore plays an important role in circulatory stasis. TNF-α is a potent chemoattractant for neutrophils and also increases their adherence to the endothelium. In resting macrophages, it induces the synthesis of IL-1 and PGE₂. It also stimulates phagocytosis and the synthesis of SOD in macrophages.

Mathiesen et al. (228) showed that on admission of patients suffering from SAH, CSF levels of TNF-α were similar to control patients (means 25–52 pg/ml), and that in patients with an unfavorable prognosis, i.e., Glasgow outcome scale 1 to 3, CSF levels of TNF-α were increased between days 4 and 10 to the range 200–300 pg/ml. As for IL-1ra, the pattern of TNF-α levels fits with the time course of symptomatic VS or DID (delayed ischemic deficit) in humans. TNF-α probably mediates some of the brain damage caused by SAH: it specifically mediates myelin, oligodendrocyte and neuronal damage; increases BBB permeability to molecules and white blood cells; and induces astrocytic proliferation. However, in canine spastic artery with increased expression of genes related to inflammation, mRNA for TNF-α is not increased, suggesting no influence of this cytokine in cerebral vascular tissue with respect to sustained contraction (222).

IL-2

IL-2 and IL-2ra have been little studied in the context of SAH. CSF soluble IL-2ra levels moderately increase soon after SAH, between days 1 and 3, probably through an intrathecal rather than a systemic source (230). The results of these authors suggest that a causal relationship may exist between IL-6 and IL-2ra. Other results are not all concordant and do not so far point to a clear role for IL-2 with respect to VS.

IL-8 and MCP-1

IL-8 and MCP-1, members of the CXC and CC families of chemokines, are characterized by potent neutrophil and monocyte-chemotactic properties. IL-8 and MCP-1 can be produced by several cell types including macrophages, lymphocytes, neutrophils, fibroblasts and endothelial cells in response to a variety of stimuli such as other cytokines like IL-1 and TNF-α.

Increased levels of IL-8 and MCP-1 can be detected in CSF of all patients suffering from SAH, from the early stage (day 3) to near the end stage (day 12). The concentrations are highest between day 3 and day 9, then decrease while remaining elevated until days 12–14 (220, 225, 233). On the contrary, serum concentration of IL-8 remains extremely low compared to those obtained in CSF at the same time. This suggests local release into the subarachnoid space of IL-8 and MCP-1, presumably by blood cells forming the SAH clot, endothelial cells or perhaps astrocytes, which may secrete IL-6, IL-8 and MCP-1 if stimulated by IL-1β or TNF-α (234, 235). A very strong correlation exists between IL-6, IL-8 and MCP-1, suggesting simultaneous or sequential secretion, which in turn, attract and activate neutrophils resulting in aggregation and adhesion to endothelium. IL-8 could play a role in the pathophysiology of VS by stimulating the COX cascade. IL-8 has been shown to induce a COX-2-like protein in canine basilar artery within 12 h after intracisternal injection (114) and to stimulate human aortic SMCs to produce PGE₂. Moreover, canine spastic basilar arteries showed an increased mRNA expression for IL-8 (222), but IL-8 alone seems unable to generate vasoconstriction (220).

4. Studies on anti-inflammatory agents

A large number of tests have been performed on the capacity of anti-inflammatory drugs to prevent VS and, in humans, to improve patient outcome. A certain proportion of the animal studies have also been directed towards some more specific endpoints directly related to inflammatory reactions. Considerable efficiency of such drugs has been observed in animal studies whereas the results in human studies show less strikingly effective therapy or VS prophylaxis. Nonetheless, certain of the large-scale clinical
trials do suggest that some amelioration can be expected with the lazaroid, tirilazad mesylate.

4-1. Superoxide radical scavengers

The critical role of superoxide is suggested by the numerous studies which have shown appropriate improvement with superoxide scavengers. Human recombinant Cu-Zn SOD has been found to prevent VS at 2 – 4 days in a rabbit SAH model and to prevent the appearance of endothelial lesions (236). In a cisternal talc injection model which induced strong VS at 3 – 7 days, it was found that SOD significantly inhibited the VS and almost abolished the associated myonecrosis and cell junction detachment (237). However, a study in monkeys using clot-containing agarose gel to simulate SAH did not reveal significant differences in VS compared to placebo (though different from control) when SOD and catalase were administered, and no significant change in CSF malondialdehyde (indicator of lipid peroxidation) was noted between treated and untreated oxyHb groups (62). That SAH really causes the production of superoxide was demonstrated recently by histochemical methods: the authors showed amber deposits indicative of superoxide presence at 2 and 7 days after SAH, mostly around degenerating erythrocytes and (less) near macrophages and neutrophils (238). Transgenic mice overexpressing SOD have been found to be protected from VS at day 3 (239) and to show less damage to neocortical cells after subarachnoid hemolysate injection (240). The endpoint in the latter study, the protection of neocortical cells, suggests that the overall inflammatory reaction extends to the brain tissue itself, although the exact cause of cell death was not verified in this study.

4-2. Lipid peroxidation and lazaroids

Two papers in the 1980s (241, 242) demonstrated large increases in the malondialdehyde concentrations in CSF in dogs and in humans and in cerebral arteries in dogs. Simultaneously, there were oppositely changing concentrations of protective enzymes, SOD and glutathione peroxidase, so that a solid argument appeared in favor of post-hemorrhagic increased lipid peroxidation due to the presence of excess ROSs. The development of 21-aminosteroid compounds (lazaroids) with anti-oxidant and anti-lipid peroxidation properties thus rapidly led to their being tested in SAH models, especially tirilazad mesylate. In two studies in monkeys, this agent was tested for its capacity to reduce the amplitude of VS (243) and to reduce malondialdehyde concentrations in the clot (244). Both parameters were effectively reduced, although curiously the most favorable concentration was the lowest (0.3 mg/kg). The basic result on VS was confirmed in dogs (245), and again the best dose (0.5 mg/kg) was not the highest. More recently, a study in monkeys found that VS at 7 days was attenuated by tirilazad at 0.3 mg/kg and that phosphatidylcholine hydroperoxide levels in cerebral arteries were reduced to undetectable levels (246).

Several large-scale clinical trials have been performed to determine the value of treatment by tirilazad of patients with SAH. The earlier studies from North America (247) and Europe/Australia/New Zealand (248) included doses from 0.6 – 6 mg/kg per day and both placebo- and tirilazad-treated patients received oral nimodipine. Evaluated on all patients, the first study did not find a significant difference in incidence or severity of VS or any improvement in overall outcome, in the tirilazad groups. The second study did find an improvement in mortality and frequency of good outcome at 3 months but a reduction in VS frequency that was considered non-significant ($P = 0.048$). On the basis of statistical analysis applied to subpopulations, it was concluded that men were better protected than women and that for patients with a poor neurological grade at admission (IV or V), tirilazad treatment resulted more frequently in improvement (at 6 mg/kg).

The results of two high dose (15 mg/kg) studies in women were published in 1999 (249, 250). The North American study again revealed differences only when subgroups comprising poorly graded patients were analyzed (endpoint of mortality at 3 months). In the Euro/southern hemisphere study, positive results with tirilazad could be found especially in the incidence of delayed ischemia and the frequency and severity of symptomatic VS. However, a bias may have been introduced by the more frequent use of hyperdynamic therapy in placebo patients.

Overall, these trials hint at some beneficial effect of treatment with this type of anti-inflammatory agent. Considering the problem of gender differences, presumably due to pharmacokinetic differences, and the fact that in several animal studies, the most favorable dose was low, one can speculate that some kind of unfavorable side effect could be at work, limiting benefit for better graded patients.

4-3. Iron chelators and other anti-oxidants

In view of the known property of hemoglobin to release iron during its degradation, it has long been hypothesized that iron-catalyzed production of free radicals via the Haber-Weiss and the Fenton reactions could contribute to SAH pathology (251). In fact, studies in animals have been directed exclusively to the endpoint of VS reduction which, although important, may not represent the only criterion of interest. Several VS studies on deferoxamine in the rat femoral artery model (252, 253), deferoxamine or deferoxiprone in rabbit cerebral arteries (254, 255), and 2,2'-dipyridyl in primate cerebral arteries (256) have added evidence for an important activity of iron chelators against the development of VS. According to Horky et al. (256), the chelator 2,2'-dipyridyl should be more efficient since
the catalytic activity concerns the ferrous and not the ferric form of iron. Certain data also suggested that the route of administration could be critical since deferoxamine is hydrophilic and might not attain the site necessary for effective action.

Anti-oxidant treatment after SAH with ebselen in the monkey (257) and ginkgo biloba extract in the dog (258) also reduced VS significantly, and treatment with ascorbate could reduce VS of the rat femoral artery (252). Ebselen seems also to exert an acute anti-contractile activity in rabbit basilar arteries in vitro, partly by inhibiting PKC activation and partly by radical scavenging (259).

4-4. NSAIDs and glucocorticoids

In recent years, interest for NSAIDs has rather receded except in seeking their specific activity as antiaggregants. Two such studies can be mentioned which suggest that such activity may be of some benefit to patients. In a large scale screening of patients’ intake of NSAIDs before admission for the SAH (before and after hemorrhage), it was calculated that the risk for patients to suffer from delayed cerebral ischemia was much lower (0.4) in those who had salicylates in their urine (at admission) relative to those who did not (260). Another smaller study concluded, on the basis of 50 patients given aspirin suppositories (100 mg) for 21 days after the surgery for aneurysm clipping (at 4 days maximum), that there could be some long-term benefit in the treatment, but a larger trial was necessary to demonstrate it. In experiments on femoral arteries, early application (at <6 h) of ibuprofen to the adventitia inhibited the VS probably by decreasing the perivascular interstitial macrophage and granulocyte count (261). Older animal studies have also shown normalizing effects of ibuprofen and methylprednisolone on VS and CSF PG concentrations (262) and on vasoreactivity of cerebral arteries removed from animals subjected to SAH (263).

Aside from the latter two studies, methylprednisolone has been found to offer certain benefits in both human and animal studies. According to Hall (264), one of the major mechanisms of its ability to offer protection at high doses is due to its property as an inhibitor of free radical-induced lipid peroxidation. A study of 21 patients with high-dose methylprednisolone found that a good outcome occurred twice as often and that the mortality rate and the incidence and severity of delayed cerebral ischemia were reduced (265). Other human studies have also found appreciable benefits: intracisternal irrigation of methylprednisolone (1 mg/ml) after surgery before day 3 improved the clinical results compared to placebo (266), and a similar study from the same center found very favorable outcome figures and low symptomatic VS incidence with such treatment coupled with further daily intracisternal injections (267). A study with hydrocortisone (268) also showed benefits of this type but its association with diltiazem and dextran precludes any definite conclusion on the effects of corticosteroid treatment. Animal investigations have confirmed VS inhibition and prevention of artery morphological changes (269), and determined reduction by intracisternal methylprednisolone of lipoperoxidation at days 4 and 7 (270). In rats treated every 8 h after SAH with high-dose methylprednisolone, the in vitro production of eicosanoids by brain slices, normally raised by SAH, was reduced (271).

4-5. Other treatments

It has been suggested through experiments on dog and rat SAH models that RNA synthesis inhibitors can prevent the occurrence of VS, especially if treatment begins early (272). It was not demonstrated which protein synthesis was specifically responsible. Serine protease inhibitors have been found in some trials to provide benefit. A trial involving 23 patients treated with FUT-175 versus 22 untreated patients found decreased VS incidence and especially reduced frequency of delayed ischemic neurological deficit in treated patients (55% in controls, 13% in the FUT-175 group) (273). The effectiveness of FUT-175 in antagonizing VS in rabbits was also shown (209), and more recent work has shown its efficacious action against symptomatic VS (187). Its action is presumed to include inhibition of complement.

5. Conclusions

The potentiality of the blood-CSF mixture as an inflammatory system for large cerebral arteries seems considerable when examining the components. The timing of the various phenomena triggered is important and the role of feedback control of inflammation may be critical: if control is insufficient, then excessive lesions and peroxidation may deteriorate the system, probably including nonvascular cells (neurons, glia, plexus). There is growing evidence in favor of modulated protein expression, frequently characteristic of inflammation (COX-2, HO-1, NOS-2 and cytokines).

Concerning small-vessel lesions, there is little data, but it may be important in conjunction with microcirculation platelet aggregation (194) and vasoreactivity (274). Some possible mechanisms of microvessel constriction have been evoked in a recent review (275). That intraparenchymal vessels constrict after SAH has been recently demonstrated in morphometric and corrosion cast studies (276, 277). Two inhibitors of mitogen-activated protein kinases prevented the development of clinical symptoms of VS, and also the constriction and lesions of penetrating arteries, although one of them did not reduce the angiographic VS of the basilar artery (277). To date, however, there have been
no studies on inflammatory markers in the cerebral microcirculation.

This survey also revealed interesting evidence on the implication of proteases involved in the coagulation cascade (complement, thrombin), and a possible role for PDGF, both induced in the artery wall and platelet-derived, which has vasospastic properties (193). There are well-known interactions between platelets, coagulation factors and the endothelium, the latter becoming notably prothrombotic and pro-inflammatory when physically or functionally perturbed (278). In this respect, more effort should perhaps be directed towards investigation of the detailed, chronological alterations of the state of the endothelium.

Although the early inflammation seems to favor the development of VS (279), it may be that VS is an important but not a sufficient condition in the induction of neurological lesions. As we report, there are a number of anti-inflammatory agents that seem to act efficiently in animal models, but the endpoint usually regarded is the angiographic VS, which in humans does not always reflect the clinical condition. Some cases reveal clearly a discrepancy (e.g., ref. 144). There is a problem of reproducibility in human trials and of significant and sensitive neurological tests in animals.

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Inflammation in Subarachnoid Hemorrhage 249

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