Potential Role of Granulocyte–Monocyte Colony-Stimulating Factor in the Progression of Intracranial Aneurysms

Nohra Chalouhi,¹ Thana Theofanis,¹ Robert M. Starke,² Mario Zanaty,¹ Pascal Jabbour,¹
Sarah A. Dooley,³ and David Hasan³

Macrophages play a central role in the inflammatory response leading to aneurysm formation, progression, and rupture. The purpose of this study was to determine whether granulocyte–monocyte colony-stimulating factor (GM-CSF) plays a role in the progression of human intracranial aneurysms. Specifically, we investigated whether there was a correlation between the aneurysm size and the concentration of GM-CSF in the lumen of intracranial aneurysms. The concentrations of GM-CSF in blood samples drawn from the lumen of 15 human unruptured saccular intracranial aneurysms of 14 consecutive patients were compared. The aneurysm size was 10.3 ± 9 mm on average. The mean plasma concentration of GM-CSF was 27.9 ± 3.1 pg/mL in the lumen of intracranial aneurysms. The mean plasma concentration of GM-CSF was significantly higher in aneurysms larger than 7 mm (30.1 ± 2.8 pg/mL) compared with aneurysms smaller than 7 mm (26.4 ± 2.4 pg/mL; $p = 0.02$). There was a significant positive correlation between the aneurysm size and the plasma concentration of GM-CSF (Spearman's $\rho = 0.55$; $p = 0.04$). There is a significant positive correlation between the aneurysm size and the plasma concentration of GM-CSF in aneurysm lumens. This suggests that GM-CSF, through its stimulatory function on macrophages, may promote aneurysm progression and may be a possible therapeutic target.

Introduction

IN RECENT YEARS, there has been mounting evidence that intracranial aneurysms are not passively enlarging vascular structures but exhibit prominent features of inflammation (Chalouhi *et al.*, 2012). Several constituents of the inflammatory response appear to be involved, including leukocytes, vascular smooth muscle cells, cytokines, growth factors, reactive oxygen species, and matrix metalloproteinases (MMPs) (Starke *et al.*, 2013b). Macrophages appear to play a central role in the inflammatory response leading to aneurysm formation, progression, and rupture (Chalouhi *et al.*, 2013b). The tissue-infiltrating macrophages release proinflammatory cytokines that lead to the recruitment of additional inflammatory cells and release MMPs that digest arterial wall extracellular matrix.

Granulocyte–monocyte colony-stimulating factor (GM-CSF) is known to be a major regulator involved in the control of granulocyte and macrophage lineage populations at all stages of maturation (Fleetwood *et al.*, 2005). Evidence also implicates a central role for GM-CSF in the local activation, recruitment, and survival of macrophage lineage cells, contributing to macrophage proliferation at local sites of inflammation (Hamilton, 2002, 2008; Hamilton and Anderson, 2004; Fleetwood *et al.*, 2005). Although the role

of GM-CSF in several local inflammatory diseases has been established, little is known about the contribution of GM-CSF in intracranial aneurysms. In this study, we sought to determine whether GM-CSF plays a role in the progression of human intracranial aneurysms. Specifically, we investigated whether there was a correlation between the aneurysm size and the concentration of GM-CSF in the lumen of intracranial aneurysms.

Materials and Methods

The study protocol was approved by the University Institutional Review Board. All enrolled patients presented to the Department of Neurosurgery at the University of Iowa Hospital and Clinics between November and December 2012.

Consecutive patients harboring unruptured intracranial aneurysms who were candidates for coil embolization were enrolled in the study. Patients taking corticosteroids, aspirin, anti-inflammatory medications, or immunosuppressant therapy were excluded. A total of 14 patients harboring 15 aneurysms were enrolled.

Cerebral angiography and blood sampling

In each patient, arterial access was obtained through femoral puncture by the use of the Seldinger technique,

¹Department of Neurosurgery, Jefferson Hospital for Neuroscience, Thomas Jefferson University, Philadelphia, Pennsylvania.

²Department of Neurosurgery, University of Virginia School of Medicine, Charlottesville, Virginia.

³Department of Neurosurgery, Carver College of Medicine, University of Iowa, Iowa City, Iowa.

followed by an insertion of a 7F arterial sheath. The guiding catheter was navigated into each studied vessel and the aneurysm was identified. A microcatheter was subsequently advanced over a microguidewire and placed in the aneurysm lumen. A blood sample (5 mL) was taken from the aneurysm lumen before coil deployment, centrifuged, and the plasma was stored at -80°C until analysis. All samples were immediately frozen after collection. The plasma concentration of GM-CSF was quantified with the Luminex-based immunoassay.

Statistical analysis

Data are presented as mean and range for continuous variables, and as frequency for categorical variables. Statistical analysis of categorical variables was carried out using the Fisher exact test. As values were not normally distributed, comparison of means was carried out using the Wilcoxon rank-sum test and the correlation was assessed with the Spearman rank correlation. p -Values of ≤ 0.05 were considered statistically significant. Statistical analysis was carried out with Stata 10.0 (College Station, TX).

Results

Of the 14 patients, 11 were women and 3 were men with a mean age of 57.8 ± 12 years. The aneurysm size was 10.3 ± 9 mm on average. Eleven aneurysms were located in the anterior circulation and three in the posterior circulation.

The mean systemic plasma concentration of GM-CSF was 28.4 ± 3.8 pg/mL. The mean plasma concentration of GM-CSF was 27.9 ± 3.1 pg/mL in the lumen of intracranial aneurysms (Table 1). The mean plasma concentration of GM-CSF was significantly higher in aneurysms larger than 7 mm (30.1 ± 2.8 pg/mL) compared with aneurysms smaller than 7 mm (26.4 ± 2.4 pg/mL; $p = 0.02$). Likewise, there was a statistically significant positive correlation between the aneurysm size and the plasma concentration of GM-CSF (Spearman's $\rho = 0.55$; $p = 0.04$; Fig. 1). There was no dif-

FIG. 1. Correlation between the aneurysm size and granulocyte-monocyte colony-stimulating factor (GM-CSF) plasma concentration.

ference in the concentration of GM-CSF between anterior (27.9 ± 3.3 pg/mL) and posterior circulation aneurysms (27.8 ± 2.4 pg/mL; $p = 0.9$).

Discussion

Aneurysm formation is thought to occur following hemodynamic insult, which elicits a series of proinflammatory changes in arterial walls (Chalouhi *et al.*, 2013a). The inflammatory reaction is characterized by the infiltration, activation, and proliferation of monocytes with the release of MMPs and free radicals, which ultimately results in aneurysm wall weakening and rupture.

Macrophages are invariably noted in human aneurysm samples and represent the main constituent of the inflammatory response in aneurysm walls (Chalouhi *et al.*, 2013a). There is a localized increase in the concentration of macrophage chemoattractants in the lumen of intracranial aneurysms. Macrophage depletion has been shown to halt aneurysm formation in mice, whereas macrophage infiltration correlates with the risk of aneurysm rupture in humans (Kanematsu *et al.*, 2011; Hasan *et al.*, 2012). Aoki *et al.* (2007) showed that the expression of macrophages and macrophage-derived MMPs was closely associated with aneurysm growth and that selective inhibition of these MMPs blocked aneurysm progression. Likewise, the inhibition of monocyte chemoattractant protein-1, a chemokine regulating the migration and infiltration of macrophages, halted intracranial aneurysm formation in mice (Aoki *et al.*, 2009). Therefore, therapies targeting macrophage activation and infiltration in aneurysm walls may potentially halt aneurysm formation, progression, and rupture.

Many cytokines have been found to be involved in the pathogenesis of cerebral aneurysms, most prominently interleukin (IL)1, IL6, and tumor necrosis factor- α (TNF- α) (Chalouhi *et al.*, 2012; Starke *et al.*, 2013a, 2013b). There have been no reports regarding the role of GM-CSF in the pathogenesis of intracranial aneurysms. In the present study, we found that patients with aneurysms larger than 7 mm had higher intraluminal plasma concentrations of GM-CSF compared with smaller aneurysms. There was also a significant positive correlation between the aneurysm size and

TABLE 1. CONCENTRATION OF GRANULOCYTE-MONOCYTE COLONY-STIMULATING FACTOR IN THE LUMEN OF INTRACRANIAL ANEURYSMS

Location of aneurysm	Aneurysm size	GM-CSF
MCA	5	23.9
PCOM	5	23.9
MCA	7	25.1
BTA	6	25.4
ICAB	6	25.8
MCA	7	26.1
ACOM	9	27.4
ACOM	6	27.6
BTA	14	27.7
CAVERNOUS	43	28.8
BTA	3	30.2
ICAB	7	30.2
MCA	9	30.5
ACOM	7.5	30.8
CAVERNOUS	20	35.3

ACOM, anterior communicating artery; BTA, basilar tip aneurysm; GM-CSF, granulocyte-monocyte colony-stimulating factor; ICAB, internal carotid artery bifurcation; MCA, middle cerebral artery; PCOM, posterior communicating artery.

the plasma concentration of GM-CSF in aneurysm lumens. These findings suggest that GM-CSF, a known regulator of monocyte and granulocyte function, may play an important role in the progression of human intracranial aneurysms. We suspect that higher local levels of GM-CSF promote macrophage infiltration and activation in arterial walls, driving aneurysm progression. These findings may also explain the observation in large trials that aneurysms larger than 7 mm have a higher risk of rupture (International Study of Unruptured Intracranial Aneurysms Investigators, 1998).

GM-CSF is primarily a hematopoietic growth factor that regulates the production, differentiation, and function of granulocytes and monocyte/macrophages (Hamilton, 2002, 2008; Hamilton and Anderson, 2004; Fleetwood *et al.*, 2005). A major source of GM-CSF is activated lymphocytes, monocytes/macrophages, neutrophils, eosinophils, and epithelial cells. GM-CSF is thought to be a key mediator of inflammation, especially at the local level. In fact, upon stimulation, GM-CSF promotes the local activation, recruitment, and survival of macrophages as well as neutrophils (Hamilton, 2002; Hamilton and Anderson, 2004). Intraperitoneal administration of GM-CSF caused a marked recruitment of macrophages, and rheumatoid disease flared during GM-CSF treatment (Hamilton, 2002). GM-CSF has a profound effect on several functions of macrophages and granulocytes, including stimulation of chemotaxis and cellular adhesion, free radicals production, and production of proinflammatory cytokines, such as IL6 and TNF- α (Hamilton, 2002, 2008; Hamilton and Anderson, 2004; Fleetwood *et al.*, 2005). These functions, as discussed above, are particularly relevant with regard to intracranial aneurysms. Therefore, it is possible that GM-CSF inhibitors may have therapeutic implications to prevent aneurysm progression. Classic GM-CSF inhibitors include Suramin, the side effects of which are vomiting, dermatitis, and adrenal cortical toxicity (Doukas *et al.*, 1995). Recently, inhibition of the effects of GM-CSF has been achieved by blockade of either the soluble cytokine with MOR103 or the GM-CSF receptor mavrilimumab. MOR103 is a high-affinity recombinant human IgG1 antibody that binds to a GM-CSF epitope resulting in blockade of cytokine-receptor interaction and receptor activation (Behrens *et al.*, 2014). It is well tolerated by patients, with the most common side effects being nasopharyngitis, fatigue, and lung toxicity (Behrens *et al.*, 2014). On the other hand, mavrilimumab is a fully human monoclonal antibody targeting the alpha subunit of GM-CSF receptor (Burmester *et al.*, 2013). Adverse events to mavrilimumab are generally mild in intensity and include nasopharyngitis, upper respiratory tract infections, and lung toxicity (Burmester *et al.*, 2013).

Limitations

This study is limited by the small number of patients enrolled, but sufficient data were obtained to demonstrate the overexpression of GM-CSF in the lumen of larger human cerebral aneurysms. Further studies are necessary to determine if concentrations of GM-CSF are significantly elevated in ruptured versus unruptured aneurysms. The exact mechanism through which GM-CSF drives aneurysm progression remains to be determined. It would be interesting to determine if systemic GM-CSF blockade can result

in lower levels of macrophages in aneurysm walls and whether this could potentially prevent aneurysm progression. We did not compare the levels of GM-CSF in patients with aneurysms with the levels in normal controls undergoing angiography for other reasons. However, based on the literature, the systemic levels of GM-CSF vary between 1.4 and 6.0 pg/mL in normal individuals (Dincol *et al.*, 2000). In our study, the mean systemic plasma concentration of GM-CSF was 28.4 ± 3.8 pg/mL.

Conclusion

In this study, we found a significant correlation between the luminal concentration of GM-CSF and the size of intracranial aneurysms. This suggests that GM-CSF, through its stimulatory function on macrophages, may promote aneurysm progression. GM-CSF may thus be a possible therapeutic target of medical treatment for the prevention of cerebral aneurysm progression and rupture.

Disclosure Statement

The authors declare there are no conflicts of interest regarding the publication of this article.

References

- Aoki, T., Kataoka, H., Ishibashi, R., Nozaki, K., Egashira, K., and Hashimoto, N. (2009). Impact of monocyte chemoattractant protein-1 deficiency on cerebral aneurysm formation. *Stroke* **40**, 942–951.
- Aoki, T., Kataoka, H., Morimoto, M., Nozaki, K., and Hashimoto, N. (2007). Macrophage-derived matrix metalloproteinase-2 and -9 promote the progression of cerebral aneurysms in rats. *Stroke* **38**, 162–169.
- Behrens, F., Tak, P.P., Ostergaard, M., Stoilov, R., Wiland, P., Huizinga, T.W., *et al.* (2014). MOR103, a human monoclonal antibody to granulocyte-macrophage colony-stimulating factor, in the treatment of patients with moderate rheumatoid arthritis: results of a phase Ib/IIa randomised, double-blind, placebo-controlled, dose-escalation trial. *Ann Rheum Dis*. DOI: 10.1136/annrheumdis-2013-204816.
- Burmester, G.R., Weinblatt, M.E., McInnes, I.B., Porter, D., Barbarash, O., Vatutin, M., *et al.* (2013). Efficacy and safety of mavrilimumab in subjects with rheumatoid arthritis. *Ann Rheum Dis* **72**, 1445–1452.
- Chalouhi, N., Ali, M.S., Jabbour, P.M., Tjoumakaris, S.I., Gonzalez, L.F., Rosenwasser, R.H., *et al.* (2012). Biology of intracranial aneurysms: role of inflammation. *J Cereb Blood Flow Metab* **32**, 1659–1676.
- Chalouhi, N., Hoh, B.L., and Hasan, D. (2013a). Review of cerebral aneurysm formation, growth, and rupture. *Stroke* **44**, 3613–3622.
- Chalouhi, N., Jabbour, P., and Hasan, D. (2013b). Inflammation, macrophages, and targeted imaging in intracranial aneurysms. *World Neurosurg* **81**, 206–208.
- Dincol, D., Akbulut, H., Buyukcelik, A., and Icli, F. (2000). Diurnal variations of serum GM-CSF levels. *Cytokine* **12**, 1151–1155.
- Doukas, M., Chavan, A., Gass, C., Nickel, P., Boone, T., and Haley, B. (1995). Inhibition of granulocyte-macrophage colony-stimulating factor (GM-CSF) activity by suramin and suramin analogues is correlated to interaction with the GM-CSF nucleotide-binding site. *Cancer Res* **55**, 5161–5163.

- Fleetwood, A.J., Cook, A.D., and Hamilton, J.A. (2005). Functions of granulocyte-macrophage colony-stimulating factor. *Crit Rev Immunol* **25**, 405–428.
- Hamilton, J.A. (2002). GM-CSF in inflammation and autoimmunity. *Trends Immunol* **23**, 403–408.
- Hamilton, J.A. (2008). Colony-stimulating factors in inflammation and autoimmunity. *Nat Rev Immunol* **8**, 533–544.
- Hamilton, J.A., and Anderson, G.P. (2004). GM-CSF biology. *Growth Factors* **22**, 225–231.
- Hasan, D., Chalouhi, N., Jabbour, P., Dumont, A.S., Kung, D.K., Magnotta, V.A., *et al.* (2012). Early change in ferumoxytol-enhanced magnetic resonance imaging signal suggests unstable human cerebral aneurysm: a pilot study. *Stroke* **43**, 3258–3265.
- International Study of Unruptured Intracranial Aneurysms Investigators. (1998). Unruptured intracranial aneurysms—risk of rupture and risks of surgical intervention. *N Engl J Med* **339**, 1725–1733.
- Kanematsu, Y., Kanematsu, M., Kurihara, C., Tada, Y., Tsou, T.L., van Rooijen, N., *et al.* (2011). Critical roles of macrophages in the formation of intracranial aneurysm. *Stroke* **42**, 173–178.
- Starke, R.M., Ali, M.S., Jabbour, P.M., Tjoumakaris, S.I., Gonzalez, F., Hasan, D.M., *et al.* (2013a). Cigarette smoke modulates vascular smooth muscle phenotype: implications for carotid and cerebrovascular disease. *PLoS One* **8**, e71954.
- Starke, R.M., Chalouhi, N., Ali, M.S., Jabbour, P.M., Tjoumakaris, S.I., Gonzalez, L.F., *et al.* (2013b). The role of oxidative stress in cerebral aneurysm formation and rupture. *Curr Neurovasc Res* **10**, 247–255.

Address correspondence to:
David Hasan, MD
Department of Neurosurgery
Carver College of Medicine
University of Iowa
200 Hawkins Drive, JCP 1616
Iowa City, IA 52242

E-mail: david-hasan@uiowa.edu

Received for publication July 15, 2014; accepted August 15, 2014.