Long-term Elevated Siglec-10 in Cerebral Spinal Fluid Heralds Better Prognosis for Patients with Aneurysmal Subarachnoid Hemorrhage

Sen Gao

Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital

Zheng Peng

Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital

Xiang-Xin Chen

Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital

Xun-Zhi Liu

Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital

Han Wang

Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital

Yue Lu

Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital

Zong Zhuang

Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital

Chun-Hua Hang

Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital

Wei Li (✉ lwzlw@126.com)

Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital <https://orcid.org/0000-0002-9258-3500>

Research article

Keywords: Aneurysmal subarachnoid hemorrhage, Siglec-10, protection, DAMP, Inflammation

Posted Date: June 2nd, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-29769/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

The presence of aneurysmal subarachnoid hemorrhage (aSAH) is usually accompanied by excessive inflammatory response leading to the destruction of central nervous system, while the sialic acid-binding Ig-like lectin 10 (Siglec-10) is a factor recognized as having the ability to modify the inflammatory microenvironment, indicating a potential protective effect in aSAH.

Methods

We collected the cerebrospinal fluid (CSF) of control group and aSAH patients at different time points, measured the Siglec-10 concentration by the enzyme-linked immunosorbent assay (ELISA), and evaluated the changes of Glasgow Outcome Scale (GOS) and Glasgow Coma Scale (GCS) in the disease progression.

Results

Our data showed that the Siglec-10 level in CSF could respond to aSAH and quickly rose to a peak, then fell back to a stable range slightly higher than the normal range. Severely impaired patients were likely to maintain high levels of Siglec-10 for longer periods. Generally, having high-level and long-term Siglec-10 indicated a better prognosis.

Conclusions

These results suggested that Siglec-10 could possibly reduce the degree of inflammatory response related to the damage-associated molecular patterns (DAMPs) by regulating the balance between pro-inflammatory and anti-inflammatory, thereby improving the patient's prognosis. These findings might provide new treatment perspectives for aSAH and other inflammation-related diseases.

Background

Aneurysmal subarachnoid hemorrhage (aSAH) refers to the extravasation of blood into the subarachnoid space caused by the rupture of an aneurysm, as is the most common type in SAH[1, 2]. After aSAH occurs, a series of complex pathophysiological changes will give rise to the impairment of the central nervous system, including inflammation, ionic disbalance, oxidative stress, ischemia, hypoxia and so on[3]. Unfortunately, the mechanism of aSAH is so unintelligible that the mortality rates still range from 8–67% with significant morbidity among survivors[4, 5]. Consequently, there is an urgent demand to explore factors that can improve the prognosis of aSAH.

The sialic acid-binding Ig-like lectins (Siglecs) are members of the immunoglobulin superfamily expressed on the cell surface. They are recognized as having the function of negatively regulating cells in the innate and adaptive immune system[6, 7], which depends on the structure called immunoreceptor tyrosine-based inhibitory motifs (ITIMs)[8, 9]. Siglec-10 is a member of the Siglec family with such ITIMs. It is expressed on human leucocytes, including B cells, eosinophils, monocytes, dendritic cells, T cells and NK cells[10–14]. Studies have shown that Siglec-10 plays an important role in regulating the immune balance of inflammatory diseases. For example, the interaction of Siglec-10 and CD24 reduces the inflammatory response associated with the damage-associated molecular patterns (DAMPs) by inhibiting the activation of high mobility group box 1 (HMGB1) and NF- κ B[15–18]. The interaction between Siglec-10 expressed on human DC and pseudaminic acid can increase the expression of IL-10 through MyD88 and p38 MAPK signaling pathway to promote anti-inflammatory function[19]. Several other reports also confirmed its ability to influence the inflammatory microenvironment[20–23]. Research on tumors indicates that tumors achieve immune escape through the inhibition of NK cells and T cells and reduction of the tumor-killing ability of macrophages, which comes out from up-regulating the expression of Siglec-10[24, 25]. In contrast, blocking Siglec-10 can increase phagocytosis to tumors by macrophages[26, 27]. Besides, Siglec-10 also plays a role in the establishment of immune tolerance[28].

Since the inflammatory response is an important progress in aSAH, in which excessive reaction usually causes serious harm[18], Siglec-10, an anti-inflammatory factor, may affect the recovery of aSAH patients by adjusting the balance of pro-inflammatory and anti-inflammatory. This has not been proved in vivo or in vitro. To test the hypothesis, we designed this clinical experiment to investigate whether Siglec-10 is involved in the pathophysiological process after aSAH, and what the relationship there is between Siglec-10 and the prognosis of patients.

Methods

All the methods used for human trials were made in line with the Helsinki Declaration and approved by the medical institutional review board (NO. 2020-041-01) at Nanjing Drum Tower Hospital. All clinical samples were obtained with the agreement of patients.

Patients selection

The selection criteria of the experimental group were: (1) Hunt-Hess grade I or II, (2) no other CNS disease, (3) patients of the experimental group were admitted and received interventional therapy within two days after aSAH, (4) no diabetes, malignant tumors, connective tissue diseases, or other systemic diseases. The patients' condition of the experimental group was shown in Table 1. The control group was from patients who needed surgeries with lumbar subdural anesthesia, and they had no SAH or any other CNS disease.

Table 1
Injury status of aSAH Patients

Case	Gender	Age	Aneurysm location	Hunt-Hess	GCS on admission	GOS on admission
1	male	42	V4 segment of left vertebral artery	II	13	3
2	female	57	Anterior communicating artery	II	15	3
3	female	57	Right posterior communicating artery	II	13	3
4	male	49	The bifurcation of right middle cerebral artery	I	15	4
5	female	51	Anterior communicating artery	II	10	3
6	female	76	Anterior communicating artery	I	15	4
7	female	46	Posterior communicating artery	II	12	3
8	female	48	Apex of basilar artery	II	15	3
9	female	65	Left anterior choroidal artery	I	15	4
10	male	52	The bifurcation of middle cerebral artery	I	15	4
11	female	65	Left posterior communicating artery	I	15	5
12	female	49	Right posterior communicating artery	I	13	4
13	male	68	Right anterior choroidal artery	II	13	3
14	female	45	Apex of basilar artery	I	15	4

Sample collection and detections

The cerebrospinal fluid (CSF) samples in the experiment were from the experimental group (n = 14) and the control group (n = 11). CSF of the experimental group was extracted from patients on day 3, day 7, and day 9 after aSAH. And CSF from control group patients was set as control. All clinical samples were centrifuged (3000 g, 5 min) in a sterile tube and the supernatant was taken and stored at -80 °C.

The enzyme-linked immunosorbent assay (ELISA) was then used to determine the protein levels of Siglec-10 in CSF when we had collected enough samples. We used the commercial human ELISA kit (cat# MBS2509371, MyBioSource) in this study with the manufacturer's instructions. We first equilibrated the samples and reagents to room temperature and set the blank well, control well and sample well. 50 µl of the standard sample was added to the control well. In the sample well, we added 40 µl of sample dilution and 10 µl of sample and then mixed the samples. After sealing the plate, we placed the plate at 37 °C and

incubated for 30 minutes. Then we peeled off the plate, discarded the liquid, added the washing liquid after drying, and washed for 30 seconds for 5 times. We added 50 µl of the enzyme-labeled reagent to each well except the blank well. After incubation and washing (same methods as above), we added the developer, mixed the wells, and developed the color at 37 °C in the dark for 15 minutes. The microplate turned blue and 50 µl of stop buffer was added to stop the reaction. Then the microplate turned yellow. The optical density (OD) was measured of each well at 450 nm. Then we drew the standard curve with the concentrations as the abscissa and the OD values as the ordinate and calculated the regression equation of the standard curve. Finally, we used the actual OD values and dilution values of the samples to calculate the Siglec-10 concentrations.

Assessment of recovery status and grouping

We evaluated the Glasgow Outcome Scale (GOS) and Glasgow Coma Scale (GCS) of patients on admission, day 3, day 7, day 9 and at discharge. All patients were admitted and received interventional therapy within two days after aSAH. Therefore, GOS and GCS evaluated on admission are used as a benchmark for comparing changes.

To explore the correlation between Siglec-10 and the prognosis of patients, a feasible method was to group patients according to whether the GOS score was higher than that on admission, then compare the differences between Siglec-10 levels of different groups. As shown in Table 2, only one patient's GOS increased on day 3 and all patients' GOS increased to 5 points at discharge, for which the data cannot be grouped by whether the GOS changes on day 3 or at discharged. Still, we can make reasonable groupings based on whether the GOS score changes on day 7 and day 9. In this way, patients were divided into GOS-elevated and unchanged groups. Another way to investigate their correlation was to analyze whether the GOS at admission was related to Siglec-10 levels on the following days.

Table 2
GOS of aSAH patients at different time points

Case	Admission	Day 3	Day 7	Day 9	Discharge
1	3	3	3	4	5
2	3	3	4	4	5
3	3	3	3	4	5
4	4	3	4	4	5
5	3	3	4	4	5
6	4	3	4	4	5
7	3	3	4	4	5
8	3	3	4	4	5
9	4	3	4	4	5
10	4	4	4	5	5
11	5	5	5	5	5
12	4	4	4	4	5
13	3	3	4	4	5
14	4	5	5	5	5

The GCS of the patients is shown in Table 3. It was difficult to group by whether the score changes to make qualitative analysis because only one patient, among the patients with not full GCS on admission, had no GCS change on day 3, meanwhile, all patients reached the same 15 points since day 7. Considering the potential protective effects of Siglec-10, we directly analyzed the relationship between Siglec-10 levels and GCS changes, including the correlation between Siglec-10 levels on day 3 and GCS changes on day 3 or day 7 and the correlation between Siglec-10 levels on day 7 and GCS changes on day 7. Besides, the correlation between GOS at admission and Siglec-10 levels on the following days was also examined.

Table 3
GCS of aSAH patients at different time points

Case	Admission	Day 3	Day 7	Day 9	Discharge
1	13	13	15	15	15
2	15	15	15	15	15
3	13	15	15	15	15
4	15	15	15	15	15
5	10	12	15	15	15
6	15	15	15	15	15
7	12	14	15	15	15
8	15	15	15	15	15
9	15	15	15	15	15
10	15	15	15	15	15
11	15	15	15	15	15
12	13	15	15	15	15
13	13	15	15	15	15
14	15	15	15	15	15

Data analysis

We used SPSS 24.0 and GraphPad Prism7.0 to analyze the data. Unpaired Student's t-test was used to analyze the differences between continuous variables of different groups. There was a statistically significant difference when $P < 0.05$. Data were expressed as mean $\pm$ SEM. Pearson correlation coefficient was used to evaluate the simple correlation between continuous variables. It was used to evaluate the correlation between Siglec-10 levels and the changes of GOS and GCS at different time points after aSAH.

Results

Siglec-10 levels in CSF increased rapidly after aSAH

We observed that patients in different groups and at different time points had different Siglec-10 levels (Fig. 1). The expression of Siglec-10 in the control group was at an extremely low level. Meanwhile, Siglec-10 of aSAH patients quickly reached its peak on day 3 ($P < 0.05$), then entered a relatively stable plateau period from day 7, during which the concentration was not as high as day 3 but still higher than that of the control group ($P < 0.05$).

Longer maintenance of high-level Siglec-10 indicated better GOS improvements

Whether Siglec-10 played a role in the process of aSAH can be clarified by group comparison. When grouped by whether GOS elevated on day 7, there was no significant difference in Siglec-10 levels at any time point (Fig. 2A). However, when grouped by whether GOS elevated on day 9, different groups showed a significant difference of Siglec-10 levels on day 7 (Fig. 2B, $P < 0.05$), but not on day 3 or day 9. This meant that the difference between the two groups was not the peak value reached on day 3, but whether Siglec-10 has been maintained at a high level for a longer time, which was reflected by the Siglec-10 level on day 7. Having higher Siglec-10 levels on day 7, the patients with elevated GOS on day 9 had longer maintenance of high-level Siglec-10 and fell back later than those whose GOS had not changed.

There was not only a qualitative relationship but also a quantitative correlation between Siglec-10 and GOS changes. Our results showed that the amount of GOS changed from admission to day 9 was significantly positively correlated with the Siglec-10 levels on day 7 (Fig. 3A, $r = 3.597$, $p = 0.0172$). The amount of GOS changed from admission to discharge conformed to the same pattern (Fig. 3B, $r = 3.178$, $p = 0.0040$). As the level on day 7 reflected whether Siglec-10 maintained high concentration for a long time, it can be summarized that the longer the Siglec-10 can be maintained at a high level, the better the recovery would be in the following days.

The high-level Siglec-10 of severely injured patients lasted longer compensatively

To further explore the probable role of Siglec-10 in aSAH, we analyzed the relationship between GOS at admission and Siglec-10 levels at each time point. The results showed that Siglec-10 levels on day 7 was significantly correlated with the GOS on admission. Patients with lower GOS on admission had higher Siglec-10 levels on day 7 (Fig. 4B, $r = -3.178$, $p = 0.0040$). No similar phenomenon was observed on day 3 (Fig. 4A, $r = -6.857$, $p = 0.1268$) or day 9 (Fig. 4C, $r = -0.143$, $p = 0.9364$).

Siglec-10 was not significantly related to the changes of GCS

The association between GCS and Siglec-10 could not be clearly observed. On the one hand, there was no significant correlation between the GCS at admission and Siglec-10 levels, whether on day 3 (Fig. 5A, $r = -0.1945$, $p = 0.9207$), day 7 (Fig. 5B, $r = -0.387$, $p = 0.4913$) or day 9 (Fig. 5C, $r = 0.9658$, $p = 0.2428$). On the other hand, the results showed that there was no correlation between the changes of GCS on day 3 and Siglec-10 levels on day 3 (Fig. 5D, $r = -0.6947$, $p = 0.8207$), or the changes of GCS on day 7 and Siglec-10 levels on day 3 (Fig. 5E, $r = 0.1945$, $p = 0.9207$) or day 7 (Fig. 5F, $r = 0.3687$, $p = 0.4913$).

Discussion

After binding to the ligand, the tyrosine contained in the ITIM domain of Siglec-10 is phosphorylated and serves as a binding site for proteins containing the SH2 domain (SHP phosphatase for instance). This leads to the dephosphorylation of cytoplasmic proteins, thereby downregulating the stimulating signaling pathways[29]. By negatively regulating the activation of high mobility group box 1 (HMGB1) and inhibiting the activation of NF- κ B, Siglec-10 can reduce the damage of DAMP-related inflammatory response[15]. Through MyD88 and p38 MAPK signaling pathways, Siglec-10 can increase the expression of IL-10 to promote anti-inflammatory function[19]. Siglec-10 can also inhibit the function of NK cells and T cells and to interact with HSP70, HSP90, VAP-1 to reduce inflammation[15, 20]. There are many consistent contents in the research area of aSAH, such as MyD88 / NF- κ B Pathway, HMGB1 and DAMPs[30–33]. Our results showed that Siglec-10 rose to peak rapidly after aSAH and remained at a high concentration afterward, indicating that Siglec-10 did participate in the pathophysiological process after aSAH, and the possible mechanisms might be the ones mentioned above. However, these results alone cannot clarify the specific role of Siglec-10 because many factors can increase after aSAH due to the influence of bleeding and other reasons, so we conducted the analysis below.

In our experiment, patients with Siglec-10 maintained at high levels for a longer period had better GOS improvement, which was approved by both qualitative and quantitative analysis. This finding strongly confirms that high-level and long-term Siglec-10 in CSF indicates better outcomes for aSAH patients. Another finding that can verify the role of Siglec-10 indirectly is that whether Siglec-10 can be maintained at a high level for a longer period is significantly related to the GOS on admission. We know that when damaged, the host usually activates protective mechanisms as the stress response. Naturally, the heavier the host is injured, the more significant the protective mechanism will be stimulated. This phenomenon is consistent with the performance of Siglec-10 in our experiment. Another noteworthy question is why Siglec-10, generally considered to be a membrane receptor, appeared in CSF. We suspect that this is due to the infiltration of inflammation in the tissues after aSAH so that the inflammation-related cells expressing Siglec-10 could enter the CSF through the destructed blood-brain barrier, which is also a consequence of aSAH. This also explains why the Siglec-10 levels of the control group were so low.

Interestingly, we could find that Fig. 3B and Fig. 4B have the symmetrical correlation coefficients, which is because the GOS of patients all reached a perfect score of 5 on discharge. Presume that if not all patients had a full score when they discharged, then Fig. 4B would remain the same, but Fig. 4B would show a different correlation coefficient. Therefore, this situation will not affect our argumentation. Furthermore, if there were data of more time points such as day 8, day 10, day 11, new results would support our conclusion more accurately.

We also discussed GCS in the same way as analyzing GOS. Regrettably, the connection between GCS and Siglec-10 was not found. Many reasons may cause this. For example, patients in our experiment were mildly injured with almost all GCS above 12 points, which may result in a less significant comparison between different groups; most patients had recovered to the full score on day 3, causing difficulties in grouping. These errors could be reduced by recruiting more severely injured patients, increasing the frequency of evaluating GCS and expanding the sample size. That is also where future research can be

improved. Besides, the different focus of GOS and GCS can also be a reason. Though both used to assess the condition of aSAH patients, GCS tends to characterize the degree of coma at a certain time, while GOS is the more accurate descriptor of the patient's prognosis.

Conclusions

Siglec-10 is recognized as having the potential to modify the inflammatory microenvironment, and our experiments have shown that Siglec-10 can indeed protect aSAH patients. By analyzing the Siglec-10 levels in CSF of 14 patients at different time points after aSAH and the control data of 11 patients, we found that the Siglec-10 level in CSF will respond to aSAH and quickly rise to a peak, then fall back to a stable range which is slightly higher than normal. Patients injured severely are likely to maintain high-level Siglec-10 for a longer period compensatorily. In general, having high-level and long-term Siglec-10 usually indicates faster recovery, which is probably due to the anti-inflammatory effects avoiding the host's excessive inflammatory response to damage. The exploration of the role of Siglec-10 in aSAH may provide new methods for the treatment of aSAH and other inflammation-related diseases.

Abbreviations

aSAH: Aneurysmal subarachnoid hemorrhage; Siglec-10: Sialic acid-binding Ig-like lectin 10; CSF: Cerebrospinal fluid; ELISA: Enzyme-linked immunosorbent assay; GOS: Glasgow Outcome Scale; GCS: Glasgow Coma Scale; DAMPs: Damage-associated molecular patterns; ITIMs: Immunoreceptor tyrosine-based inhibitory motifs; HMGB1: High mobility group box 1.

Declarations

Ethics approval and consent to participate

All methods used for human trials were based on the Helsinki Declaration and approved by the medical institutional review board (NO. 2020-041-01) at Nanjing Drum Tower Hospital. Written informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors have no personal or financial relationships which could influence the findings of this study.

Funding

This work was supported by grants from the National Natural Science Foundation, China (NO. 81870922 for W. Li, NO. 81771291 and NO. 81971122 for C.H. Hang), Key Project supported by Medical Science and technology development Foundation, Nanjing Department of Health (NO. JQX18001 for W. Li), and the Fundamental Research Funds for the Central Universities (NO. 021414380361 for W. Li). These funders were the corresponding authors of this article.

Authors' contributions

SG and ZP analyzed the data and wrote the manuscript; XXC assessed the patients' condition (GOS and GCS); YL collected the cerebrospinal fluid; HW, XZL and ZZ conducted enzyme-linked immunosorbent assay; WL and CHH contributed to the design and revised the manuscript. All authors approved the final version of the study.

Acknowledgements

Thanks to the anesthesiology department of Nanjing Drum Tower Hospital for helping us collect the CSF of the control group.

References

1. Abraham MK, Chang WW. Subarachnoid Hemorrhage. *Emerg Med Clin North Am.* 2016;34 4:901-16; doi: 10.1016/j.emc.2016.06.011.
2. Macdonald RL, Schweizer TA. Spontaneous subarachnoid haemorrhage. *The Lancet.* 2017;389 10069:655-66; doi: 10.1016/s0140-6736(16)30668-7.
3. van Lieshout JH, Dibue-Adjei M, Cornelius JF, Slotty PJ, Schneider T, Restin T, et al. An introduction to the pathophysiology of aneurysmal subarachnoid hemorrhage. *Neurosurg Rev.* 2018;41 4:917-30; doi: 10.1007/s10143-017-0827-y.
4. Nieuwkamp DJ, Setz LE, Algra A, Linn FHH, de Rooij NK, Rinkel GJE. Changes in case fatality of aneurysmal subarachnoid haemorrhage over time, according to age, sex, and region: a meta-analysis. *The Lancet Neurology.* 2009;8 7:635-42; doi: 10.1016/s1474-4422(09)70126-7.
5. Rivero Rodriguez D, Scherle Matamoros C, Cue LF, Miranda Hernandez JL, Pernas Sanchez Y, Perez Nellar J. Predictor's of Mortality in Patients with Aneurysmal Subarachnoid Haemorrhage and Rebleeding. *Neurol Res Int.* 2015;2015:545407; doi: 10.1155/2015/545407.
6. Crocker PR, Paulson JC, Varki A. Siglecs and their roles in the immune system. *Nat Rev Immunol.* 2007;7 4:255-66; doi: 10.1038/nri2056.
7. Macauley MS, Crocker PR, Paulson JC. Siglec-mediated regulation of immune cell function in disease. *Nat Rev Immunol.* 2014;14 10:653-66; doi: 10.1038/nri3737.
8. Whitney G, Wang S, Chang H, Cheng KY, Lu P, Zhou XD, et al. A new siglec family member, siglec-10, is expressed in cells of the immune system and has signaling properties similar to CD33. *Eur J*

- Biochem. 2001;268 23:6083-96; doi: 10.1046/j.0014-2956.2001.02543.x.
9. Tsubata T. Role of inhibitory BCR co-receptors in immunity. *Infect Disord Drug Targets*. 2012;12 3:181-90; doi: 10.2174/187152612800564455.
  10. Li N, Zhang W, Wan T, Zhang J, Chen T, Yu Y, et al. Cloning and characterization of Siglec-10, a novel sialic acid binding member of the Ig superfamily, from human dendritic cells. *J Biol Chem*. 2001;276 30:28106-12; doi: 10.1074/jbc.M100467200.
  11. Munday J, Kerr S, Ni J, Cornish AL, Zhang JQ, Nicoll G, et al. Identification, characterization and leucocyte expression of Siglec-10, a novel human sialic acid-binding receptor. *Biochem J*. 2001;355 Pt 2:489-97; doi: 10.1042/0264-6021:3550489.
  12. Toh BH, Kyaw T, Tipping P, Bobik A. Immune regulation by CD52-expressing CD4 T cells. *Cell Mol Immunol*. 2013;10 5:379-82; doi: 10.1038/cmi.2013.35.
  13. Escalona Z, Alvarez B, Uenishi H, Toki D, Yuste M, Revilla C, et al. Molecular characterization of porcine Siglec-10 and analysis of its expression in blood and tissues. *Dev Comp Immunol*. 2015;48 1:116-23; doi: 10.1016/j.dci.2014.09.011.
  14. Tsubata T. Inhibitory B cell co-receptors and autoimmune diseases. *Immunol Med*. 2019;42 3:108-16; doi: 10.1080/25785826.2019.1660038.
  15. Chen GY, Tang J, Zheng P, Liu Y. CD24 and Siglec-10 selectively repress tissue damage-induced immune responses. *Science*. 2009;323 5922:1722-5; doi: 10.1126/science.1168988.
  16. Bandala-Sanchez E, Zhang Y, Reinwald S, Dromey JA, Lee BH, Qian J, et al. T cell regulation mediated by interaction of soluble CD52 with the inhibitory receptor Siglec-10. *Nat Immunol*. 2013;14 7:741-8; doi: 10.1038/ni.2610.
  17. Zhao Y, Su H, Shen X, Du J, Zhang X, Zhao Y. The immunological function of CD52 and its targeting in organ transplantation. *Inflamm Res*. 2017;66 7:571-8; doi: 10.1007/s00011-017-1032-8.
  18. Bandala-Sanchez E, N GB, Goddard-Borger ED, Ngui K, Naselli G, Stone NL, et al. CD52 glycan binds the proinflammatory B box of HMGB1 to engage the Siglec-10 receptor and suppress human T cell function. *Proc Natl Acad Sci U S A*. 2018;115 30:7783-8; doi: 10.1073/pnas.1722056115.
  19. Stephenson HN, Mills DC, Jones H, Milioris E, Copland A, Dorrell N, et al. Pseudaminic acid on *Campylobacter jejuni* flagella modulates dendritic cell IL-10 expression via Siglec-10 receptor: a novel flagellin-host interaction. *J Infect Dis*. 2014;210 9:1487-98; doi: 10.1093/infdis/jiu287.
  20. Kivi E, Elima K, Aalto K, Nymalm Y, Auvinen K, Koivunen E, et al. Human Siglec-10 can bind to vascular adhesion protein-1 and serves as its substrate. *Blood*. 2009;114 26:5385-92; doi: 10.1182/blood-2009-04-219253.
  21. Liu YC, Yu MM, Chai YF, Shou ST. Sialic Acids in the Immune Response during Sepsis. *Front Immunol*. 2017;8:1601; doi: 10.3389/fimmu.2017.01601.
  22. Thornhill SI, Mak A, Lee B, Lee HY, Poidinger M, Connolly JE, et al. Monocyte Siglec-14 expression is upregulated in patients with systemic lupus erythematosus and correlates with lupus disease activity. *Rheumatology (Oxford)*. 2017;56 6:1025-30; doi: 10.1093/rheumatology/kew498.

23. Jellusova J, Duber S, Guckel E, Binder CJ, Weiss S, Voll R, et al. Siglec-G regulates B1 cell survival and selection. *J Immunol.* 2010;185 6:3277-84; doi: 10.4049/jimmunol.1001792.
24. Zhang P, Lu X, Tao K, Shi L, Li W, Wang G, et al. Siglec-10 is associated with survival and natural killer cell dysfunction in hepatocellular carcinoma. *J Surg Res.* 2015;194 1:107-13; doi: 10.1016/j.jss.2014.09.035.
25. Li Y, Zhou J, Zhuo Q, Zhang J, Xie J, Han S, et al. Malignant ascite-derived extracellular vesicles inhibit T cell activity by upregulating Siglec-10 expression. *Cancer Manag Res.* 2019;11:7123-34; doi: 10.2147/cmar.S210568.
26. CD24 Is a "Don't Eat Me" Signal That Promotes Tumor Immune Escape. *Cancer Discov.* 2019;9 9:1156; doi: 10.1158/2159-8290.Cd-rw2019-123.
27. Barkal AA, Brewer RE, Markovic M, Kowarsky M, Barkal SA, Zaro BW, et al. CD24 signalling through macrophage Siglec-10 is a target for cancer immunotherapy. *Nature.* 2019;572 7769:392-6; doi: 10.1038/s41586-019-1456-0.
28. Sammar M, Siwetz M, Meiri H, Fleming V, Altevogt P, Huppertz B. Expression of CD24 and Siglec-10 in first trimester placenta: implications for immune tolerance at the fetal-maternal interface. *Histochem Cell Biol.* 2017;147 5:565-74; doi: 10.1007/s00418-016-1531-7.
29. Pillai S, Netravali IA, Cariappa A, Mattoo H. Siglecs and immune regulation. *Annu Rev Immunol.* 2012;30:357-92; doi: 10.1146/annurev-immunol-020711-075018.
30. Zhu XD, Chen JS, Zhou F, Liu QC, Chen G, Zhang JM. Relationship between plasma high mobility group box-1 protein levels and clinical outcomes of aneurysmal subarachnoid hemorrhage. *J Neuroinflammation.* 2012;9:194; doi: 10.1186/1742-2094-9-194.
31. Yan H, Zhang D, Wei Y, Ni H, Liang W, Zhang H, et al. Inhibition of myeloid differentiation primary response protein 88 provides neuroprotection in early brain injury following experimental subarachnoid hemorrhage. *Sci Rep.* 2017;7 1:15797; doi: 10.1038/s41598-017-16124-8.
32. Chaudhry SR, Hafez A, Rezai Jahromi B, Kinfe TM, Lamprecht A, Niemela M, et al. Role of Damage Associated Molecular Pattern Molecules (DAMPs) in Aneurysmal Subarachnoid Hemorrhage (aSAH). *Int J Mol Sci.* 2018;19 7; doi: 10.3390/ijms19072035.
33. Wu LY, Ye ZN, Zhuang Z, Gao Y, Tang C, Zhou CH, et al. Biochanin A Reduces Inflammatory Injury and Neuronal Apoptosis following Subarachnoid Hemorrhage via Suppression of the TLRs/TIRAP/MyD88/NF- κ B Pathway. *Behav Neurol.* 2018;2018:1960106; doi: 10.1155/2018/1960106.

Figures

Figure 1

Siglec-10 in CSF measured by ELISA. The protein levels of Siglec-10 in CSF of patients on the 3rd, 7th, 9th days after the occurrence of aSAH and the control group. All data were expressed as the mean $\pm$ SEM. * $P < 0.05$ compared with control group; # $P < 0.05$ compared with day 3.

Figure 2

Differences in Siglec-10 levels between GOS-elevated and unchanged groups. (A) Grouped according to whether GOS was higher than admission on day 7, there was no significant difference in Siglec-10 content at each time point. (B) Grouped according to whether GOS was higher than admission on day 9, the Siglec-10 levels in CSF on day 7 showed a difference ($P < 0.05$ versus indicated groups). All data were expressed as the mean $\pm$ SEM.

Figure 3

Relationship between Siglec-10 levels on day 7 and changes of GOS on the following days. (A) The relationship between the Siglec-10 levels on day 7 and the changes of GOS on day 9 was significantly correlated. (B) The relationship between the Siglec-10 levels on day 7 and the changes of GOS at discharge was significantly correlated.

A

B

C

Figure 4

Relationship between GOS at admission and Siglec-10 levels at various time points. (A) There was no significant correlation between GOS at admission and Siglec-10 levels on day 3. (B) GOS at admission was significantly correlated with Siglec-10 levels on day 7. (C) There was no significant correlation between the GOS at admission and Siglec-10 levels on day 9.

A

B

C

D

E

F

Figure 5

Relationship between Siglec-10 levels at different time points and the GCS levels and changes. (A) Relationship between GCS at admission and Siglec-10 levels on day 3. (B) Relationship between GCS at admission and Siglec-10 levels on day 7. (C) Relationship between GCS at admission and Siglec-10 levels on day 9. (D) Relationship between the changes of GCS on day 3 and Siglec-10 levels on day 3. (E)

Relationship between the changes in GCS on day 7 and Siglec-10 levels on day 3. (F) Relationship between the changes in GCS on day 7 and Siglec-10 levels on day 7.