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Full Length Article

The origin and nature of the complex autoantibody profile in cerebrospinal fluid

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ABSTRACT

The present study demonstrates, using human protein microarrays and plasma and cerebrospinal fluid samples obtained pre-surgically and simultaneously from 46 hip fracture repair patients, that CSF exhibits an extraordinarily complex IgG autoantibody profile composed of thousands of autoantibodies. We show that the pattern of expression levels of individual autoantibodies in CSF closely mimics that in the blood, regardless of age, gender or the presence or absence of disease, indicative of a blood-based origin for CSF autoantibodies. In addition, using five longitudinal serum samples obtained from one healthy individual over a span of nine years, we found that blood autoantibody profiles are remarkably stable over a long period of time, and that autoantibody profiles in both blood and CSF show features that are common among different individuals as well as individual-specific. Lastly, we demonstrate that an elevated CSF/plasma autoantibody ratio is more common in elderly hip fracture repair patients that experienced post-operative delirium than in non-delirium subjects, thus highlighting the crucial role that blood-brain and/or blood-CSF barrier compromise may play in the development of post-operative delirium.

1. Introduction

The immune system is the body's primary defense mechanism against pathological invasion. Recent research has expanded this role to include maintenance of a microenvironment within the body (Avrameas et al., 2018; Cohen, 2007; Norris and Kipnis, 2019). In the past, the general consensus was that the appearance of autoantibodies (aABs) in the blood was a relatively rare event linked to some type of pathology, as was most clearly shown in various autoimmune diseases (Ippolito et al., 2011; Scott et al., 2010; Wanleenuwat and Iwanowski, 2019; Wielosz et al., 2014). More current research, however, now challenges the idea of central tolerance, with evidence supporting the ubiquitous presence of thousands of self-reactive aABs in the blood, even in the absence of pathology (Avrameas and Ternynck, 1995; Nagele et al., 2013). The idea of production of self-recognizing aABs in the absence of pathology supports a homeostatic role for this part of the immune system, including the response to sterile injury, immunosurveillance of cancer, facilitating wound resolution and tissue regrowth, and cell and tissue debris clearance. The specific nature of the response to pathology has led to exploration into the potential utility of aABs as biomarkers for a number of diseases (DeMarshall et al., 2015b, 2016, 2017; Han et al., 2012; Nagele et al., 2011a; Norris and Kipnis, 2019). In addition, the ubiquitous presence of such complex aAB profiles in the blood is likely to trigger a whole new field of endeavor, with the goal of elucidating details of its role in the maintenance of body-wide homeostasis as well as in the body response to the presence of disease.

Immune privilege is an active mechanism that allows immune tolerance of certain tissues such as the brain via the presence of a physical

Abbreviations: aAB, autoantibody; CSF, cerebrospinal fluid; HFR, hip fracture repair.

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barrier that restricts leukocyte and often antibody access (Shechter et al., 2013). It is generally believed that the purpose of immune privilege in the central nervous system is to minimize neural damage and cell death that might otherwise be caused by such access, although it could also be the passive consequence of the requirement of the brain for strict maintenance of homeostasis through blocked entry of plasma components. There are three main barriers that separate the brain from the products of the peripheral immune system: the Blood-Brain Barrier (BBB), the Blood-CSF Barrier (BCSFB) and the arachnoid epithelium forming the middle layer of the meninges covering the brain (Abbott et al., 2003; Shechter et al., 2013). At all three barrier sites, tight junctions limit the para-cellular permeability of constituents of plasma, such as albumin and immunoglobulin (Abbott et al., 2003). Under conditions of pathology, the structural and functional integrity of any and all of these barriers can be disrupted to various degrees, resulting in infiltration of plasma components into the brain parenchyma. In the case of BBB disruption, such as that which occurs in stroke, traumatic brain injury, Alzheimer's disease and many other neurodegenerative disorders, the binding of brain-reactive aABs to neurons and glia may contribute to escalation of local pathology, with the nature and extent of the pathology dependent on the site and extent of the BBB breach as well as the identity and relative abundance of brain-reactive aABs (Clifford et al., 2007; Nagele et al., 2011b).

The CSF also contains immunoglobulins, although little is known about the number and type of immunoglobulins, their origin, and whether or not aABs are also abundantly present in CSF as in blood (Regeniter et al., 2009; Riddoch and Thompson, 1970). In the present study, we have used highly sensitive human protein microarrays to detect aABs in CSF, shed light on their prevalence within the population and get a better understanding of the complexity of individual CSF aAB profiles and their relationship to blood aAB profiles within the same individual.

2. Methods

This study was approved by the Rowan University Institutional Review Board (IRB) and the Johns Hopkins University IRB.

2.1. Matched CSF and plasma from STRIDE participants ($n = 46$) (Table 1)

Plasma and CSF were obtained pre-surgically from hip fracture patients enrolled in the randomized clinical trial "A Strategy to Reduce the Incidence of Postoperative Delirium in Elderly Patients" (STRIDE) (Sieber et al., 2018). A detailed description of the STRIDE study has been published previously (Li et al., 2017; Sieber et al., 2018). Briefly, inclusion criteria were age $\geq$65, pre-operative MMSE score $\geq$15, and eligible for spinal anesthesia. Main exclusion criteria were pre-operative delirium, stage IV congestive heart failure, or severe chronic obstructive pulmonary disease. Informed consent was obtained from patients or appropriate legal representatives for patients unable to give informed consent due to cognitive impairment. STRIDE HFR subjects were cognitively assessed (Sieber et al., 2018) prior to surgery using both a mini-mental status exam (MMSE) (Folstein et al., 1975) and a modified CDR as described previously (Oh et al., 2018). Both blood and CSF were taken pre-surgically and simultaneously using standard procedures; CSF was obtained during administration of spinal anesthesia. Plasma and CSF were aliquotted and stored at −80 °C until use.

2.2. Longitudinal serum samples from a healthy control subject (Table 1)

Five longitudinal serum samples were obtained from a single healthy individual from the New Jersey Institute for Successful Aging at Rowan University. A total of five serum samples were collected over the course of 9 years (in 2006, 2007, 2010, 2013, and 2015).

2.3. Human protein microarrays for detection of autoantibodies (aABs)

Invitrogen's ProtoArray v5.1 Human Protein Microarrays (Cat. No. PAH0525020, Invitrogen, Carlsbad, CA, USA), each containing 9486 unique human protein antigens (www.invitrogen.com/protoarray), were used for aAB detection. All proteins were expressed as glutathione s-transferase (GST) fusion proteins in insect cells, purified under native conditions, and spotted in duplicate onto nitrocellulose-coated glass slides. Arrays were probed with serum/plasma/CSF, processed and scanned according to the manufacturer's instructions. Briefly, microarrays were blocked using Blocking Buffer (Cat. No. PA055, Invitrogen) and each was incubated with either serum/plasma diluted to 1:500 in washing buffer, or CSF diluted 1:10 in washing buffer. After washing, arrays were probed with anti-human IgG (H + L) conjugated to Alexa-Fluor 647 (Cat. No. A-21445, Invitrogen) diluted 1:2000 in washing buffer. Arrays were then washed, dried, and immediately scanned with a GenePix 4000B Fluorescence Scanner (Molecular Devices, Sunnyvale, CA, USA).

Fluorescence data were acquired by aligning the Genepix Array List onto the microarray using the Genepix Pro analysis software. The resulting Genepix results files were imported into Invitrogen's *Prospector* 5.2.3 microarray analysis software for analysis. The "group characterization" and "two-group comparison" features in the Immune Response Biomarker Profiling (IRBP) toolbox within *Prospector* enabled M-statistical analysis of aAB expression among plasma and CSF in the 46 STRIDE subjects. The "group characterization" feature was used to examine serum samples from the same subject. *Prospector* was used to compare blood and CSF autoantibody profiles in all subjects. Out of 9486 potential protein target antigens, 676 antigens with negative and/or zero aAB RFU values were excluded from analysis, resulting in a total of 8810 useable protein targets for analysis.

2.4. CSF/plasma aAB index to detect increased blood-brain barrier permeability

STRIDE participants were organized into two groups: 22 subjects that experienced post-operative delirium following surgery, and 24 subjects who did not experience delirium following surgery. CSF/plasma ratios were calculated by first dividing the CSF RFU value by the plasma RFU value for every reactive aAB target on the microarray, and then averaging these ratios to obtain a single CSF/plasma ratio for each individual, encompassing all reactive aAB targets. CSF/plasma ratios representing the non-delirium control and delirium patient groups were calculated by determining the average CSF/plasma ratio of all members of each group. A two-tailed, unequal variance *t*-test was used to determine if the difference between the means of the two CSF/plasma ratio was significant.

3. Results

3.1. CSF, like blood, has a complex aAB profile that includes thousands of natural IgG autoantibodies

Pre-surgical plasma and CSF were obtained simultaneously from individual elderly hip fracture repair (HFR) patients and used to probe human protein microarrays. These microarrays contain 9486 native human proteins representing roughly one-third of the total human proteome. Each protein on the array serves as a potential protein target for aABs present in plasma or CSF. Plasma was diluted 500x with buffer, whereas CSF was diluted only 10X with the same buffer to compensate for the much lower levels of IgG in CSF and to bring aAB concentrations in CSF to levels that fall within the dynamic range of sensitivity of the microarrays. This facilitated direct comparison of plasma and CSF aAB profiles obtained from the same as well as different subjects.

The relative abundance of aABs directed against each protein target was measured using Relative Fluorescence Units (RFUs). For this calculation, positive aABs were determined by a Z-Factor >0.4 and a minimum

signal intensity of 1500 RFU. Using this approach, aABs in plasma and CSF at the specified dilutions were found to be immunoreactive to thousands of protein targets. An outlier test was performed on the percent overlap data, leading to exclusion of two subjects. On average, there were more aABs detected in plasma (3149) than in CSF (1025). Thus, aABs are immunoreactive with a substantial fraction (33.2% for plasma and 10.8% for CSF) of the total number (9,486) of potential protein targets represented on the array (Table 2). However, as is evident in the RFU histograms shown below in Figs. 1 and 2, our attempt to adjust the relative dilutions of plasma and CSF so that aAB titers fall within the dynamic range of sensitivity of the microarray did not yield a perfect match, leading to persistently lower RFUs for CSF aABs. Of course, this across-the-board reduction would lead to fewer CSF aABs reaching the threshold of detection, thus making it invalid to directly compare the number of aABs in CSF and serum using this data. Nevertheless, this approach is useful to clearly demonstrate that CSF aAB profiles are extremely complex, likely containing thousands of aABs.

3.2. Essentially all aABs detected in CSF are also present in the blood

We next sought to determine if the expression patterns of aABs in CSF correlate with that in plasma in the same individual. To test this, we compared the identity of the immunoreactive protein targets on protein microarrays probed with CSF and plasma to determine the degree of overlap (Table 2). On average, an exceptionally high percentage (94.5 ± 2.8%, n = 44) of aABs detected in CSF were also detected in plasma. This high percentage of overlap between highly expressed aABs provides further evidence that the source of the vast majority of aABs in CSF is likely to be plasma.

3.3. aAB profiles in CSF mirror that in the blood within the same individual

We next used human protein microarrays to compare aAB profiles in plasma and CSF obtained simultaneously and pre-surgically from individual subjects. To accomplish this, blocks of 75 potential protein targets on microarrays were randomly selected, and RFU values reflecting the extent of aAB binding at each protein spot are shown in Fig. 1. The left and right panels compare aAB profiles of plasma and CSF obtained from an 84 year-old female non-delirium control subject and an 84 year-old male non-delirium control subject, respectively. RFU values are considered to be proportional to the relative number of aABs from plasma or CSF that are bound to each protein spot on the array, with higher RFUs in most cases linked to higher levels of expression (or titer) of that particular aAB in the blood or CSF. Since the dilution of the plasma working solution is 50-fold higher than CSF, it is clear that the expression of aABs in blood far exceeds that in CSF. When histograms representing the RFUs from each of the 75 protein target spots on the microarrays are aligned, the observed patterns of RFUs derived from plasma and CSF are closely matched. This data also suggests that neither advanced age nor gender has an influence on this relationship, since the closely similar pattern of aAB expression in plasma and CSF is demonstrated in eight randomly selected blocks of 75 proteins (four from a female subject, and four from a male subject) (Fig. 1).

Table 1
Patient demographic information.

	STRIDE Subjects			Healthy Control
	Total (n = 46)	Non-Delirium Control (n = 24)	Delirium Patients (n = 22)	Longitudinal Samples (n = 5)
Age, years				
Mean (SD)	81.6 (8.1)	77.1 (7.5)	86.6 (5.5)	n/a
Range	65–96	65–91	76–96	53–62
Sex, n (%)				
Male, n (%)	12 (26)	6 (25)	6 (27)	1 (100)

The number of individuals (n), age and gender are listed for each group, as well as the 5 longitudinal samples from a single healthy control individual.

Table 2
Average number and overlap of aABs in plasma and CSF.

	Number of aABs in plasma, mean (SD)	Number of aABs in CSF, mean (SD)	CSF to plasma overlap, mean (SD)	CSF to plasma overlap percentage, mean (SD)
HFR Patients (n = 44)	3149 ± 1251	1025 ± 346	971 ± 347	94.52 ± 2.8

The average number of aABs detected in the specified dilutions of CSF and plasma obtained from STRIDE hip fracture repair patients (n = 44) using human protein microarrays was determined using a Z-factor > 0.4 and a minimum signal intensity of 1500 RFU. An outlier test was performed that resulted in the exclusion of 2 patient samples. Many more aABs were detected in plasma than in CSF and a very high percentage of aABs detected in CSF were also found in plasma.

3.4. aAB profiles of plasma and CSF are unique to each individual

Closer examination of aAB profiles described above suggests that each individual possesses a unique aAB profile. To further examine this possibility, RFUs from the same randomly selected block of 75 protein targets were plotted for each of the four subjects. In Fig. 2, each of the four plots demonstrates the same closely matched aAB expression pattern between CSF and plasma in a single individual. However, the pattern of each clearly differs from person to person, with no two people sharing an identical pattern. Although individuals share many common peaks corresponding to specific protein targets (e.g., see protein target 25), the amplitude of many other RFU peaks displays a wide range of variability, presumably reflecting marked variations in aAB titers between different individuals.

3.5. Blood aAB profiles show a remarkable degree of fidelity over time

To examine how and to what extent aAB profiles vary over time, five longitudinal blood samples were obtained from a single healthy individual spanning a period of 9 years, and the plasma was used to probe human protein microarrays. The aAB profiles for four randomly selected groups of 50 protein targets are shown in Fig. 3. Remarkably, aAB profiles remained essentially unchanged in this individual over the 9 year period, suggesting that the relative titers, as reflected by the measured RFUs, can remain relatively constant in a healthy individual over a long period of time. This provides strong evidence that, in the absence of pathology, each individual has a unique and stable baseline aAB profile in the blood that demonstrates a high degree of fidelity over time.

3.6. An elevated CSF/plasma aAB reactivity ratio suggests increased risk for post-operative delirium (POD) in elderly hip fracture repair patients via blood-brain barrier (BBB) compromise

A number of studies in humans and animal models have suggested that increased BBB permeability may be linked to an elevated risk of POD in the elderly (Yang et al., 2017). Here, we tested this possibility using pre-surgical, simultaneously drawn plasma and CSF samples from the same individuals. With the RFUs from each protein target spot on the microarray corresponding to the relative abundance of aABs binding to it, we calculated a CSF/Plasma (CP) aAB ratio for each protein target on the array. As the relative titer of CSF aABs increases as indicated by elevated CSF RFU values, the CP aAB ratio also increases, presumably reflecting the severity and/or extent of BBB compromise. An outlier test was performed on the CP aAB Ratio data, leading to exclusion of three non-delirium control samples and one delirium patient sample. As shown

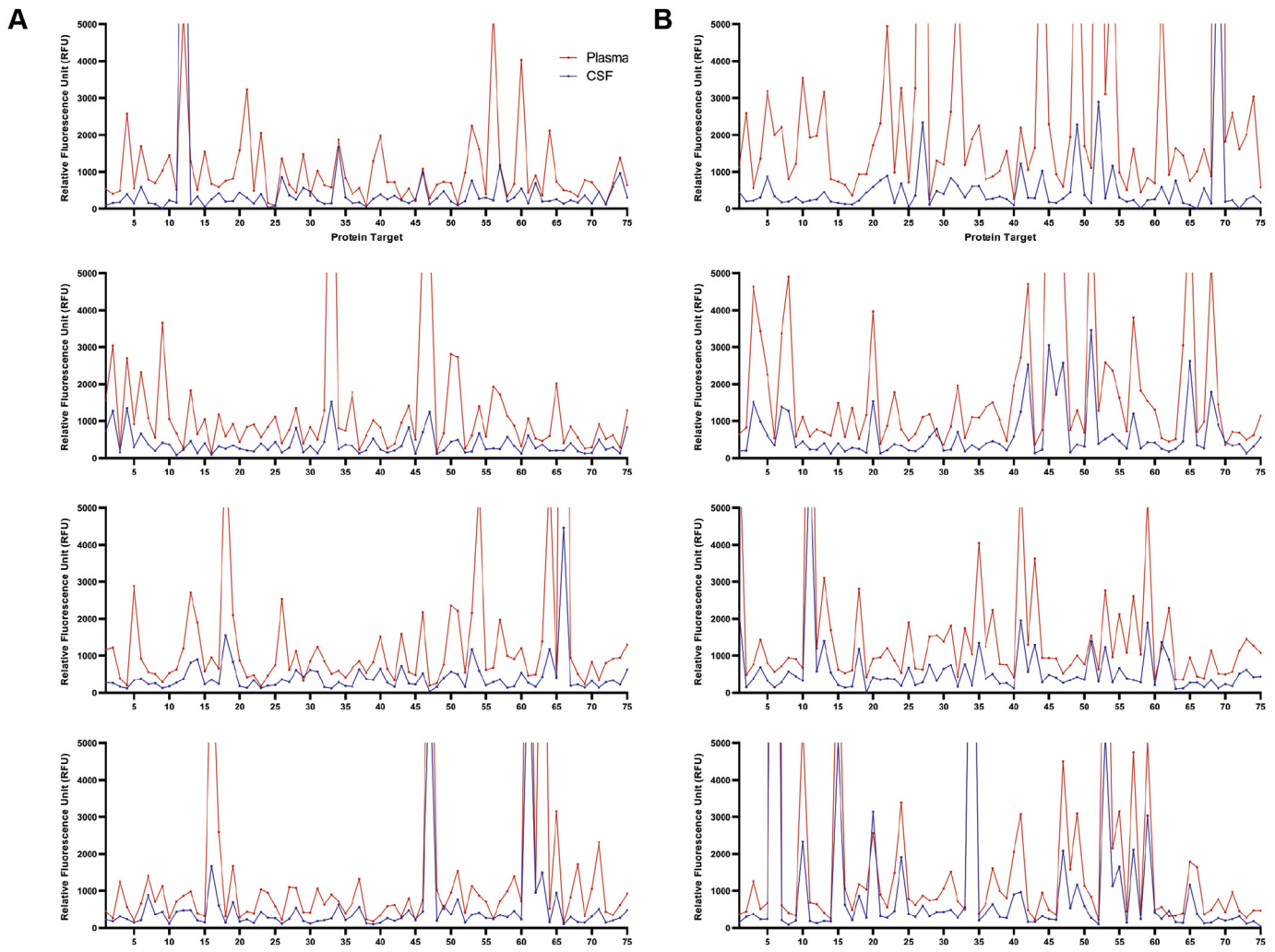

Fig. 1. Comparison of aAB reactivity in plasma and CSF obtained from female and male non-delirium control subjects.
Blocks of 75 proteins on human protein microarrays that were probed with diluted plasma and CSF were randomly selected to generate plasma (red line) and CSF (blue line) aAB profiles derived from an 84 y/o female non-delirium control subject (A) and an 84 y/o male non-delirium control subject (B). When the histograms representing the RFUs from each of the 75 protein targets in plasma and CSF are aligned, the observed pattern of RFUs in plasma and CSF is closely matched, and neither advanced age nor gender was found to influence this relationship.

in Table 3, the mean CP aAB ratio for non-delirium controls was 0.57 ± 0.2, whereas that for the delirium patients was 0.96 ± 0.63. Using a Student's t-test, the difference between the mean CP aAB ratio of delirium vs. non-delirium subjects was shown to be significant ($p = 0.013$), thus supporting a link between increased BBB permeability, higher CSF aAB titers and risk for POD. In support of this link, when patients were ranked according to their day 1 post-op Delirium Rating Scale-R-98 (DRS-R-98), five out of the six patients who had a CP aAB ratio higher than one as shown in Fig. 4 were in the top half, indicating that they had more severe symptoms of delirium.

4. Discussion

In the present study, we have used human protein microarrays to determine if CSF, like blood, exhibits a similarly complex IgG aAB profile and, if so, examine its characteristics and investigate the possible origin of these aABs. The subjects for this study were elderly hip fracture repair (HFR) patients, and the blood and CSF used were obtained pre-surgically and simultaneously. This study has six major findings. First, we show that CSF, like blood, has an extremely complex aAB profile composed of thousands of different aABs, although the levels of their expression in CSF are far lower than that in blood. Second, essentially all aABs detected in CSF are also present in the blood, suggesting the likely possibility that aABs in the CSF originate from blood, presumably through the blood-brain and blood-CSF barriers. Third, in strong support for a blood-based origin for CSF aABs, we found that levels of expression of individual aABs in the CSF mimic expression levels of the same aABs in the blood, regardless of age, gender or the presence or absence of disease. Fourth, aAB profiles in both blood and CSF have some features that are unique to each individual, collectively contributing to an individual's "aAB fingerprint". Fifth, we show that individual blood aAB profiles are remarkably consistent over long periods of time in overall healthy individuals, raising the possibility that each aAB has its own "set point" of constitutive expression in the absence of disease and other body stressors. Lastly, in elderly individuals undergoing hip fracture repair surgery, an elevated CSF/plasma aAB ratio, suggestive of increased blood-brain and/or blood-CSF barrier permeability, is more common in patients that experience post-operative delirium (POD), suggesting that barrier compromise is an important risk factor for POD.

Historically, the appearance and detection of aABs in the blood has been attributed to immune dysregulation that facilitated their production (DeMarshall et al., 2015a). The most well-known instances of this are the autoimmune diseases, hallmarked by the presence and often over-expression of specific aABs that are somehow linked to the pathology

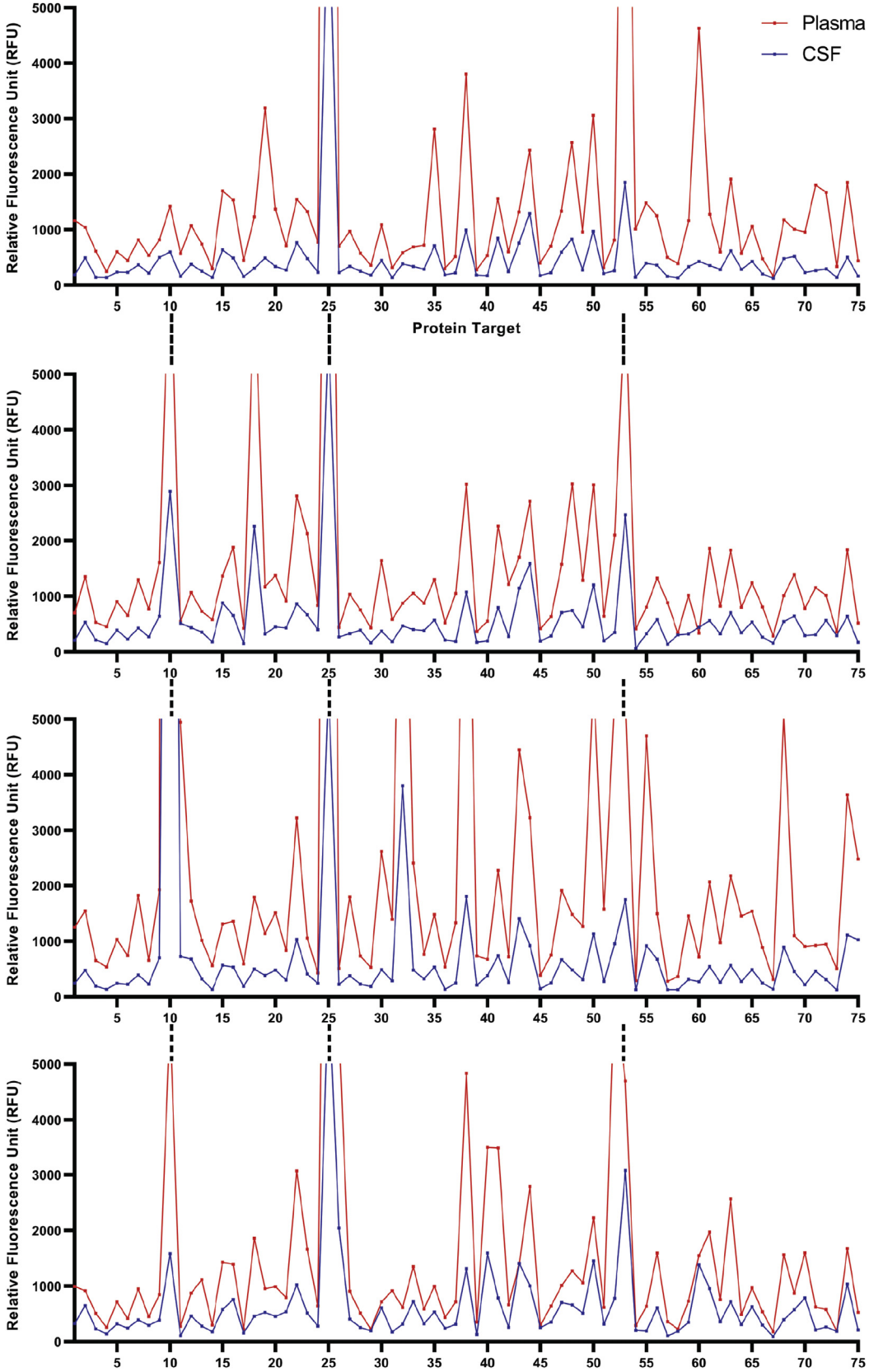

Fig. 2. Comparison of aAB reactivity in plasma and CSF among four different individuals.
RFUs from an identical block of 75 randomly selected proteins were plotted for four individual subjects to demonstrate the close matching of plasma (red line) and CSF (blue line) aAB profiles. Dashed lines indicate peaks representing aABs that are common among the four individuals.

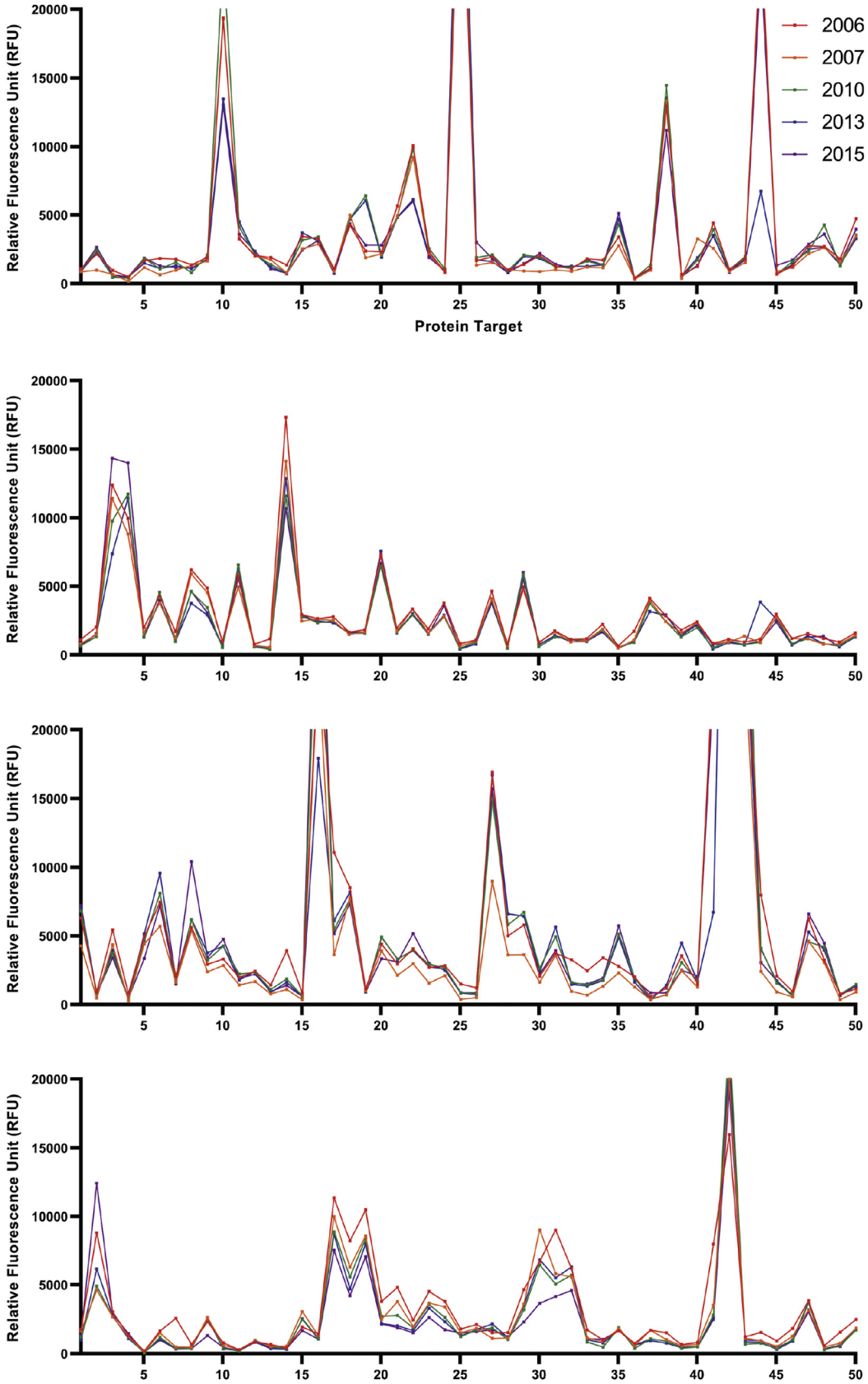

Fig. 3. Fidelity of aAB profiles in a single individual over a period of 9 years.
Four different blocks of 50 randomly selected proteins on human protein microarrays that were probed with diluted plasma (red line) and CSF (blue line) showing aAB profiles of a single healthy individual spanning a period of 9 years. aAB profiles remained essentially unchanged over the 9 year period, providing strong evidence that, in the absence of pathology, each individual has a unique and stable baseline aAB profile in the blood that demonstrates a high degree of fidelity over time.

Table 3
Comparison of CSF/Plasma (CP) aAB ratios in hip fracture repair patients with and without post-operative delirium.

	Non-Delirium Control Patients	Delirium Patients
n	21	21
mean (SD)	0.57 (0.2)*	0.96 (0.63)*

The CP ratio was calculated for delirium vs. non-delirium patient groups, with delirium patients showing higher CP ratios than non-delirium subjects (unpaired *t*-test, $p = 0.013$). *significant at $p < 0.05$.

driving the phenotype. The expression of these aABs is not exclusive to one disorder, and each disorder usually has more than just one aAB associated with it. The expression of such aABs has been used as biochemical confirmation of the presence of these diseases, in essence making them biomarkers for their associated disorders (DeMarshall et al., 2015a). These aABs have not only been used to screen for pathology, but fluctuations in their titers often correlate with the course and severity of disease. An increasing number of aABs are showing promise for use as biomarkers for various cancers, including lung, colon, breast, prostate, ovarian and head and neck (Broodman et al., 2017; Chatterjee et al., 2006; Luna Coronell et al., 2018; Qiu et al., 2018; Smith et al., 2008; Ummanni et al., 2015). aABs are also hypothesized to be involved in cancer immunosurveillance, responding to insults such as mutation, degradation, overexpression of proteins and misfolded proteins (DeMarshall et al., 2015a).

Our previous studies using highly sensitive human protein microarrays have shown that, contrary to the well-established principle of self-tolerance, a central tenet of immunology, thousands of different aABs typically populate the blood. These aABs comprise an extremely complex profile in the blood, the nature of which is strongly influenced by age, gender and presence of disease (DeMarshall et al., 2015a, 2015b; Nagele et al., 2013). Self-reactive immunoglobulins have been detected in a variety of biological fluids, including blood, colostrum, saliva and CSF (DeMarshall et al., 2015a). In the present study we have shown that, like blood, CSF typically contains thousands of aABs. With only roughly one-third of the human proteome represented on the protein microarrays used in the present study, it is necessary to multiply the number of aABs detected on these microarrays by a factor of three to estimate the actual number of aABs present in blood and CSF. In the population of elderly HFR patients who were the subjects of this study, the mean number of aABs detected in CSF and blood using this approach was 1025 and 3149, respectively, which translates to a total number of 3075 for CSF and 9447 for blood. Although it appears that blood seems to possess significantly more aABs above the minimum signal intensity of 1500 RFU than CSF, is it important to point out that this disparity could potentially be due to the difference in baseline IgG titer and final working dilutions used to probe the microarrays. Furthermore, taking these differences into consideration, it is very likely that the vast majority of aABs in the blood and CSF would demonstrate significant overlap in identity, as well as total number, which would account for the symmetry observed between CSF and plasma profiles from the same individuals displayed as shown here. It is also important to note that this study has focused only on the IgG subtype of immunoglobulins, and that we recognize that other immunoglobulin subtypes may also be involved, which will likely increase the total number of aABs in both CSF and blood. Further studies will be needed to investigate potential aAB profiles involving other Ig subtypes.

We have proposed that one function of this extraordinarily complex aAB system is the maintenance of body-wide homeostasis via cell/tissue debris clearance (DeMarshall et al., 2015a; Nagele et al., 2013). As a result of normal "wear and tear" in otherwise healthy individuals, it is expected that the body generates a certain amount of soluble debris every day that eventually makes its way into the various body fluids, especially the blood. Since this cell-derived debris would include the potential release of many thousands of different proteins, there would be a constant need for an extremely complex aAB profile to facilitate their daily removal. In the case of disease, the increased amount of debris released from the site of pathology would be expected to be at least somewhat disease-specific, since its composition would depend on the tissues/organs affected. If aAB profiles are truly involved in debris clearance and adaptive, one would expect a shift in aAB profiles reflecting the increased production of those aAB working to clear this debris. This concept has been the basis for our biomarker discovery strategy. We have already demonstrated the utility of using human protein microarrays to identify panels of aAB that are differentially expressed during disease that can be used as diagnostic indicators or biomarkers of several neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease and multiple sclerosis (DeMarshall et al., 2015b, 2016, 2017; Han et al., 2012; Nagele et al., 2011a).

We demonstrate here that essentially all aABs detected in CSF are also present in the blood (94.5 ± 2.8% overlap). Furthermore, the aAB profiles of CSF, which include the expression levels of individual aABs as relative RFUs, mirror that in the blood. Together, this provides strong evidence that the source of the vast majority of aABs in CSF is the blood. There are several potential avenues of entry for aABs into CSF from the blood, the most important being through the blood-brain and blood-CSF barriers. Although we recognize blood as the main source of CSF aABs, there are also a very few aABs that appear in CSF that are not detected in blood using the cutoff of RFUs mentioned above, e.g., antibodies directed against ROBO3 and KCNAB3 proteins. The identity of these few

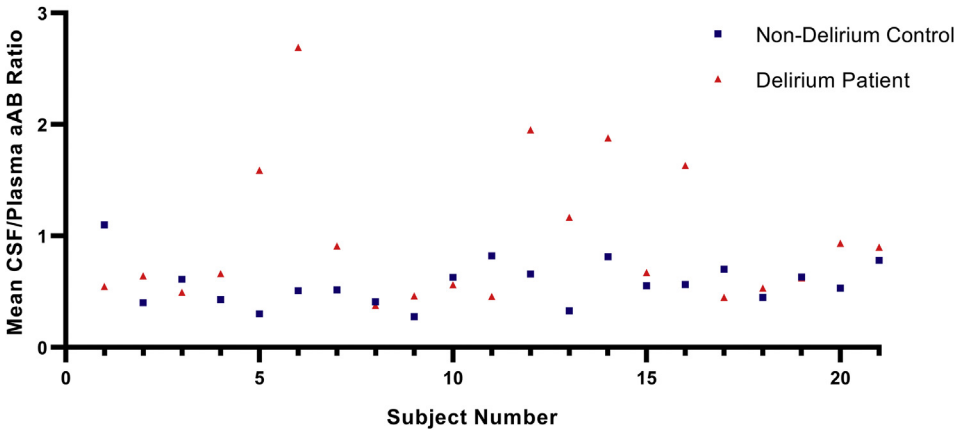

Fig. 4. Comparison of mean CSF/Plasma (CP) aAB ratios in hip fracture repair patients with and without post-operative delirium.
Comparison of individual CP aAB ratios of delirium patients (n = 21, red triangle) and non-delirium controls (n = 21, blue square). An outlier test was performed excluding a total of 4 patient samples. Note that the highest CP aAB ratios are among delirium patients.

aABs present exclusively in CSF seem to vary from one individual to the next, although it is possible that their low expression in CSF in combination with our RFU>1500 cutoff contributes to this variability. Nevertheless, their presence supports the possibility that this small subset of CSF-specific aABs may originate from an intrathecal immune system present in the brain of these individuals, which has been reported in previous studies (Anthony et al., 2003; Bouras et al., 2005; Petzold, 2013).

Comparison of aAB profiles among different individuals has revealed that many aABs show common patterns of expression in blood and CSF across the population. This was determined by comparing the aAB RFU profiles of randomly selected blocks of protein targets on the microarrays. We also found that, in addition to many aABs with common levels of expression, many aABs also show individual-specific alterations in aAB expression profiles, providing what amounts to an "aAB fingerprint" that is unique to each individual. Importantly, we also show here that, in otherwise healthy individuals, aAB profiles remain remarkably stable over long periods of time, as demonstrated in Fig. 3, with samples spanning nearly a decade. We hypothesize that, in the absence of pathology, significant body growth and renovation or physical decline associated with advanced age, aAB profiles of healthy individuals remain stable. We refer to this stable profile as the "baseline aAB profile" which, in the absence of stressors to the body, is maintained in each individual. This baseline aAB profile in each subject is composed of two conceptual subsets of aABs: a larger subset with levels that are comparable among multiple individuals and a much smaller subset with levels significantly higher or lower than the norm, thus making these aAB "stand out" as peculiar to this individual (i.e., contributing to an individual "autoantibody fingerprint"). Taken together, this suggests the presence of an "aAB setpoint" for each individual that is linked to the current status of the body. Deviation from this baseline or set point could be triggered by a number of events and body stressors, such as surgery, infections, menstruation, pregnancy, sudden weight loss and other short- and long-term pathologies.

We also investigated whether or not elevated risk for post-surgical delirium is associated with increased barrier permeability in elderly HFR patients from the STRIDE study. In an effort to test this, we calculated the pre-surgical CSF/Plasma (CP) ratio for each aAB detected and then the average CP aAB ratio representing all aABs detected for each individual, where a higher CP aAB ratio would imply an increased barrier permeability. Using this approach, the mean CP aAB ratio for patients experiencing delirium was significantly higher than that for non-delirium controls, suggesting that increased barrier permeability increases risk for POD. In previous *in vitro* and *in vivo* studies in mice, we provided evidence that, under conditions of blood-brain barrier compromise, aABs gain access to the brain interstitium and can bind to exposed cognate targets on the surfaces of neurons and glia (Acharya et al., 2013; Levin et al., 2010; Nagele et al., 2011b). Indeed, in the brains of patients with Alzheimer's disease, the same neurons showing particular vulnerability to AD pathological changes, including intraneuronal beta-amyloid deposition, are also the cells that are the most IgG-positive (Nagele et al., 2011b). This raises the possibility that the chronic binding of IgG to neuronal surfaces under conditions of blood-brain barrier breach may play a key role in POD following surgery in the short-term, as well as the pathogenesis of neurodegenerative diseases, such as AD, PD, and MS, among others, in the long-term.

This study has several strengths. The first is the availability of simultaneous pre-surgical blood and CSF samples from a cohort of elderly HFR patients. This enabled us to determine that each individual possesses their own unique CSF and blood aAB profiles. Another is the availability of multiple longitudinal serum samples from the same healthy individual over a 9 year period, which allowed us to demonstrate the remarkable stability of aAB profiles over time. Finally, a significant strength of this study was the use of Invitrogen's Protoarray human protein microarray as a platform. Encompassing roughly one-third of the human proteome, these microarrays allowed us to survey nearly 10,000 full-length native-folded protein targets using a high-throughput, sensitive, and unbiased protocol. This study also has several weaknesses, the most significant being the small sample size. Another limitation is the demographic homogeneity of the patient group, which limits the data to this small cohort of elderly individuals receiving hip fracture repair surgery. Although intended as a "proof of concept" study, we acknowledge the need for additional studies in larger community-based populations to further expand the scope of this work. An additional limitation of this study is the absence of matching CSF samples to accompany the longitudinal serum samples from the single healthy individual over the span of 9 years. Due to the invasive nature and risk of infection, obtaining serial lumbar punctures from this individual was not possible. As a result, although we expect to see maintenance of the same closely matching pattern of CSF and blood aAB profiles, we have not confirmed the fidelity of CSF aABs longitudinally over this time period.

5. Conclusion

We demonstrate, for the first time, evidence for the ubiquitous presence of thousands of individual aABs in human CSF, and also show that their profile closely mirrors that in the blood. Furthermore, we have found that while individuals may share common features, each person possesses their own unique CSF/blood aAB profile, akin to an "aAB fingerprint". Due to the striking similarity in pattern between the CSF and blood of every individual studied, we speculate the origin of these aABs in the CSF to be a result of blood-CSF and blood-brain barrier penetration, the latter allowing the infiltration of aABs into the brain parenchyma as a possible driver of subsequent neuropathology. However, the contribution of an intrathecal immune system in the brain cannot be ruled out, although it would only account for a very small fraction of total CSF aABs. We also demonstrated that in otherwise healthy individuals, aAB profiles remain remarkably stable over long periods of time, suggesting a production "set point" for each aAB that is linked to the "current status" of the body. Lastly, data in this study suggests that an increased CSF/plasma aAB ratio is indicative of increased blood-brain barrier (BBB) permeability in elderly HFR patients experiencing greater risk for post-operative delirium (POD). Based on our previous studies, we have demonstrated that both age and the presence of disease cause specific alterations in aAB profiles that have utility in the diagnosis and staging of various neurodegenerative diseases, with the potential for early or preclinical disease detection and disease monitoring during pharmacological treatment. Taken together, our data lead us to suggest that the observed extensive production of aABs for the purpose of soluble debris removal from the various body fluids involves a separate component of the adaptive immune system. Much additional work is needed to determine its origin, features and the detailed mechanisms associated with its function.

Author contributions

RN conceived and designed the experiments. RK, CD, and RN wrote the first draft of the article. Microarrays were processed and analyzed by CD and RK. All authors contributed to manuscript writing/revision. Statistical analysis was performed by RK and CD. Hip fracture repair patient plasma and CSF samples were provided by FS and EO.

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Declaration of competing interest

None.

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