Oligoclonal immune response in cerebral cavernous malformations

Laboratory investigation

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Object. Mechanisms of cerebral cavernous malformation (CCM) pathogenesis include genetic predisposition in some cases, but other factors are likely to be involved in lesion proliferation and clinical manifestations. Given the unique antigenic milieu of CCMs, there may be a characteristic immune response in these lesions. We hypothesize that the immunoglobulin (Ig) fraction in CCMs reflects an oligoclonal immune response not present in paired sera from the same patients or in other types of cerebrovascular malformations.

Methods. Surgically excised lesions from five patients with CCMs, three patients with arteriovenous malformations (AVMs), and four normal brain control specimens obtained at autopsy were homogenized and extract tested for IgG clonality by isoelectric focusing in parallel with each patient’s serum.

Results. The authors detected B cells in all three lesions examined, and plasmacytes in two out of three lesions examined. Four of five extracts of homogenized CCMs showed an oligoclonal pattern of IgG distinct from the polyclonal pattern seen in those patients’ sera. Immunoglobulin G oligoclonality was not seen in AVMs or control brain specimens.

Conclusions. The results of isoelectric focusing studies showed that CCM lesions had oligoclonal patterns of IgG unrelated to peripheral blood contamination, indicating selective synthesis of IgG within the lesions. This finding probably reflects a clonal expansion of B cells and/or plasmacytes in CCMs, an event that might be antigen-driven or a potential marker of inflammation. (DOI: 10.3171/JNS-07/11/1023)

KEY WORDS • arteriovenous malformation • cavernous angioma • cerebral cavernous malformation • immune response • isoelectric focusing • oligoclonality

Cerebral cavernous malformations affect more than one in 200 individuals in the general population, exposing them to a lifetime risk of hemorrhagic stroke and epilepsy.2 The CCMs are characterized by a phenotype of dilated “leaky capillaries” and angiogenic proliferation within the lesions.10 The abnormal vascular phenotype is likely mediated by genetic predisposition and/or somatic mutation of cytoskeleton-associated protein.14 The proliferative potential of individual lesions is highly variable, and other factors are probably involved in lesion growth and clinical manifestations. Using microarrays, our group documented differential upregulation of 13 Ig-related genes in CCMs.11 Normally, Igs are not synthesized within the CNS, but when B cells enter the CNS—if they proliferate in response to local antigen stimulation—they may undergo clonal expansion and differentiate into plasma cells. Each resulting plasma cell clone will secrete a unique Ig. Because there are only a relatively small number of plasma cell clones within the CNS (in contrast to the number in peripheral tissues), the resulting Ig may be of restricted heterogeneity, manifesting itself as “oligoclonal banding” on electrophoretic analysis. This differs from the usual peripheral Ig synthesis, which is polyclonal and shows a heterogeneous or polyclonal electrophoretic pattern. A potential role of the immune response in CCMs has not been postulated previously. Our objectives are to investigate the immune response in CCMs through evaluation of local synthesis and oligoclonality of IgG in these lesions.

Materials and Methods

This study included patients undergoing surgical excision of CCMs for clinical indications unrelated to the research study. The study was approved by our institution’s institutional review board and the participants gave informed consent for the processing of a portion of their surgical specimen and a blood sample for research purposes.

Immunohistochemical staining of three archived CCM specimens was performed at the Evanston Northwestern Healthcare Department of Pathology to evaluate CCM lesions for the presence of B cells and/or plasma cells. Anti-CD20 (L26, human B-cell specific) and anti-CD138 (BC/B-B4, human plasma-cell specific) antibodies, obtained from Biocare Medical, were used to stain 5-μm sections from CCM specimens embedded in paraffin blocks. Antigen retrieval was accomplished by treating the specimens in a pressure cooker

Abbreviations used in this paper: AVM = arteriovenous malformation; CCM = cerebral cavernous malformation; CNS = central nervous system; CSF = cerebrospinal fluid; Ig = immunoglobulin.
Macrophage inflammatory cells have long been recognized to infiltrate CCM lesions, especially in reaction to acute bleeding.\(^{14}\) Macrophages were also recently noted in histological analysis of the first CCM lesions produced in transgenic mice (\(\text{Ccml}^{-/-}\text{Trp53}^{-/-}\)).\(^{9}\) Nevertheless, the presence of other inflammatory cells, especially those involved in an antigenic response has not, to our knowledge, been examined previously in these lesions. Our findings of B cells and plasma cells in CCM lesions provide preliminary evidence for the existence of an intrallesional humoral immune response. The preliminary observations of antibody-producing cells in a limited number of lesions will need to be confirmed in a larger number of cases, with specific correlation to recent lesion behavior. Such a study is already underway in our laboratory. The findings reported in the current study and previous results showing upregulation of several Ig genes\(^{11}\) in CCM lesions motivate us to further investigate the nature of this immune response. The demonstration of IgG of restricted heterogeneity within CCM lesions suggests that the immune response is produced by a limited number of plasma cell clones.

Oligoclonal IgG bands can be identified in the CSF of patients with a variety of infectious and inflammatory conditions involving the nervous system. These include multiple sclerosis, acute disseminated encephalomyelitis, Guillain–Barré syndrome, subacute scribing panencephalitis, progressive rubella panencephalitis, and acute viral encephalitis.\(^{12}\) Oligoclonal IgG bands are also present in the CSF of a number of patients with structural CNS lesions, in whom no evidence for an infectious or inflammatory condition affecting the CNS can be found.\(^{1}\) Oligoclonal IgG bands are also detected in cases of acute subarachnoid hemorrhage and stroke, often in both CSF and blood from these patients, probably representing a systemic response to these diseases.\(^{13}\) In addition, oligoclonal bands have also been described specifically in the CSF of patients with sphenoidal arteriovenous fistulae, frequently associated with venous hypertension myelopathy. To our knowledge, oligoclonal IgG bands have never been reported in cerebrovascular lesions themselves.

The clinical course of CCMs remains highly unpredictable because biological mechanisms underlying proliferative lesion phenotypes have not been elucidated. This is the first documentation of oligoclonal IgG in CCM lesions. The CCM lesions probably contain a significant amount of thrombus at various stages of organization in sequestered caverns. Although some of the lesional thrombus may simply reflect peripheral blood contamination, there may be differences in the gamma globulin composition within CCMs and peripheral blood, including the particular Igs associated with sequestered thrombus. The absence of identical oligoclonal bands in paired sera from the same cases effectively excludes blood contamination as a source of the intrallesional oligoclonal IgG and suggests focal synthesis in the CCM lesions.

Oligoclonal IgG was absent in AVMs, which are phenotypically discrete cerebrovascular abnormalities. The AVMs indeed lack the leaky endothelial cell layer and repetitive hemorrhages associated with CCM, or the clustering of organized clot in brain parenchyma.\(^{13,18}\) Such features of CCMs may reflect the characteristic immune response apparently associated with CCM but not with AVM. The absence of IgG oligoclonality in brain AVMs appears to contradict a previous report of CSF oligoclonal bands in three of 11 patients with spinal AVMs,\(^{3}\) but this difference in findings is probably due to the different nature of spinal AVMs and cerebral AVMs. Spinal AVMs indeed represent dural fistulae and cause ischemic myelopathy from secondary venous hypertension, and associated CSF oligoclonal bands could reflect this secondary myelopathy. Oligoclonal IgG has never been reported in AVM lesions themselves.

Oligoclonal IgG bands were also absent from normal brain and blood vessels in four control cases, even when they were present in the serum in two of these cases. Their presence in the serum was probably due to the specific B-
cell clonal expansion in one patient with multiple myeloma, and to inflammation from multiple organ dysfunction after coronary bypass surgery in the second patient. The absence of the oligoclonal IgG bands in these patients’ brains was probably due to the integrity of their blood–brain barrier.

The demonstration of oligoclonal IgG in four of five CCM lesions provides evidence for a characteristic focal immune response. A focal immune response with Ig oligoclonality has been associated with abnormal vascular proliferation in a number of pathological conditions.\textsuperscript{5,6,8} An IgG response to infection is associated with proliferative hemorrhagic capillaries, similar in histological characteristics to CCMs.\textsuperscript{7} The apparent local synthesis and oligoclonality of IgG in CCM lesions probably suggests clonal expansion of B cells and/or plasma cells in CCM lesions, an event that might be antigen-driven. The CCM phenotype predisposes to vascular leakage and accumulation of blood products in adjacent brain tissue. This phenomenon may create a special milieu for antigenic challenge and immune response and in turn contribute to lesion proliferation and clinical manifestations.

The sequestered blood and chronic accumulation of blood breakdown products within CCM lesions may be the trigger of this humoral immune response. The blood from acute brain hemorrhages is absorbed over time, and may not have an opportunity to trigger a similar response. \textsuperscript{9}

\textsuperscript{8}German and Schlichter\textsuperscript{15} examined the inflammatory response to intracerebral hemorrhage in a rat model, and described microglial and macrophage infiltration but no antibody-producing B cells or plasma cells. A unique aspect of CCM lesions is repetitive hemorrhage, which results in longstanding deposition of blood products as well as the presence of sequestered blood in caverns. These features may be responsible, in part, for the immune response that we observed. The question of whether an oligoclonal immune response is evoked in relation to the duration and type of blood product deposition in the brain should be examined in future studies.

A focal immune response in CCMs could explain, in part, why some CCM lesions remain biologically dormant, whereas others proliferate with serious clinical consequences. Or it could represent a reaction to and potential marker of lesion proliferation and hemorrhage. Studies are underway in our laboratory, with an aim of correlating inflammatory response in CCM with recent lesion proliferation and hemorrhage. Future studies are planned to confirm oligoclonality of the immune response by assessing the distribution of lengths of the third complementarity-determining regions (CDR3s) of the Ig heavy-chain genes in mRNA isolated from pooled plasma cells and B cells laser-captured from CCMs, in comparison with peripheral lymphocytes from the blood of the same patients. Identification of an autoimmune or extrinsic antigenic trigger could possibly con-
Conclusions

Because macrophages have already been found in CCM lesions, we searched for other cells that may contribute to inflammation within CCMs. Our discovery that B cells and plasmacytes are present within CCM lesions suggests an oligoclonal humoral immune response within CCMs. A more systematic analysis of inflammatory cell distribution in these lesions is already underway in our laboratory, including correlation with recent lesion behavior. We demonstrated, by isoelectric focusing, that there are indeed oligoclonal patterns of IgG in CCM lesions that are independent of peripheral blood contamination. These findings, which indicate selective production of IgG within the lesions, constitute the first demonstration of oligoclonal IgG in CCMs. This possible local clonal expansion of B cells and/or plasma cells in CCMs might be an antigen-driven event. A partial explanation for why some CCM lesions remain biologically quiescent whereas others proliferate with grave clinical consequences may result from the specific characteristics of this immune response. This immune response could also be a reaction to and potential marker of lesion proliferation and hemorrhage in CCMs. Future work may lead to identification of the antigenic trigger for the characteristic immune response in CCMs.

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