Thrombotic processes in multiple sclerosis as manifestation of innate immune activation

Tatiana Koudriavtseva*

Unit of Neurology, Multiple Sclerosis Center, Regina Elena National Cancer Institute, Rome, Italy
*Correspondence: koudriavtseva@ifo.it

INTRODUCTION

Multiple sclerosis (MS) is a chronic demyelinating inflammatory disease of the central nervous system (CNS) affecting prevalently young adults (1, 2). The pathogenesis of MS has long been attributed to self-reactive T cells but recently the relevant role of B cells has also been recognized (2, 3). Furthermore, it has been demonstrated that innate immunity has a pivotal role in the beginning and in advanced stages of MS (4–6).

Innate immunity represents the immediate non-specific defense against infections and dangerous agents acting through its essential arms such as inflammation and blood coagulation (7–10). The coagulant processes are normally balanced by the natural anti-coagulant system needed to limit the host damage, and their imbalance leads to venous thrombosis (7, 8).

Many evidences reported below support a significant presence of local and systemic thrombotic events in MS confirming the global over-stimulation of innate immunity for both its inflammatory and coagulant components.

BACKGROUND

VENOUS THROMBOEMBOLISM AND CEREBRAL THROMBOSIS IN MS

Several extensive studies have shown the association between venous thromboembolism and MS from its early stages. In a large population-based cohort study evaluating data from a 30-year Danish National Registry, MS was associated with increased long-term risk of venous thromboembolism (11). Another paper, based on the review of one cohort study and three cross-sectional studies, found that early MS was

associated with an increased risk of pulmonary embolism, deep venous thromboembolism, and stroke, both the latter persisting over time (12). Other large studies have shown an increased risk of venous thromboembolism in MS as well as in several other immune-mediated illnesses (13–15).

Cases of cerebral thrombosis have been also reported in MS, mainly attributed to the effect of lumbar puncture and methylprednisolone therapy, however, in some cases no pre-disposing condition was found (16–19).

Moreover, a collaborative genome-wide association study screen in MS patients, not only implicated a multitude of genes coding for cytokine pathway, co-stimulatory, and signal transduction molecules of immunological relevance, but also for a vascular cell adhesion molecule (VCAM1) mediating leukocyte–endothelial cell adhesion and signal transduction (20). Interestingly, thrombin is able to induce expression of functional forms of VCAM1 on endothelial cells (21) and this expression can be inhibited by the specific thrombin inhibitor, hirudin. In my opinion, these data may further support the association between coagulation system and inflammation as well as between components of innate and adaptive immunity in MS.

PRO-THROMBOTIC CONDITIONS AND VASCULAR DYSFUNCTIONS IN MS

There are several studies confirming platelet (22–24) and complement (25–27) involvement in MS, both essential for the innate immune response by linking inflammation and coagulation. Moreover, many vascular dysfunctions have been reported

in MS. They have been largely resumed in the review of D'Haeseleer and colleagues (28) facilitating their discussion in the restricted space of this work.

Vascular risk factors in MS

Interestingly, in the review of D'Haeseleer and colleagues, all the vascular risk factors reported to be associated with MS such as reduced physical activity, smoking, endothelial dysfunction, platelet activation, thrombophilia, and hyper-homocysteine are essentially pro-thrombotic conditions. Conversely, other vascular risk factors such as atrial fibrillation, flutter, arterial hypertension, diabetes mellitus, dyslipidemia, and obesity have not been associated with MS (28).

Cerebral hypoperfusion in MS

Decreased cerebral blood volume and flow, as well as its prolonged brain mean transit time, were demonstrated in all forms of MS and even in clinically isolated syndrome, both in white and gray matter, by SPECT, PET, and dynamic susceptibility contrast-enhanced MRI (28). In my opinion, the widespread cerebral hypoperfusion in MS may be prevalently determined by the blood flow deceleration in the venous bed due to the inflammatory-thrombotic processes. This consequently leads to decreased arterial flow with reduced tissue oxygenation and energy metabolism clarifying the similarities between ischemic and MS lesions for both the histopathological features and enhanced expression of hypoxia-inducible factors mentioned by D'Haeseleer et al. This explanation of cerebral hypoperfusion appears to be more effective compared to

both reduced axonal activity and decreased astrocyte energy metabolism proposed in the review as the possible explanation for hypoperfusion.

Venous blood drainage

According to D'Haeseleer et al., I think that MS could unlikely be caused by the obstructed extracranial venous drainage. The CCSVI theory explains the perivenular pattern of MS lesions by extravasation and degradation of erythrocytes due to blocked venous drainage with consequent iron deposition causing the inflammatory demyelinating lesions. However, the perivenular distribution of MS lesions, named "Dawson's fingers," prevalently around the ventricle-based brain veins, has long been thought to be the result of inflammation and coagulation around the major axis of medular veins (29).

Following the first single center study (30), many groups have tried to replicate their findings, but largely without success. Two recent studies from Italy (31) and Canada (32) provide further evidence to refute the idea of CCSVI as a cause of MS.

Even the short-lasting improvements reported by many operated patients that were considered placebo effect by the authors of the review (28), may be partly attributable to the intra-operatory intra-venous and 3-week subcutaneous low-weight heparin therapy according to Zamboni' protocol (33), since heparin has been used with some success in MS in the 60s (34, 35). However, further studies are needed in order to confirm this hypothesis.

Nevertheless, in my opinion, several studies carried out to test this theory have indirectly demonstrated the presence of the widespread endothelial damage likely due to the persistence of inflammatory-thrombotic processes. The emerging evidences on the link of jugular venous reflux with several CNS diseases have not clarified their cause–effect relationship (36), however, the venous dysfunctions seem to be the common consequence of inflammatory processes on the vascular endothelium rather than the unique anatomic cause of different disorders. Interestingly, a reduction in venous vasculature visibility on susceptibility-weighted imaging venography was already demonstrated, thus suggesting a correlation with both

a decrease in metabolism and venous morphological alterations in the brain (37). These manifestations could correlate to endothelial injury determined by long-term inflammatory-thrombotic processes rather than to the stenosis of extracranial veins. The latter should determine brain venous engorgement in contrast with reduced visibility of venous vasculature.

More convincingly, Kirk and colleagues much earlier found the microvascular endothelial tight junctions abnormalities in vessels of any size, especially in active lesions as compared to normal appearing white matter, supporting a causative role of diffusible inflammatory mediators (38). In fact, the inflammatory factors increase the endothelial permeability and the expression of adhesion molecules determining the extravasation of immune cells and erythrocytes (7, 8).

ROLE OF COAGULATION SYSTEM IN MS AND IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

Many evidences in literature support the role of the coagulation system as an expression of innate immune activation both in MS and in experimental allergic encephalomyelitis (EAE).

In 1935, Putnam already proposed the pivotal role of venule thrombosis in MS (39). Another pathological study in acute MS reported fibrin deposition on endothelial cells in many thin veins and capillaries in areas without myelin damage or reactive parenchymal changes, with some thrombosed vessels or even intra-cerebral hemorrhage (40). Moreover, coagulation proteins were detected within chronic active plaques in MS (41).

Chapman highlighted the relevance of thrombin in inflammatory brain diseases (42, 43). Thrombin has numerous hormone-like functions modulating, among other, microglia and astrocytes (44). The activity of thrombin in the brain is regulated by thrombin inhibitors such as serum antithrombin III and brain protease nexin-1 secreted by glial cells and neurons that confirms the relevance of thrombin regulation in the brain (42, 43). An increase of brain protease nexin-1 levels was shown at early pre-clinical stages in EAE (45) while the plasma thrombin–antithrombin complexes increased immediately prior to

EAE symptoms and decreased in relation to their improvement (46).

The activated microglia, a cellular component of innate immunity in the brain, along with fibrin deposition, were both found in acute/early MS and in rat EAE lesions, representing a first stage of tissue injury before active demyelination and massive T-cell infiltration (47). Fibrin deposition preceded and regulated inflammatory demyelination, as well as genetic or pharmacological fibrin depletion ameliorated both clinical symptoms and inflammatory response in EAE (48). Likewise, hirudin or recombinant activated protein C improved EAE and suppressed pro-inflammatory T-helper1 and T-helper17 cytokines in astrocytes and immune cells (41). The suppression of EAE by dermatan sulfate (49) or low doses of heparins (50) has been long demonstrated.

All reported here data highlight that the activation of coagulation system, linked to innate immunity, is a mandatory process during the early immune reaction toward the tissue damage of any kind. This reaction is independent of the underlying cause of injury and precedes a specific adaptive immune response in both MS and EAE.

DISCUSSION

Although the histopathological studies have only rarely found cerebral venous thrombosis in MS, there is convincing evidence supporting the presence of inflammatory-thrombotic processes during the overall course of the disease, from its early phases. This might confirm the relevant role of innate immunity in MS not only for its inflammatory component (4–6, 47), but also for the coagulant one. These manifestations are present both in the brain and at peripheral level reflecting a systemic stimulation of innate immunity by unknown exogenous factors that may be represented by viral infections (51). In fact, even if the etiology of MS still remains unexplained, viral infections are considered the main environmental factors related to MS as suggested by their association with disease exacerbations (52) as well as by many epidemiological studies and the common presence of oligoclonal IgG in the cerebrospinal fluid of MS patients (51) and by their relevance as non-heritable factors in gene–environment interactions (53).

The early systemic activation of innate immunity suggests a direct triggering by pathogens rather than by an indirect autoimmune CNS reaction. The innate immunity not only stimulates and modulates adaptive immunity at the beginning of disease but also mediates the neurodegenerative changes in progressive phase of MS (5, 6). While many efforts are aimed to better define the function of the innate immune cells in order to modulate their potential therapeutic action in MS (5, 6), the coagulant component of innate immunity, well studied in animals, is not sufficiently evaluated in humans.

The physiological innate immune response to infections does not usually determine the thrombotic manifestations due to an effective action of the natural anti-coagulant system. However, thrombotic manifestations can take place when the natural anti-coagulant system fails to counterbalance the prolonged or intense coagulation processes. These conditions may occur occasionally in immune-competent people during particularly severe infections, or may manifest persistently during chronic latent infections occurring in individuals with unregulated immunity and genetic or environmentally induced impairment of anti-coagulant system. MS and other immune-mediated disorders associated with thromboembolism may fall into this last category. However, there is no doubt that if the activation of innate immunity is directly triggered by viral infection, playing a protective role against infection, our therapeutic intervention should be not greater than necessary to compensate the reduced anti-coagulant capacity.

CONCLUSION

Several evidences support persistent inflammatory-thrombotic processes in MS confirming the prolonged activation of innate immunity, likely by chronic latent infections. Experimental evidences coming from the literature here reported may open new therapeutic perspectives for MS patients. In fact, it is already possible to interfere with the coagulation system at various levels of the cascade, and clinical trials trying to transfer the promising results on EAE to humans are needed.

REFERENCES

1. Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple Sclerosis. *N Engl J Med* (2000) 343(13):938–52.

2. Sospedra M, Martin R. Immunology of multiple sclerosis. *Annu Rev Immunol* (2005) 23:683–747. doi:10.1146/annurev.immunol.23.021704.115707

3. Disanto G, Morahan JM, Barnett MH, Giovannoni G, Ramagopalan SV. The evidence for a role of B cells in multiple sclerosis. *Neurology* (2012) 78(11):823–32. doi:10.1212/WNL.0b013e318249f6f0

4. Weiner HL. A shift from adaptive to innate immunity: a potential mechanism of disease progression in multiple sclerosis. *J Neurol* (2008) 255(Suppl 1):3–11. doi:10.1007/s00415-008-1002-8

5. Gandhi R, Laroni A, Weiner HL. Role of the innate immune system in the pathogenesis of multiple sclerosis. *J Neuroimmunol* (2010) 221(1–2):7–14. doi:10.1016/j.jneuroim.2009.10.015

6. Mayo L, Quintana FJ, Weiner HL. The innate immune system in demyelinating disease. *Immunol Rev* (2012) 248(1):170–87. doi:10.1111/j.1600-065X.2012.01135.x

7. Esmon CT. Interactions between the innate immune and blood coagulation systems. *Trends Immunol* (2004) 25(10):536–42. doi:10.1016/j.it.2004.08.003

8. Esmon CT, Xu J, Lupu F. Innate immunity and coagulation. *J Thromb Haemost* (2011) 9(Suppl 1):182–8. doi:10.1111/j.1538-7836.2011.04323.x

9. Esmon CT. Protein C anticoagulant system – anti-inflammatory effects. *Semin Immunopathol* (2012) 34(1):127–32. doi:10.1007/s00281-011-0284-6

10. Danckwardt S, Hentze MW, Kulozik AE. Pathologies at the nexus of blood coagulation and inflammation: thrombin in hemostasis, cancer, and beyond. *J Mol Med (Berl)* (2013) 91(11):1257–71. doi:10.1007/s00109-013-1074-5

11. Christensen S, Farkas DK, Pedersen L, Miret M, Christiansen CF, Sørensen HT. Multiple sclerosis and risk of venous thromboembolism: a population-based cohort study. *Neuroepidemiology* (2012) 38(2):76–83. doi:10.1159/000335496

12. Christiansen CF. Risk of vascular disease in patients with multiple sclerosis: a review. *Neurol Res* (2012) 34(8):746–53. doi:10.1179/1743132812Y.0000000051

13. Ramagopalan SV, Wotton CJ, Handel AE, Yeates D, Goldacre MJ. Risk of venous thromboembolism in people admitted to hospital with selected immune-mediated diseases: record-linkage study. *BMC Med* (2011) 9:1. doi:10.1186/1741-7015-9-1

14. Zöller B, Li X, Sundquist J, Sundquist K. Autoimmune diseases and venous thromboembolism: a review of the literature. *Am J Cardiovasc Dis* (2012) 2(3):171–83.

15. Ocak G, Vossen CY, Verduijn M, Dekker FW, Rosendaal FR, Cannegieter SC, et al. Risk of venous thrombosis in patients with major illnesses: results from the MEGA study. *J Thromb Haemost* (2013) 11(1):116–23. doi:10.1111/jth.12043

16. Vandenberghe N, Debouverie M, Anxionnat R, Clavelou P, Bouly S, Weber M. Cerebral venous thrombosis in four patients with multiple sclerosis. *Eur J Neurol* (2003) 10(1):63–6. doi:10.1046/j.1468-1331.2003.00513.x

17. Maurelli M, Bergamaschi R, Candeloro E, Todeschini A, Micieli G. Cerebral venous thrombosis and demyelinating diseases: report of a case in a clinically isolated syndrome suggestive of multiple sclerosis onset and review of the literature. *Mult Scler* (2005) 11(2):242–4. doi:10.1191/1352458505ms1125cr

18. Kalanie H, Harandi AA, Alidaei S, Heidari D, Shahbeigi S, Ghorbani M. Venous thrombosis in multiple sclerosis patients after high-dose intravenous methylprednisolone: the preventive effect of enoxaparin. *Thrombosis* (2011) 2011:785459. doi:10.1155/2011/785459

19. Presicci A, Garofoli V, Simone M, Campa MG, Lamanna AL, Margari L. Cerebral venous thrombosis after lumbar puncture and intravenous high dose corticosteroids: a case report of a childhood multiple sclerosis. *Brain Dev* (2013) 35(6):602–5. doi:10.1016/j.braindev.2012.09.001

20. International Multiple Sclerosis Genetics Consortium, Wellcome Trust Case Control Consortium, Sawcer S, Hellenthal G, Pirinen M, Spencer CC, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* (2011) 476(7359):214–9. doi:10.1038/nature10251

21. Kaplanski G, Marin V, Fabrigoule M, Boulay V, Benoliel AM, Bongrand P, et al. Thrombin-activated human endothelial cells support monocyte adhesion in vitro following expression of intercellular adhesion molecule-1 (ICAM-1; CD54) and vascular cell adhesion molecule-1 (VCAM-1; CD106). *Blood* (1998) 92(4):1259–67.

22. Horstman LL, Jy W, Ahn YS, Zivadinov R, Maghzi AH, Etemadifar M, et al. Role of platelets in neuroinflammation: a wide-angle perspective. *J Neuroinflammation* (2010) 7:10. doi:10.1186/1742-2094-7-10

23. Nurden AT. Platelets, inflammation and tissue regeneration. *Thromb Haemost* (2011) 105(Suppl 1):S13–33. doi:10.1160/THS10-11-0720

24. Behari M, Shrivastava M. Role of platelets in neurodegenerative diseases: a universal pathophysiology. *Int J Neurosci* (2013) 123(5):287–99. doi:10.3109/00207454.2012.751534

25. Horstman LL, Jy W, Ahn YS, Maghzi AH, Etemadifar M, Alexander JS, et al. Complement in neurobiology. *Front Biosci* (2011) 16:2921–60. doi:10.2741/3890

26. Veerhuis R, Nielsen HM, Tenner AJ. Complement in the brain. *Mol Immunol* (2011) 48(14):1592–603. doi:10.1016/j.molimm.2011.04.003

27. Ingram G, Hakobyan S, Hirst CL, Harris CL, Loveless S, Mitchell JP, et al. Systemic complement profiling in multiple sclerosis as a biomarker of disease state. *Mult Scler* (2012) 18(10):1401–11. doi:10.1177/1352458512438238

28. D'Haeseleer M, Cambron M, Vanopdenbosch L, De Keyser J. Vascular aspects of multiple sclerosis. *Lancet Neurol* (2011) 10:657–66. doi:10.1016/S1474-4422(11)70105-3

29. Fog T. The topography of plaques in multiple sclerosis with special reference to cerebral plaques. *Acta Neurol Scand Suppl* (1965) 1965(15):1–161.

30. Zamboni P, Galeotti R, Menegatti E, Malagoni AM, Tacconi G, Dall'Ara S, et al. Chronic cerebrospinal venous insufficiency in patients with multiple

sclerosis. *J Neurol Neurosurg Psychiatry* (2009) **80**(4):392–9. doi:10.1136/jnnp.2008.157164

31. Comi G, Battaglia MA, Bertolotto A, Del Sette M, Ghezzi A, Malferrari G, et al. Observational case-control study of the prevalence of chronic cerebrospinal venous insufficiency in multiple sclerosis: results from the CoSMo study. *Mult Scler* (2013) **19**(11):1508–17. doi:10.1177/1352458513501231

32. Traboulsee AL, Knox KB, Machan L, Zhao Y, Yee I, Rauscher A, et al. Prevalence of extracranial venous narrowing on catheter venography in people with multiple sclerosis, their siblings, and unrelated healthy controls: a blinded, case-control study. *Lancet* (2014) **383**(9912):138–45. doi:10.1016/S0140-6736(13)61747-X

33. Salvi F, Bartolomei I, Buccellato E, Galeotti R, Zamboni P. Venous angioplasty in multiple sclerosis: neurological outcome at two years in a cohort of relapsing-remitting patients. *Funct Neurol* (2012) **27**(1):55–9.

34. Courville CB. The effects of heparin in acute exacerbations of multiple sclerosis. Observations and deductions. *Bull Los Angel Neuro Soc* (1959) **24**:187–96.

35. Maschmeyer J, Shearer R, Lonser E, Spindle DK. Heparin potassium in the treatment of chronic multiple sclerosis. *Bull Los Angel Neuro Soc* (1961) **26**:165–71.

36. Zivadinov R. Is there a link between the extracranial venous system and central nervous system pathology? *BMC Med* (2013) **11**:259. doi:10.1186/1741-7015-11-259

37. Zivadinov R, Poloni GU, Marr K, Schirda CV, Magnano CR, Carl E, et al. Decreased brain venous vasculature visibility on susceptibility-weighted imaging venography in patients with multiple sclerosis is related to chronic cerebrospinal venous insufficiency. *BMC Neurol* (2011) **11**:128. doi:10.1186/1471-2377-11-128

38. Kirk J, Plumb J, Mirakhur M, McQuaid S. Tight junctional abnormality in multiple sclerosis white matter affects all calibres of vessel and is associated with blood-brain barrier leakage and active demyelination. *J Pathol* (2003) **201**(2):319–27. doi:10.1002/path.1434

39. Putnam TJ. Studies in multiple sclerosis: encephalitis and sclerotic plaques produced by venular obstruction. *Arch Neurol Psychiatry* (1935) **33**:929–40. doi:10.1001/archneurpsyc.1935.02250170015002

40. Wakefield AJ, More LJ, Difford J, McLaughlin JE. Immunohistochemical study of vascular injury in acute multiple sclerosis. *J Clin Pathol* (1994) **47**:129–33. doi:10.1136/jcp.47.2.129

41. Han MH, Hwang SI, Roy DB, Lundgren DH, Price JV, Ousman SS, et al. Proteomic analysis of active multiple sclerosis lesions reveals therapeutic targets. *Nature* (2008) **451**(7182):1076–81. doi:10.1038/nature06559

42. Chapman J. Thrombin in inflammatory brain diseases. *Autoimmun Rev* (2006) **5**(8):528–31. doi:10.1016/j.autrev.2006.02.011

43. Chapman J. Coagulation in inflammatory diseases of the central nervous system. *Semin Thromb Hemost* (2013) **39**(8):876–80. doi:10.1055/s-0033-1357482

44. Coughlin SR. Thrombin signalling and protease-activated receptors. *Nature* (2000) **407**:258–64. doi:10.1038/35025229

45. Beilin O, Karussis DM, Korczyn AD, Gurwitz D, Aronovich R, Hantai D, et al. Increased thrombin inhibition in experimental autoimmune encephalomyelitis. *J Neurosci Res* (2005) **79**(3):351–9. doi:10.1002/jnr.20270

46. Inaba Y, Ichikawa M, Inoue A, Itoh M, Kyogashima M, Sekiguchi Y, et al. Plasma thrombin-antithrombin III complex is associated with the severity of experimental autoimmune encephalomyelitis. *J Neurol Sci* (2001) **185**:89–93. doi:10.1016/S0022-510X(01)00468-3

47. Marik C, Felts PA, Bauer J, Lassmann H, Smith KJ. Lesion genesis in a subset of patients with multiple sclerosis: a role for innate immunity? *Brain* (2007) **130**(Pt 11):2800–15. doi:10.1093/brain/awm236

48. Akassoglou K, Adams RA, Bauer J, Mercado P, Tseveleki V, Lassmann H, et al. Fibrin depletion decreases inflammation and delays the onset of demyelination in a tumor necrosis factor transgenic mouse model for multiple sclerosis. *Proc Natl Acad Sci USA* (2004) **101**:6698–703. doi:10.1073/pnas.0303859101

49. Inaba Y, Ichikawa M, Koh CS, Inoue A, Itoh M, Kyogashima M, et al. Suppression of experimental autoimmune encephalomyelitis by dermatan sulfate. *Cell Immunol* (1999) **198**:96–102. doi:10.1006/cimm.1999.1588

50. Lider O, Baharav E, Mekori YA, Miller T, Naparstek Y, Vlodavsky I, et al. Suppression of experimental autoimmune diseases and prolongation of allograft survival by treatment of animals with low doses of heparins. *J Clin Invest* (1989) **83**(3):752–6. doi:10.1172/JCI113953

51. Owens GP, Gilden D, Burgoon MP, Yu X, Bennett JL. Viruses and multiple sclerosis. *Neuroscientist* (2011) **17**(6):659–76. doi:10.1177/1073858411386615

52. Sibley WA, Bamford CR, Clark K. Clinical viral infections and multiple sclerosis. *Lancet* (1985) **1**(8441):1313–5. doi:10.1016/S0140-6736(85)92801-6

53. Mechelli R, Umeton R, Policano C, Annibali V, Coarelli G, Ricigliano VA, et al. A "candidate-interactome" aggregate analysis of genome-wide association data in multiple sclerosis. *PLoS One* (2013) **8**(5):e63300. doi:10.1371/journal.pone.0063300