ANNALS of Clinical and Translational Neurology
Open Access
ANA American Neurological Association since 1875

EDITORIAL

Sequencing the immunopathologic heterogeneity in multiple sclerosis

Jack P. Antel, Samuel K. Ludwin & Amit Bar-Or

Neuroimmunology Unit, Montreal Neurologic Institute, McGill University, Montreal, Quebec, Canada

Annals of Clinical and Translational Neurology 2015; 2(9): 873–874

doi: 10.1002/acn3.230

Plenas et al. are to be congratulated on the application of cutting edge cell and molecular biologic techniques to address central issues regarding the immune-pathogenesis multiple sclerosis (MS) of lesions.[1] This was a demanding study with regard to acquisition of patient material, experimental design, quality and quantity of labor involved, and cost. As emphasized in this report, a challenge in MS is to define the immunologic events ongoing in actual lesions and then determine whether and how these may be reflected or monitored by analysis of more readily available tissue samples such as cerebrospinal fluid (CSF) or blood. The authors specifically address the concept of there being distinct phenotypes of early lesions among MS patients, categorized as patterns 1–4.[2] Pattern 2 lesions have been described to feature immunoglobulin (Ig) and complement deposition in addition to inflammatory cell infiltration and myelin destruction and were the most frequent lesion pattern observed in the initial combined biopsy and autopsy sample. Data supporting the distinct features of pattern 2 lesions include therapeutic responses to plasma exchange therapy[3] and potentially presence of unique serum autoantibody signatures.[4] The authors acknowledge the controversy that exists regarding this concept, providing rationale for generating more direct evidence as in this study.

This study evolves from an in depth molecular analysis, using next-generation sequencing techniques, of lesions found at autopsy in a case with an extremely aggressive course of MS. The core of the cellular immune data presented is derived from one very active lesion that shows Ig/complement deposition characteristic of pattern 2. Data from two less active lesions are in keeping with observations that the disease process is homogenous in individual cases. The data confirm previous studies based on T-cell receptor (TcR) phenotyping that CD8 T cells are the most frequent T-cell type present in such lesions and that they have a restricted repertoire suggesting that they are responding to a specific antigen. This study now identifies CD4 T cells with identical CDR3 TcR sequences but using different Vβ segments indicating that CD4 T cells are also responding to specific antigen, although no mutations in DNA sequences were detected.

The authors derive further information about these clones by identifying the presence of such clones in the CSF of the patient based on having identical CDR3 sequences. They were able to expand these CSF clones non-selectively using phytohemagglutinin and thus derive sufficient cell numbers to examine extensively their cellular properties. As a result of the large amount of work involved, they could identify six CD4 T cell clones (TCCs) in the CSF that based on sequencing results, were also present in lesions. Not all lesion clones, including the most common ones, were present in the CSF. The authors suggest this could reflect that the most active clones are exhausted (limiting the capacity to expand them), technical issues such as availability of full range of regents to select all clones, and of particular clinical relevance that "CSF was not an ideal surrogate for the central nervous system (CNS)". Ongoing work should further clarify how representative CSF-derived clones are of the entire array present in the MS lesions.

A provocative postulate arising from this study is that Th2-biased CD4 clones are present in pattern 2 MS lesions and play a role in lesion pathogenesis by supporting antigen-specific B cell/Ig responses. When re-visiting the original Witebsky criteria to establish causality in autoimmune disease, Rose and Bona[5] stated that direct evidence requires adoptive transfer of disease; this, however, is still not readily achievable for a primary human-cell-mediated disorder. Indirect evidence includes "isolation of autoantibodies or self reactive T cells from the organs which represent the major target of autoimmune disease" and reproduction in an animal model (see later comment). The current data indicate that three of

the six CD4 clones expanded from the CSF and that corresponded to clones found in the lesions were of the Th2 phenotype; the others, however, did have Th1 properties. Clones derived from another clinical case in which a biopsy showed Type III pathology were all Th1. This study does not address the issue of antigen specificity of the clones. Recent data indicate that although frequency of myelin basic protein (MBP) reactive CD4 T cells in the systemic compartment may not differ between MS patients and controls, their functional properties are distinct with regard to production of proinflammatory cytokines (Th1 and Th17).[6] Now recognized is that T-cell support of antibody production extends beyond the initial Th2 versus Th1 paradigms with identification of multifunctional T cells such as Th1 cells coexpressing IFN-γ and IL-10, the identification of follicular helper T cells (Tfh) as a predominant CD4(+) T helper subset for B cells, and the inherent plasticity of different CD4(+) T cells."[7] Furthermore, not all CNS autoreactive antibodies need impact disease expression; these may be part of a person's normal humoral repertoire[8] and even contribute to tissue protection or repair. Autoreactive CD4 TCCs could still themselves induce tissue injury through bystander mechanisms mediated by production of effector molecules or by acquisition under inflammatory conditions of promiscuous cytotoxic capability linked to expression of NKG2 molecules that interact with corresponding ligands on the target cells within the lesions.[9]

The limited access to active MS lesions and scope of work involved makes confirming the results of this study a formidable task. This study could not detect cytokines in the CSF indicating the need for focus on cell analysis, a technically demanding task using the limited number of cells available, with concerns that all the cells will be expanded. Since the type 2 lesion phenotype is common, one asks whether existent reports describing properties of expanded T-cell lines derived from MS patient CSF should have already indicated this Th2 bias. Reproducing the Th1 bias seen in the single pattern 3 case including in those with a Balo's phenotype, would be welcome. This study also puts forth the challenge to those using the animal model experimental autoimmune encephalomyelitis to re-produce the findings reported here in the actual human disease, fulfilling one of the criteria for autoimmunity mentioned above. One notes a parallel of the type of approach used in this study with studies in neuromyelitis optica that initially identified the presence of Ig in the lesions leading to subsequent identification of a specific pathogenic antibody; hopefully similar successes will be achieved in MS.

Overall, the current report illustrates the complexities and challenges of applying cutting edge cell and molecular biologic techniques to actual case material but also the potential of using these approaches to answer the important questions that arise from the study.

Conflict of Interest

None declared.

References

1. Planas R. Central role of Th2/Tc2 lymphocytes in pattern II multiple sclerosis lesions. Ann Clin Transl Neurol DOI: 10.1002/acn3.218.
2. Lucchinetti C, Bruck W, Parisi J, et al. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol 2000;47:707–717.
3. Keegan M, Konig F, McClelland R, et al. Relation between humoral pathological changes in multiple sclerosis and response to therapeutic plasma exchange. Lancet 2005;366:579–582.
4. Quintana FJ, Farez MF, Viglietta V, et al. Antigen microarrays identify unique serum autoantibody signatures in clinical and pathologic subtypes of multiple sclerosis. Proc Natl Acad Sci USA 2008;105:18889–18894.
5. Rose NR, Bona C. Defining criteria for autoimmune diseases (Witebsky's postulates revisited). Immunol Today 1993;14:426–430.
6. Cao Y, Goods BA, Raddassi K, et al. 2015. Functional inflammatory profiles distinguish myelin-reactive T cells from patients with multiple sclerosis. Sci Transl Med 7:287ra74.
7. Perez-Mazliah D, Langhorne J. CD4 T-cell subsets in malaria: TH1/TH2 revisited. Front Immunol 2014;5:671.
8. Ligocki AJ, Lovato L, Xiang D, et al. A unique antibody gene signature is prevalent in the central nervous system of patients with multiple sclerosis. J Neuroimmunol 2010;226:192–193.
9. Zaguia F, Saikali P, Ludwin S, et al. Cytotoxic NKG2C+ CD4 T cells target oligodendrocytes in multiple sclerosis. J Immunol 2013;190:2510–2518.