Natalizumab and PML

To the editor:

Recently, *Nature Neuroscience*[1] highlighted progress and complications in drug treatments for multiple sclerosis (MS) arising from trials with natalizumab (Tysabri)®, a drug that seemed highly effective but was associated in 0.1% of recipients with progressive multifocal leukoencephalopathy (PML), a serious, often fatal infection of CNS glia with JC virus (JCV).

The initial reaction was that natalizumab recipients lacked effective immunosurveillance of JCV, leading to unrestrained viral replication and spread of infection to the CNS, with the catastrophic consequence of PML. A recent editorial in the *New England Journal of Medicine* stated "Therefore, it appears likely that natalizumab, by preventing normal trafficking of lymphocytes, led to unbridled JC virus replication," concluding darkly, "Bad things may happen when rescuers are turned back at the gates"[2]. This line of thinking is understandable: prior PML cases invariably arose in the context of immunosuppression, and natalizumab, which blocks α4 integrins (including α4β1/VLA-4), has powerful anti-inflammatory effects, making it plausible that natalizumab-mediated immunosuppression caused PML. This conclusion now seems premature: relevant considerations include effects of natalizumab beyond inhibiting inflammation, the biology of JCV infection and mechanisms underlying CNS immunosurveillance.

The underlying question is this: was PML in natalizumab recipients an off-target adverse effect or a direct consequence of the mechanism of therapeutic action? This issue is critically important, as the answer helps shape our anti-inflammatory strategies for treating MS. If PML was a direct consequence of inhibiting leukocyte trafficking, then this extremely promising avenue for treating patients[3] must be abandoned. If PML was an off-target adverse effect, then the way forward remains open, in principle.

VLA-4 exerts widespread functions in the hematopoietic system. It is required for generating T cells and B cells from bone marrow progenitors in adult mice and for some inflammatory lymphocyte trafficking but not for entry into spleen, lymph nodes or intestinal epithelium[4]. Adult mice with inducible α4 deletion from hematopoietic cells showed circulating myeloid progenitors, doubling in blood lymphocyte counts and 20% fewer B cell progenitors in marrow[5]. Patients on natalizumab had elevated lymphocyte, basophil and eosinophil counts, with 5% showing circulating nucleated erythrocytes (according to the Tysabri package insert). Clearly, natalizumab affects bone marrow physiology by blocking VLA-4 interactions with its ligands.

Effects of natalizumab on bone marrow, and B cells in particular, are directly relevant for PML pathogenesis. JCV seropositivity, indicating virus exposure, is present in 80% of the population, with an unknown number harboring virus in renal tubular epithelial cells and bone marrow. When PML occurs, JCV transcriptional control region (TCR) sequences in bone marrow (but not kidney) strongly resemble those in CNS and confer competence to replicate in glial cells *in vitro*. Reactivated JCV is believed cross into the CNS within B lymphocytes or as cell-free virus[6,7]. It is plausible, therefore, that natalizumab mobilizes JCV-infected cells from bone marrow stores, leading to poorly controlled viral replication within infected pre-B cells deprived of contact with marrow stromal cells. A natalizumab-induced spike of JC viremia in a Crohn's disease patient[8] may have represented release of virus from marrow stores rather than uncontrolled JCV proliferation.

Natalizumab strongly suppresses inflammatory leukocyte trafficking into CNS across parenchymal microvessels and also reduces (to levels below those seen in healthy controls) the numbers of cerebrospinal fluid memory $CD4^+$ T lymphocytes in the cerebrospinal fluid, which mediate CNS immunosurveillance[9]. Natalizumab's full effects on CNS immunosurveillance remain unknown: PML was particularly shocking, as there were few if any additional infectious complications, in more than 2,000 patient-years of natalizumab/MS clinical trial experience[10]. Opportunistic infections in Crohn's disease natalizumab clinical trials are under investigation and were possibly affected by other concurrent immunosuppressives.

These considerations suggest that natalizumab-associated PML was specific to the α4-integrin target, and they prompt the following hypothesis: that natalizumab treatment led to PML by mobilizing infected bone marrow cells, possibly in combination with reduced inflammatory and surveillance trafficking to the CNS. If this hypothesis is correct, agents that do not affect bone marrow will lack this complication, whereas those affecting bone marrow and lymphoid organs (anti-CXCR4, anti-α4 integrin and FTY720, for example) require caution and surveillance. Furthermore, PML may be regarded as an off-target adverse effect, and it remains possible to treat MS patients with agents that address leukocyte trafficking to the CNS. Reduced numbers of cerebrospinal fluid $CD4^+$ T cells could indicate possibly impaired CNS immunosurveillance.

This hypothesis needs to be addressed, and many questions, including the prevalence of pathogenic JCV, a possible CNS JCV reservoir, and effects of releasing JCV-infected cells from bone marrow on TCR elements and viral replication, need to be answered. Ultimately, each leukocyte trafficking modulator will have its own liabilities, and we need to avoid being caught 'fighting the last war' against PML as we evaluate other agents. The prize will be to regain momentum in the fight for effective treatment for MS and other inflammatory diseases.

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1. *Nat. Neurosci.* **8**, 837 (2005).
2. Berger, J.R. & Koralnik, I.J. *N. Engl. J. Med.* **353**, 414–416 (2005).
3. Steinman, L. *Nat. Rev. Drug Discov.* **4**, 510–518 (2005).
4. Arroyo, A.G., Yang, J.T., Rayburn, H. & Hynes, R.O. *Cell* **85**, 997–1008 (1996).
5. Scott, L.M., Priestley, G.V. & Papayannopoulou, T. *Mol. Cell. Biol.* **23**, 9349–9360 (2003).
6. Houff, S.A. *et al. N. Engl. J. Med.* **318**, 301–305 (1988).
7. Jensen, P.N. & Major, E.O. *J. Leukoc. Biol.* **65**, 428–438 (1999).
8. Van Assche, G. *et al. N. Engl. J. Med.* **353**, 362–368 (2005).
9. Ransohoff, R.M., Kivisakk, P. & Kidd, G. *Nat. Rev. Immunol.* **3**, 569–581 (2003).
10. Sheridan, C. *Nat. Rev. Drug Discov.* **4**, 357–358 (2005).