Reviewer's report

Title: Treatment of refractory epilepsy with natalizumab in a patient with multiple sclerosis. Case report

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Reviewer: Annamaria Vezzani

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This manuscript has been improved by adding additional information of the literature relevant for discussing these data. The following clarifications need to be added in the manuscript where indicated below:

Minor Essential Revisions

1. Abstract-Background: The sentence “Recent studies…..show a key role for blood-brain barrier damage etc”-show should be substituted by “suggest”
2. Background: line 8 from bottom-Marchi et al citation (9) is not adequately quoted since the paper shows that BBB damage induced by osmotic opening with mannitol induces acute seizures in pig; human subjects with brain tumors underlying the same procedure for chemotherapeutic purposes also showed acute seizures in 25% of cases. Therefore this study does not show that leukocyte trafficking induce BBB damage leading to seizures.
3. Kim et al (11) studied a mouse model of viral meningitis leading to acute seizure-induced death. This is important to state in the background section.
4. Line 6 from bottom-“ White matter angiopathy and leukocyte accumulation into the brain parenchyma were documented in patients with refractory epilepsy” should be changed in “White matter angiopathy and increased number of activated microglia, macrophages and CD3-positive T cells in perivascular cavities were documented in a subpopulation of young patients…”
5. Discussion: Authors specify preventive and therapeutic effects of anti-VLA-4 antibody. I think that preventive is more appropriate since the treatment has been evaluated before chronic epilepsy was established in this model.
6. References 27-29 in the text should be moved after “neutrophils” since they refer to the first part of this sentence.
7. At the end of discussion, the authors should be more precise when describing the presence of leukocytes in brain specimens from epilepsy subjects: i.e. Ref 12 refers to a “subpopulation of young patients” with refractory epilepsy; Ref 31 reports “rare to sparse CD8 cells” as compared to more abundant macrophages in TS.
8. There is much literature to support that the inflammatory mechanisms now mentioned in the revised discussion and related to glia activation can also be responsible for, or contribute significantly to vascular changes. This should be at
least mentioned; as it stands now it appears like these two phenomena are independent on each others. Seizure-induced inflammation in glia as one trigger of vascular inflammation (up-regulation of adhesion molecules) is supported by experiments done in isolated guinea pig brain (ref. 30) where self-sustained seizures (and parenchymal inflammation, unpublished but see Librizzi et al, Rhodes ECE 2010) are provoked by transient arterial infusion of convulsant drugs in artificial CSF which lacks any blood component.

9. When discussing activation of inflammatory signals in brain cells such as microglia, astrocytes and neurons the ref 22 should be added to ref 21 since IL-1beta/IL-1R1, as for complement system components, are highly expressed in these cells also in cortical tubers. Moreover, the same signaling is upregulated in parenchymal brain cells in malformations of cortical development such as FCD and glioneuronal tumors (Ravizza et al Neurobiol Dis, 2006).

10. The authors report that neutrophil depletion reduce seizure generation in the animal model of pilocarpine-induced epilepsy (Fabene et al., 2008) and that myelomonocytic driven inflammation induce severe seizures in mice (Kim et al., 2009). As discussed in my former review, pilocarpine induces a phenomenon of systemic inflammation reminiscent of infection, before inducing seizures (Marchi et al, 2007) which fits with the evidence that systemic LPS (mimicking bacterial infection) decreases seizure threshold. Kim et al have indeed used a mouse model of viral meningitis to show leukocyte trafficking involvement in acute seizures. MS is an autoimmune disease therefore associated with peripheral immune cells activation which perpetuates brain inflammation. It remains unclear which would be the trigger of immune cells activation (for ex. neutrophils)/trafficking, and more in general which is the sequence of events foreseen by the authors to propose their hypothesis in epilepsy outside the context of concomitant infections or autoimmunity. A brief speculative statement in the discussion would help to clarify this pont.

11. About interactions between natalizubam and AEDs it is unclear if this aspect was investigated or not. If not, this should be clearly stated since further studies are warranted to exclude this possibility.

12. Histocompatibility complex molecules and inhibits the T cell response to several myelin antigens. In addition, it was shown to act as a T cell receptor antagonist for the 82-100MBP epitope. Thus, GA treatment causes in vivo changes of the frequency, cytokine secretion pattern and effector function of GA-specific T cells......... steroids seem to have transitory non-selective anti-adhesion effects by reducing endothelial activation, but, according to international clinical guidelines, its use has to be limited to short courses (3-5 days) of high-dose intravenous methylprednisolone in case of clinical relapse. Thus, in contrast to Natalizumab therapy, a specific and sustained anti-adhesive activity exerted by GA and steroids in the present clinical case seems rather unlikely.

Reply: the reader would benefit if these considerations are succinctly reported in the discussion.
Additional replies to authors comments:

1. The reviewer mentions that “Gd signal which should reflect leukocyte-vascular interactions and their entry into the brain”. However, to our knowledge, there are no studies showing that gadolinium is a marker of leukocyte-endothelial interactions or of leukocyte entry into the brain in multiple sclerosis.

Reply: I understand that the authors hypothesis is that BBB leakage is due to leukocyte/endothelial cell adhesion and brain extravasation; if Gd signal reflects BBB breakdown in MS, it should be also an indirect reflection of such process.

2. Gd is a contrast medium with low molecular weight (MW) molecule <1000 Da, whereas Natalizumab has a much higher MW of 149,000 Da. Thus Gd enhancement does not necessarily imply Natalizumab passage into the brain due to their different molecular weights and capacity to cross inflamed brain endothelium.

Reply: IgG complexes and albumin can also enter the leaky BBB showing that the degree of opening may allow the entry of big macromolecules. Natalizumab labelled with fluorescein isothiocyanate (FITC) could be used to test this aspect in EAE models.

3. To our knowledge no study has yet demonstrated that Natalizumab is able to “interact” with osteopontin or fibronectin.

Reply: This is a personal communication by Dr. G. Comi, University S. Raffaele and described in technical drug annexes.

4. The paper by Turrin and Rivest, 2004 was not cited as no specific cellular marker for monocyte, neutrophil or lymphocyte detection was used.

Reply: the paper reports lack of IL-12 and IFN-gamma in the brain of mice undergoing seizures.

5. Although not apparently related with the focus of our case report, the interruption of glia-neuron communication effect proposed by recent studies was mentioned in the discussion.

Reply: a valid reason for discussing these data was to suggest the plausible possibility that worsening of seizures in this patients with onset of status epilepticus can be consequent to proinflammatory signaling between glia and neurons that has been shown in various experimental models to contribute to seizure precipitation and severity.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a
statistician.

Declaration of competing interests:
I declare that I have no competing interests