LETTER TO THE EDITOR

Reply: Beneficial effect of interleukin-2-based immunomodulation in Alzheimer-like pathology

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Sir,

We are appreciative of Dansokho, Aucouturier and Dorothee’s interest in our recent article entitled ‘Interleukin-2 improves amyloid pathology, synaptic failure and memory in Alzheimer’s disease mice’ (Alves et al., 2017) and for their constructive comments. As they noted, our work showed that low-dose interleukin-2 (IL-2) rescued memory defects of mice treated at a time they have established Alzheimer’s disease, extending their own work in which they showed the prevention of Alzheimer’s disease by IL-2. They used APPPS1 mice treated at Week 6, the onset of amyloid-β deposition, and monitored the treatment effects at Month 4; we used the APPPS1ΔE9 mouse model treated at Month 8, and monitored treatment effects at Month 13. We also reported additional novel findings regarding the potential of IL-2 in Alzheimer’s disease: (i) IL-2 levels are decreased in hippocampal biopsies from Alzheimer’s disease patients; (ii) IL-2 treatment expanded regulatory T cells (Tregs) not only in the periphery (Dansokho et al., 2016), but also in the brain; and (iii) IL-2’s beneficial effects on memory could be correlated with decreased hippocampal amyloid plaques and the amyloid Aβ42/40 ratio, as well as improved synaptic plasticity and spine density (Alves et al., 2017).

Dansokho and colleagues then make comments on some of our observations that could be interpreted as being at variance with theirs or some from the literature, to which we respond below.

They first point to a study reporting an increase of IL-2 levels (129%) in hippocampal homogenates (Araujo and Lapchak, 1994), a potential discrepancy with our observation of a 50% decrease of IL-2 levels in hippocampal biopsies from severely affected Alzheimer’s disease patients (Thal 5/Braak VI). However, the study referred to used radio-immunoassays with antisera specific for IL-2, while we used a commercial assay based on monoclonal...
antibody. We observed that IL-2 decrease was heterogeneous among Alzheimer’s disease patients, with a strong decrease observed in three out of five Alzheimer’s disease patients (Fig. 1A in Alves et al., 2017). Importantly, the levels of IL-2 remained relatively homogeneous among the five control individuals analysed. Furthermore, we evaluated IL-2 levels in five Thal 5/Braak VI Alzheimer’s disease patients, while no information regarding clinical stage is mentioned in the study of Araujo and Lapchak (1994). Larger studies with Alzheimer’s disease patients at different Braak stages (Braak et al., 2006) are warranted to further analyse IL-2 levels in hippocampal biopsies of Alzheimer’s disease patients.

Dansokho and colleagues then point to the fact that we observed improved learning of IL-2-treated wild-type mice during the 5-day acquisition phase in the Morris water maze place navigation task, relative to control mice, and despite no increased IL-2 hippocampal levels (Fig. 3A in Alves et al., 2017). Even though no increased IL-2 was observed in the hippocampus, IL-2 injection in wild-type mice induced increased Tregs in the brain. Besides, Dansokho and co-workers could also have noted that, in contrast, IL-2 did not improve the performance of Alzheimer’s disease mice during the learning phase. Similar results during the acquisition/learning phase have recently been observed in the same Alzheimer’s disease mouse model (APP/PS1ΔE9), in which increased performance was found in littermates treated with AAV-APPs25 relative to littermates injected with the control vector or to Alzheimer’s disease mice injected with either vector (Fol et al., 2016). These data suggest that therapies work differentially according to the physiological or pathological context. In a physiological context, IL-2 treatment could act as a memory enhancer; in an Alzheimer’s disease context, IL-2 treatment could exert only disease-modifying effects leading to restoration of memory abilities without promnesic properties. Hence, we believe that the probe tests, which evaluate spatial reference memory after the training trials, are more relevant to the study of IL-2’s impact on Alzheimer’s disease. These tests clearly showed beneficial effects of IL-2 during the memory consolidation period (Alves et al., 2017). Importantly, this improved memory performance correlates with tissue remodelling showing improved synaptic plasticity and rescued spine density in IL-2-treated Alzheimer’s disease mice (Alves et al., 2017), which was not addressed in Dansokho et al. (2016).

Dansokho and colleagues then comment on the fact that our observed Treg increase in the brain of IL-2-treated Alzheimer’s disease mice does not tell us whether these cells are in the brain parenchyma or outside of the CNS. Indeed, we did not address this particular question, which could be answered by performing immunohistochemistry in mouse brain slices. Also, as reported in the ‘Materials and methods’ section, animals were perfused transcardially before brain extraction. Dansokho and co-workers also question the role of Tregs in Alzheimer’s disease, referring to the work of Baruch and colleagues (2015) who showed that Treg depletion improved Alzheimer’s disease in 5XFAD mice. We discussed this work in our article, pointing to the fact that the Treg depletion in this study led to ‘a marked enrichment of Tregs in the brain 3 weeks after the last Treg depletion modality’ (Alves et al., 2017).

Then, they point to the fact that we report that IL-2 is associated with a mild 18% decrease in hippocampal amyloid load in our IL-2-treated APPPS1ΔE9 mice with established pathology at the time of treatment, while they observed a ‘slight non-significant increase in total amyloid-β deposition’ in IL-2-treated APPPS1 mice. We did not stress that this mild reduction in amyloid pathology (plaques) was pivotal to explaining the improved Alzheimer’s disease-like phenotype. Actually, the interpretation of the role of amyloid plaques in the Alzheimer’s disease context remains controversial. As an example, the pharmacological targeting of CSF1R in APP/PS1 mice improved memory defects and prevented synaptic degeneration, but with no major changes in the number of amyloid-β plaques (Olmos-Alonso et al., 2016). These results are supported in a study showing the removal of plaques in patients immunized against amyloid-β, who nonetheless show a decline in cognitive abilities (Boche et al., 2008). Importantly, our data indicate that the hippocampus of IL-2-treated mice exhibit an increased amyloid-β40 trend, thus decreasing the Aβ42/Aβ40 ratio; this is in line with several studies suggesting that amyloid-β40 confers neuroprotection and inhibition in amyloid deposition (Zou et al., 2003; Kim et al., 2007). Amyloid-β40 levels were not reported in Dansokho et al. (2016).

In their article, Dansokho and colleagues suggest that the beneficial effects of IL-2 in APPPS1 mice are associated with cortical microglial recruitment to plaques, and potentially to type I interferon-dependent beneficial microglial activation (Dansokho et al., 2016). We did not study type I interferon in our model, but rather IFN-γ. It has
been reported that overexpression of IFN-γ is associated with an increased microglial and astrocytic response, leading to a decrease in amyloid-β deposition (Chakrabarty et al., 2010), and with reduced tau phosphorylation in triple transgenic Alzheimer’s disease mice (Mastrangelo et al., 2009). Besides, low doses of IFN-γ were reported to clear amyloid-β plaques in vivo by T cell-dependent mechanisms (Monsonengo et al., 2006) and to improve learning in APP mice by enhancing neurogenesis (Baron et al., 2008). Interestingly, our unpublished results show that the levels of IFN-γ in APP/PS1ΔE9 mice receiving IL-2 were slightly increased as compared with rAAV8-LUC-injected APP/PS1ΔE9 mice (P = 0.0264) (Fig. 1).

We did not observe microglial recruitment around amyloid plaques in IL-2-treated APP/PS1ΔE9 mice at late disease stages. In addition, western blot analysis with five microglial markers did not detect differences after IL-2 administration to APP/PS1ΔE9 mice. In contrast, we observed increased expression of astrocytic markers in the hippocampus of IL-2-treated APP/PS1ΔE9 mice and enhanced astrocytic recruitment around amyloid plaques (Alves et al., 2017). In fact, Dansokho and colleagues reported that IL-2 therapy ‘slightly but non-significantly’ augmented the overall magnitude of astrocytosis in APPPS1 mice (Dansokho et al., 2016).

In conclusion, there are convergent and divergent observations of IL-2’s effects obtained in two quite different Alzheimer’s disease mouse models. Importantly, the convergent observations are that IL-2 mediates preventive (Dansokho et al., 2016) as well as therapeutic (Alves et al., 2017) beneficial effects on memory loss. The apparent divergences may well be explained by the different settings investigated (different models, different treatment modalities, different time points etc). These two reports warrant additional mechanistic studies of the effects of IL-2 in experimental Alzheimer’s disease. Our observation that low dose IL-2 has therapeutic effects on established pathology (Alves et al., 2017) also warrants the investigation of IL-2’s potential in human Alzheimer’s disease.

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**References**


