THE EMBO JOURNAL

Manuscript EMBO-2015-91488

Gatekeeper role of brain antigen-presenting CD11c+ cells in neuroinflammation

Magdalena Paterka, Volker Siffrin, Jan O Voss, Johannes Werr, Nicola Hoppmann, René Gollan, Patrick Belikan, Julia Bruttger, Jerome Birkenstock, Steffen Jung, Enric Esplugues, Nir Yogev, Richard A Flavell, Tobias Bopp, Frauke Zipp

Corresponding author: Frauke Zipp, University Medical Center Mainz

Review timeline:

Submission date:	10 March 2015
Editorial Decision:	13 April 2015
Revision received:	11 August 2015
Editorial Decision:	12 September 2015
Revision received:	01 October 2015
Accepted:	07 October 2015

Editor: Karin Dumstrei

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 13 April 2015

Thank you for submitting your manuscript to The EMBO Journal. Your study has now been reviewed by two referees and their comments are provided below.

As you can see below, referee #1 finds the manuscript exciting and important. However, referee #2 is more hesitant if the novel insight provided is sufficient to consider publication here given the previous links between DCs and the development and progression of EAE. While I see referee #2's point, I also appreciate that your analysis adds new insight into our understanding of the interactions between DCs and T cells in the CNS. I have also sought external advice on the novelty issue and the advisor also finds that the analysis adds important new insight. However, there are also specific issues such as missing controls that have to be address in order to consider publication here. Should you be able to address the concerns raised in full then we would like to invite a revision. I should add that it is EMBO Journal policy to allow only a single major round of revision, and that it is therefore important to resolve the raised concerns at this stage.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: http://emboj.embopress.org/about#Transparent_Process

Thank you for the opportunity to consider your work for publication. I look forward to your

revision.

REFEREE REPORTS

Referee #1:

Siffrin & Paterka et al. study the role of CNS CD11c+ cells in EAE. Using CD11c-DTR/GFP and IL-17 reporter mice the authors show the dependence of TH-17 infiltration to the CNS parenchyma and IL-17 production on the presence of DCs in the CNS. Analysis of chemokines and corresponding chemokine receptors on both cell types supports the importance of the Th-17-DCs interplay in the CNS in the EAE model. Using advanced imaging and image analysis tools, the authors report Th-17-DC interactions, which results in a change of behavior of Th17 cells, and presence of Th-17 and DC cell clusters in the CNS.

The study is mostly descriptive but exciting and very important. The role of DCs in CNS is rather neglected and this study shows their novel, important role in the most popular and studied model of CNS pathology. Especially modification of Th17 cells behavior and formation of cell clusters in the CNS (which is interesting by itself, can be a subject of many further studies) points to the crucial role of CNS DCs in the MS pathology.

Major points
1. Why were some experiments (Fig. 1) performed in chimeras? How were the chimeras prepared?
2. Depletion of DCs before EAE induction by adoptive transfer of Th17 2d2 cells delayed, but not prevented disease pathology (fig. 1A, day 28-30). Does the pathology fully develop later (after day 30), or it never develops to the same extent?
3. What if DTx is given at the peak of the disease (e.g. day 23, based on Fig. 1A, or in the active EAE model, but at the peak of the pathology)?
4. Following this topic; how GM-CSF blockage would influence EAE and cluster formation?
Minor comments:
5. As mentioned before: the chimera's preparation method should be described in greater detail and the percentage of chimeras should be indicated.
6. Fig.3; there is no arrow or arrowhead in A. In B the vessel wall should be marked in each picture.

Referee #2:

By using two photon laser scanning microscopy, the authors analyzed the interaction of CD11c+ cells and 2D2Th17 cells in the brain in the adoptive transfer EAE model. The IL17/GM-CSF expression by T cells coincides with the CD11c+ cell accumulation in the CNS. They find that CNS DCs express proinflammatory chemokines leading to the attraction of encephalitogenic T cells into the CNS and promoting their survival.

Accumulation of monocytes and monocyte-derived DCs (moDCs)/macrophages has been previously shown to be a critical event for the development and progression of EAE. Also the interaction of T cells with local APCs has been demonstrated previously. Thus I am somewhat concerned that there is little novelty in this paper.

My major concerns are:

Figure 1
It is critical that a control group C57BL6 → C57BL6 bone marrow chimeras treated with DTX is included in this experiment to control for DTX-mediated delay in disease onset independently of the DTR transgene.

The cDCs in the CD11c-DTR model can be well depleted as shown previously. The FACS analysis in Fig.S1 is of very poor quality. To show a deletion of DCs, the authors should gate at least on CD11c and MHCII.
In addition to the spleen, which is not the relevant organ in the adoptive transfer EAE model, data should be provided on the depletion of DCs in the brain before and during EAE. The DCs in the

CNS during EAE are monocyte-derived. It is not clear whether they target both the local APCs in the perivascular space and also the monocyte-derived DCs that accumulate in the CNS during EAE. Are both effectively depleted?

Is it possible to delete DCs after disease onset? Does it have an impact on EAE progression?

Figure 1F: This is not a convincing staining for GM-CSF.

Figure 4 and Figure S5:
To draw any conclusions about chemokine receptor expression from different cell populations in an EAE-diseased brain, flow cytometry cell sorting has to be performed. As shown in Figure S5, MACS sorting of total CD11c+ cells does not reach high enough purity. For example CNS CD11c- cells, in addition to the CD4+ T cells, comprise monocytes, neutrophils, and macrophages. On the other hand, CD11c+ cells contain monocytes and monocyte-derived cells (DCs/macrophages) and maybe a contamination of activated microglia. The authors then compare the CD11c+ and CD11c- CNS cells to CD11c+ and CD11c- splenic cells. This is not a fair comparison as the composition of splenic CD11c- cells is completely different and CD11c+ splenic cells are classical DCs and not monocyte-derived DCs as in the inflamed brain.

Minor concerns/mistakes:
There's a mistake in the title

Figure 1B: At what time point after EAE induction?
Figure 1B, C, F: On what are these cells pregated?

Figure 3: A and B: Description of figure legend does not correlate with the pictures.
(arrow, arrowheads and asterisk).

1st Revision - authors' response 11 August 2015

Referee 1:

Why were some experiments (Fig. 1) performed in chimeras? How were the chimeras prepared?
Experiments that needed CD11c depletion by DTX were performed in chimera to bypass the reported problem of lethality after repetitive DTX injection in the CD11c-DTR/GFP mice (Probst and van den Broek, 2005; Zaft et al., 2005). This toxicity problem derives from ectopic expression of the transgene in non-immune cells. Therefore, this mouse model, in which only the immune system is transgenic for CD11c, is routinely used as a bone marrow chimera for DC-depletion experiments (Bennett and Clausen, 2007). More details have now been included in the manuscript (see p. 5 ll. 105-113) and we have added a description of the generation of BM chimera to the methods section of the manuscript (see p. 18 ll. 402-415).

Depletion of DCs before EAE induction by adoptive transfer of Th17 2d2 cells delayed, but not prevented disease pathology (fig. 1A, day 28-30). Does the pathology fully develop later (after day 30), or it never develops to the same extent?
DTX-mediated DC depletion is generally seen as a good model for sustained DC depletion. However, it never reaches 100%, which might explain that there is no black and white phenotype. To address the point, the proportion of EAE-unaffected mice over time is given for the referee's appreciation in the following analysis (Kaplan-Meier analysis). (Figures for referees not shown.)

The clinical pathology develops to almost the same extent after day 30. However, we do not think that this proves that DCs only delay T cell activation in the CNS. This might rather reflect that the residual DCs are just sufficient to allow the entry of encephalitogenic T cells. In this chimeric model, $CD11c^+$ depletion is very efficient but does not reach 100%, therefore we cannot answer whether $CD11c^+$ cells are essential for T cell activation/survival within the CNS or if their role can be compensated for in the long run by another population of antigen presenting cells. We included this point in the discussion (see p. 15 ll. 346-350).

What if DTx is given at the peak of the disease (e.g. day 23, based on Fig. 1A, or in the active EAE model, but at the peak of the pathology)?
As suggested by this referee, we performed new experiments to answer the question if DC depletion after onset of the disease has a similar effect. We chose the active EAE model in which the priming of encephalitogenic T cells precedes the effector phase which is marked by the onset of the disease. We started treatment when a clinical score of 1 (tail plegia) was reached, i.e., day 12 after immunization. We observed a statistically significant reduction in mean clinical score starting at day 18 (see Fig. S2). Thus, $CD11c^+$ cells still play a role in the more developed disease, yet as expected, to a slightly lesser extent, since clusters have already impacted effector T cells.

Following this topic; how GM-CSF blockage would influence EAE and cluster formation?
There are reported data, which when taken together address this point and which show the influence of GM-CSF blockage on immune cell infiltration and clusters, so we have now included a new paragraph in the discussion on this relevant topic.
GM-CSF blockade during the priming and effector phase in EAE has been shown to reduce clinical signs of active EAE (Codarri et al., 2011; El-Behi et al., 2011). This effect was independent of Th17 polarization which has been shown to be similar in *Csf2-/-* Th17 and *wild-type* Th17 cells(El-Behi et al., 2011). This is indeed a similar finding as we found by depletion of $CD11c^+$ cells. In addition, also GM-CSF deficient Th1 cells exhibited less encephalitogenicity (Codarri et al., 2011). The latter publication stressed the role of GM-CSF for the secondary recruitment of inflammatory cells in the effector phase of EAE, which supports the relevance of our findings of an increase in numbers of $CD11c^+$ cells, a continuous proportional increase of (GM-CSF-dependent) monocyte-derived $CD11c^+$ cells and the formation of $CD11c^+$ clusters as the disease progresses. It has been shown that there is a clear link between chronic CNS inflammation and induction of $CD11c^+$ cells from myeloid and microglia cells in EAE and infectious encephalitis (Fischer and Reichmann, 2001; Ponomarev et al., 2007). For the potential consequence of a lack of this $CD11c^+$ accumulation, Codarri et al. also showed that, in their model with actively immunized $Csf2^{-/-}$ mice, there is a lack of retention or accumulation of $CD4^+$ T cells in the CNS, which again is equivalent to low CNS $CD4^+$ T cell numbers in $CD11c^+$-depleted animals in our adoptive transfer EAE. We discuss these publications in the context of our findings in depth (see p. 14 ll. 312-329).

As mentioned before: the chimera's preparation method should be described in greater detail and the percentage of chimeras should be indicated. I'm looking for data concerning the percentage of chimera.
Please, see the answer to question 1 of this referee. We have referred to our previous publications which used the same protocol to generate chimeric mice (Siffrin et al., 2009) and which yielded graft-derived leukocytes in >95%. To specify this gross engraftment rate in these CD11cDTR/GFP->C57BL/6 mice we performed additional analyses to show the graft derived $CD11c^+$ cells in relation to all $CD11c^+$ cells in a new Figure (see Figure S1A/S1B). We identified a chimerism of >85% when looking at $CD11cGFP^+$ cells of $CD11c^+$ cells and >93% for CD11cDTR-depleted cells of $CD11c^+$ cells.

Fig.3; there is no arrow or arrowhead in A. In B the vessel wall should be marked in each picture.
This has been changed accordingly.

Referee 2:

Figure 1: It is critical that a control group C57BL6 → C57BL6 bone marrow chimeras treated with DTX is included in this experiment to control for DTX-mediated delay in disease onset independently of the DTR transgene.
We had included this control in the original experiment, and now, as suggested by this referee, include this control group in Fig. 1A. In addition, this control group is shown for the peak treatment in active EAE (see Fig. S2).

The cDCs in the CD11c-DTR model can be well depleted as shown previously. The FACS analysis in Fig.S1 is of very poor quality. To show a deletion of DCs, the authors should gate at least on CD11c and MHCII. In addition to the spleen, which is not the relevant organ in the

adoptive transfer EAE model, data should be provided on the depletion of DCs in the brain before and during EAE. The DCs in the CNS during EAE are monocyte-derived.
We have carried out additional EAE experiments to address this point. Please find the detailed analysis of $CD11c^+$ cells in different phases of the EAE in the new Figure 4. As suggested by the referee, we have now also included another depletion control for DTX-mediated CD11c-depletion in chimeric EAE mice. Furthermore we show spleen and CNS $CD11c^+$ cells and the depletion efficiency in both of these compartments (see Fig. S1C/S1D).

It is not clear whether they target both the local APCs in the perivascular space and also the monocyte-derived DCs that accumulate in the CNS during EAE. Are both effectively depleted?
We have now included new data on the subpopulations of CD11c-expressing cells in the course of EAE (see new Fig. 4). We see here that before onset, conventional DCs are the most frequent CD11c-expressing cells. In clinically evident EAE, we see an accumulation of monocyte-derived Ly6G/$Ly6C^{int}$ CD11c-expressing cells in the CNS whereas in the spleen the subpopulations remain stable over the different disease stages. Depletion controls reveal a strong depletion of all subsets (Fig. S1C/S1D).

Is it possible to delete DCs after disease onset? Does it have an impact on EAE progression?
As suggested by this referee we have performed peak depletion experiments in active EAE (start of CD11c-depletion at a score of 1; see Fig. S2), which showed the same result as the depletion of $CD11c^+$ cells in adoptive transfer EAE. Please also see point 3 by referee 1.

Figure 1F: This is not a convincing staining for GM-CSF.
This has been exchanged by a new and brighter GM-CSF staining which better shows these results (see Fig. 1F/1G).

Figure 4 and Figure S5: To draw any conclusions about chemokine receptor expression from different cell populations in an EAE-diseased brain, flow cytometry cell sorting has to be performed. As shown in Figure S5, MACS sorting of total CD11c+ cells does not reach high enough purity. For example CNS CD11c- cells, in addition to the CD4+ T cells, comprise monocytes, neutrophils, and macrophages. On the other hand, CD11c+ cells contain monocytes and monocyte-derived cells (DCs/macrophages) and maybe a contamination of activated microglia. The authors then compare the CD11c+ and CD11c- CNS cells to CD11c+ and CD11c- splenic cells. This is not a fair comparison as the composition of splenic CD11c- cells is completely different and CD11c+ splenic cells are classical DCs and not monocyte-derived DCs as in the inflamed brain.
As suggested by the referee, we have further characterized $CD11c^+$ cells in the CNS in EAE (see new Fig. 4). The $CD11c^+Ly6C/G^{low}$ subset in the spleen consists of approximately equal proportions of CD11b-expressing cells and CD11b-negative cells, $CD11c^+$ $Ly6C/G^{int}$ (monocyte-derived $CD11c^+$) cells were a rare subset in the spleen. We have included these data in both the Results (new section) and Discussion.
We performed additional experiments with (>90% purity) FACS-sorted subpopulations of $CD11c^+$ cells. We show that the $Ly6C/G^-$ $CD11c^+$ cells and the monocyte-derived $CD11c^+$ cells from the CNS of EAE affected mice have a similar expression pattern for the investigated chemokines (included here for the referee's appreciation).

As suggested by the referee, we have changed our analysis of chemokine/cytokine expression of different cell subsets. We now compare well-defined subpopulations (instead of $CD11c^+$ vs. non-$CD11c^+$). We now compare $CD11c^+$ cells in the CNS with microglia (CX3CR1-GFP CD45int IA_b^+) and CD4+ T cells. In the spleen, we compare $CD11c^+$ cells with monocytes/macrophages ($CD11c^-$ $CD11b^+IA_b^+$) and CD4+ T cells (see Fig. 5). (Figures for referees not shown.)

Figure 1B: At what time point after EAE induction?
The analysis was performed on day 30 after cell transfer of animals shown in Fig. 1A. This is now included in the figure legend.

Figure 1B, C, F: On what are these cells pregated?
Cells were pre-gated on lymphocyte cells (FSC SSC gate) and PI-negative (only for 1B). 1C/1F were pre-gated in addition to FSC/SSC on CD45+CD4+. This is now specified in the figure legend.

Figure 3: A and B: Description of figure legend does not correlate with the pictures. (arrow, arrowheads and asterisk).
We apologize for the mistake. This has now been corrected.

2nd Editorial Decision 12 September 2015

Thank you for submitting your revised manuscript to The EMBO Journal. Your study has now been re-reviewed by the two original referees. As you can see below, the referees appreciate the introduced changes and support publication here. There are just few remaining comments to sort out before final acceptance here. Please also take a look at our new guidelines regarding supplemental information http://emboj.embopress.org/authorguide#expandedview

You can use the link below to upload the revised version.

REFEREE REPORTS

Referee #1:

Th3 authors adequately addressed the comments made by the referees.
Regarding the title, it would be wise to replace CD11C with the function of that these cells instead of than using their immunological classification. After all EMBO J. is not immunological journal and the title should be such that it will speak to the general readership. I would also suggest to follow this recommendation with respect to the Abstract.

Referee #2:

The authors have addressed most of my concerns. I only have a few comments:

In my opinion Figure 4 does not add much to improve the MS. The increase in monocytes/moDCs in the CNS over the course of EAE has been well described previously.
What is still not quite clear to me is why the authors chose to display the spleen. The CNS is the target organ and if they would like to display a peripheral organ it would be better to show the 'immunization-site' draining lymph nodes. In addition, it would be better to gate on CD11c and MHCII and not just on a broad CD11c gate, which includes other cells than DCs/moDCs. For example a contamination of neutrophils or activated microglia could slightly shift into this gate. Why not gate on Ly6C and Ly6G separately?
My suggestion for Figure 4 is to maybe omit A) or move it to supplementary Figures but instead adding the DTx-treated CNS along the control CNS at the different time points (Fig. 4B). Thus combine Figure 4B with S1D?

Figure S1C/D: The figure legend is not clear.
What is the pre-gate? CD45+ cells including microglia?
How many days after EAE induction? (peak disease or onset of EAE)?
Adoptive transfer model or s.c. immunization with MOG/CFA?

Figure 5: Could they provide a gating strategy for the sorting (for example as part of a supplementary figure)?

Figure 1: Figure legend for A) should also be adjusted to the new Figure.

2nd Revision - authors' response 01 October 2015

Referee 1:

1. The authors adequately addressed the comments made by the referees.

Regarding the title, it would be wise to replace CD11C with the function of that these cells instead of than using their immunological classification. After all EMBO J. is not immunological journal and the title should be such that it will speak to the general readership. I would also suggest to follow this recommendation with respect to the Abstract.
As suggested by the referee we changed the title by exchanging "CD11c+ cells" by the function of these cells ("professional antigen-presenting CD11c+ cells") to appeal to a broader readership. We have also amended the abstract accordingly.

Referee 2:

2. The authors have addressed most of my concerns. I only have a few comments:
In my opinion Figure 4 does not add much to improve the MS. The increase in monocytes/moDCs in the CNS over the course of EAE has been well described previously.
Indeed, the presence of monocytes/moDCs in the CNS in EAE has been described previously (Serafini et al., 2000; Suter et al., 2000; Clarkson et al., 2014). However, our detailed description of different phases (pre-onset, peak, chronic) in combination with a flow cytometric characterization (monocyte-derived vs. CD11b+ and CD11b- conventional DCs) is, to the best of our knowledge, new. As this information is the basis for the subsequent molecular characterization of these cells and to better address the broad readership of EMBO J, who may be less familiar with these specific changes in the EAE model, we agree with the referee, and have reduced the complexity of the figure according to this referee's suggestions (see new Fig. 4).

3. What is still not quite clear to me is why the authors chose to display the spleen. The CNS is the target organ and if they would like to display a peripheral organ it would be better to show the 'immunization-site' draining lymph nodes.
We agree with the referee that the CD11c+ cells within the CNS are the major cell subset of interest, since we focus on the role of DCs in the CNS, in particular in the later stages of neuroinflammation ("effector phase"), in active EAE and adoptive transfer EAE, in which ready-made encephalitogenic T cells are transferred and distributed primarily in the spleen and CNS.
We chose to show the splenic CD11c+ cells for comparison, since this population has been characterized in depth in basic immunological studies (Steinman, 2007). We did not choose lymph node DCs, in particular not draining LNs, because these are relevant in the first days directly following immunization, but less so in the pre-onset, peak and chronic phases of EAE. For the referee's appreciation, Fig. I (Figures for referees not shown.) shows a summarizing graph from unpublished data in accordance to current knowledge, outlining fold changes in the different DC subsets (spleen vs. draining LN) at different time points post immunization. This graph shows that splenic-derived conventional DCs and mononcyte-derived DCs follow a similar course as those found in the CNS, whereas the DCs found in the dLN show different kinetics according to their local role.

Thus, we agree with the reviewer that the DCs in the LNs are of relevance, but rather in the early activation events in the first few days following immunization in active EAE ("priming phase"). However, this stage is not in the focus of our study as we aimed to elucidate the role of DCs after priming has been completed.

The following sentences in the Discussion should clarify this point:

Here, we unraveled the role of CD11c+ cells in the CNS on transferred encephalitogenic T cells and in the effector phase of active EAE, thus focusing on the admission and survival of pathogenic T cells in the CNS. The question of sustained inflammation in the CNS might be more relevant for the human disease, which is usually not elicited by vaccinations (Siffrin et al., 2007) and persists in the absence of any systemic inflammatory challenges.

4. In addition, it would be better to gate on CD11c and MHCII and not just on a broad CD11c gate, which includes other cells than DCs/moDCs. For example a contamination of neutrophils or activated microglia could slightly shift into this gate.
We performed a series of experiments with a combined staining including MHCII. As we had some trouble with "weak" CNS IAb/MHCII staining (e.g., in comparison to the spleen) and found the same ratios concerning the relevant cell populations, we did not pursue this. We are aware of this

problem from other epitopes and it is most likely due to the harsh digestion process that is needed for immune cell isolation from the CNS (see also M&M). Importantly, we did not see a major difference with or without pre-gating on MHCII (see please below). We show here the data with and without pre-gating on MHCII (Fig II) for the referee's appreciation. (Figures for referees not shown.)

5. Why not gate on Ly6C and Ly6G separately?
Concerning the Ly6C/Ly6G staining, we followed the recommendation of DC experts for a commonly used antibody that recognizes both molecules and serves for the discrimination of monocyte-derived CD11c+ cells vs. conventional DCs. We did not have more fluorescence labels left in our staining protocol for a separate staining of the two molecules. This antibody (clone RB6-8C5) has been used for discrimination of monocyte-derived CD11c+ cells in similar studies (Serbina et al., 2003).

6. My suggestion for Figure 4 is to maybe omit A) or move it to supplementary Figures but instead adding the DTx-treated CNS along the control CNS at the different time points (Fig. 4B). Thus combine Figure 4B with S1D?
As suggested by the referee, we have reduced the complexity of this figure by showing the spleen CD11c+ cells only in the supplementary information. We found the addition of DTx-treated CNS rather more complex (if shown for all stages), and therefore, we left the DTx-depleted late (CNS and spleen) time points in the Fig. S1C/D to show the reliable DTx-mediated DC depletion. However, if this referee, after this modification, still requests that S1C/D be included in the main figure, we can certainly change it.

7. Figure S1C/D: The figure legend is not clear. What is the pre-gate? CD45+ cells including microglia? How many days after EAE induction? (peak disease or onset of EAE)? Adoptive transfer model or s.c. immunization with MOG/CFA?
As suggested by the referee, we changed the figure legend to make it more comprehensible.

8. Figure 5: Could they provide a gating strategy for the sorting (for example as part of a supplementary figure)?
This is now shown in the new supplementary Figure S6.

9. Figure 1: Figure legend for A) should also be adjusted to the new Figure.
As suggested by the referee we have adjusted the figure legend to the new figure.

References
Clarkson BD, Walker A, Harris M, Rayasam A, Sandor M, Fabry Z (2014) Mapping the accumulation of co-infiltrating CNS dendritic cells and encephalitogenic T cells during EAE. J Neuroimmunol 277:39–49.
Serafini B, Columba-Cabezas S, Di Rosa F, Aloisi F (2000) Intracerebral recruitment and maturation of dendritic cells in the onset and progression of experimental autoimmune encephalomyelitis. Am J Pathol 157:1991–2002.
Serbina NV, Salazar-Mather TP, Biron CA, Kuziel WA, Pamer EG (2003) TNF/iNOS-producing dendritic cells mediate innate immune defense against bacterial infection. Immunity 19:59–70.
Steinman RM (2007) Dendritic cells: understanding immunogenicity. Eur J Immunol 37 Suppl 1:S53–S60.
Suter T, Malipiero U, Otten L, Ludewig B, Muelethaler-Mottet A, Mach B, Reith W, Fontana A (2000) Dendritic cells and differential usage of the MHC class II transactivator promoters in the central nervous system in experimental autoimmune encephalitis. Eur J Immunol 30:794–802.

