A mannan-conjugated myelin antigen inhibits the induction of CNS autoimmunity by CD11b^{+}Ly6C^{hi} monocytes

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Introduction: Immature CD11b^{+}Ly6Chi myeloid progenitors have been shown to be important for the development of experimental autoimmune encephalomyelitis (EAE). We recently showed that mannan-conjugated MOG (OM-MOG) ameliorates MOG-induced EAE by inducing a peripheral type 2 myeloid response. Here we investigate the involvement of Ly6C^{hi} and PD-L1 in OM-MOG-induced immune tolerance.

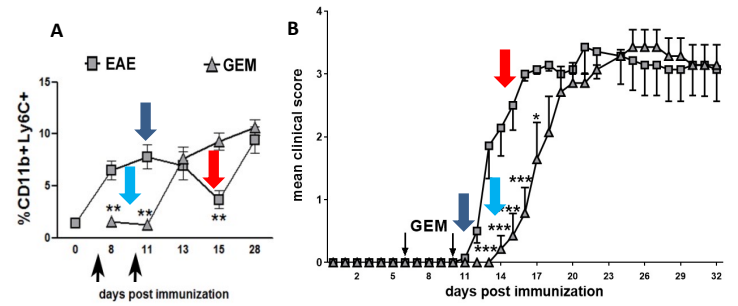
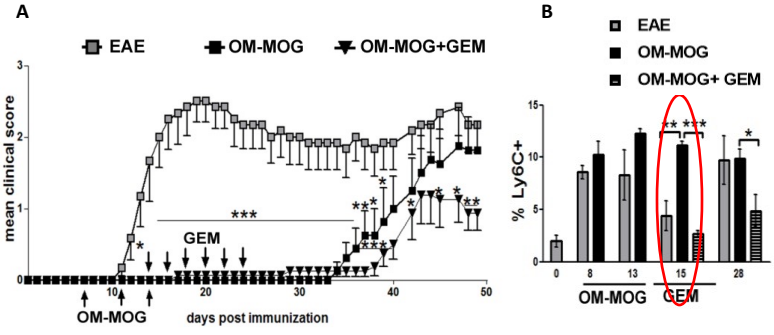


Figure 1: CD11b^{+}Ly6C^{hi} monocytes are critical for EAE onset and transiently depleted at disease peak. Gemcitabine depletes CD11b^{+}Ly6C^{hi} and delays EAE onset.
C57BL/6 mice were immunized with MOG (MOG35-55; 37μg) and treated with gemcitabine (GEM, i.p., 60mg/kg) at indicated timepoints (black arrows). **(A)** Percentages of CD11b^{+}Ly6C^{hi} cells in peripheral blood by FACS on days 0,8,11,13,15 and 28 dpi. **(B)** Mean clinical scores of EAE mice treated with GEM. Statistical analysis by Student's T test (A) and two-way ANOVA (B) is shown. (*p≤0.05, **p≤0.01, ***p≤0.001).



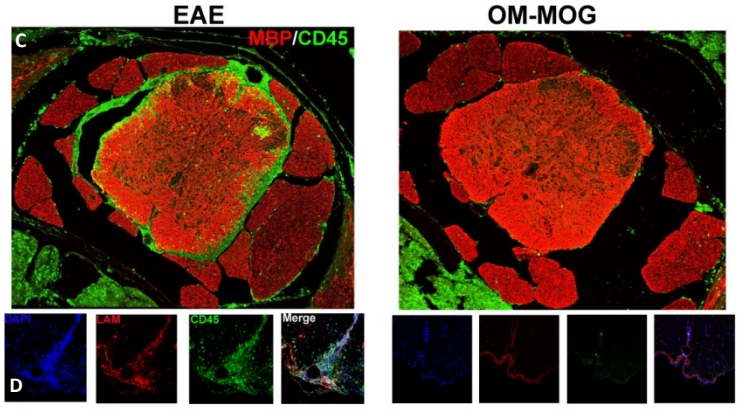


Figure 2: OM-MOG prevents spinal cord lesion formation by retaining Ly6C^{hi} monocytes in the periphery.
C57BL/6 mice were immunized with MOG and treated with OM-MOG (i.d., 30 μg, upward arrows) on days 7,11 and 14, and with GEM (i.p., 60mg/kg) at indicated timepoints (downward arrows). **(A)** Mean clinical scores of mice treated with OM-MOG or OM-MOG+GEM at indicated time points. **(B)** Percentages of Ly6C^{hi} in peripheral blood of the same mice as in A on days 0,8,11,13,15 and 28 post-immunization for EAE. **(C)** Whole mount spinal cord sections together with the surrounding bone taken at EAE peak (dpi18) and immunostained with antibodies against MBP and CD45. (D) Spinal cord sections from the same mice as in C immunostained with antibodies against laminin (LAM; red) and CD45 (green).

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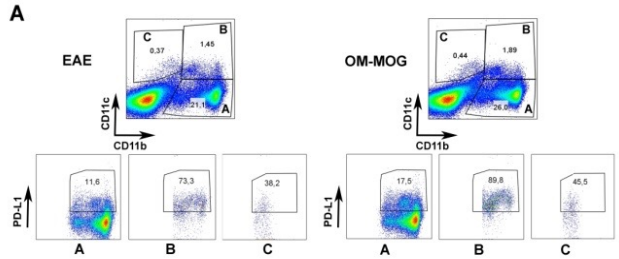

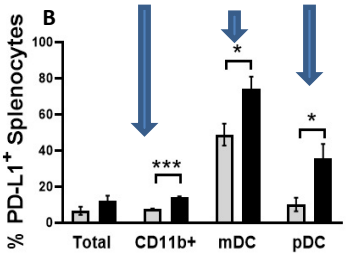

Figure 3: Therapeutic OM-MOG increases PD-L1 production by splenic myeloid cell populations.
(A) FACS plots of splenocytes, isolated after one therapeutic injection of OM-MOG (dpi 14-17). **(B)** Percentages of PD-L1+ cell populations as in A (EAE grey columns, OM-MOG black columns). Statistical analysis by Student's T test (*p≤0.05, ***p≤0.001)

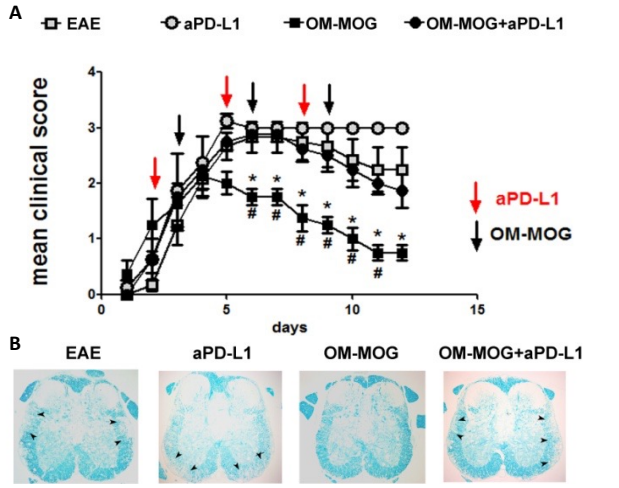

Figure 4: PD-L1 blockade reverses OM-MOG tolerance
C57BL/6 mice were immunized with MOG, untreated (grey squares) or treated with OM-MOG (i.d., 30 μg/injection) (black squares), and anti-PD-L1 antibody (i.p., 200μg/injection), or vehicle. **(A)** Mean clinical scores of mice treated with OM-MOG and/or anti-PD-L1 antibody at indicated time points. **(B)** Spinal cord sections taken from the day of sacrifice, stained with Luxol Fast Blue to show myelin (arrowheads show demyelinating lesions). Statistical analysis by Mann-Whitney test (*p≤0.05, EAE vs OM-MOG, # p≤0.05, OM-MOG vs PD-L1)

Conclusions

- Peripheral immature Ly6C^{hi} monocytes are critical for EAE and their selective depletion by gemcitabine inhibits disease onset.
- OM-MOG induces immune tolerance by retaining Ly6C^{hi} monocytes in the periphery, and is prolonged by gemcitabine.
- OM-MOG activates peripheral myeloid cells to produce PD-L1, which in turn is a critical mediator of OM-MOG immune tolerance.