Supporting Information

Mars et al. 10.1073/pnas.0809317106

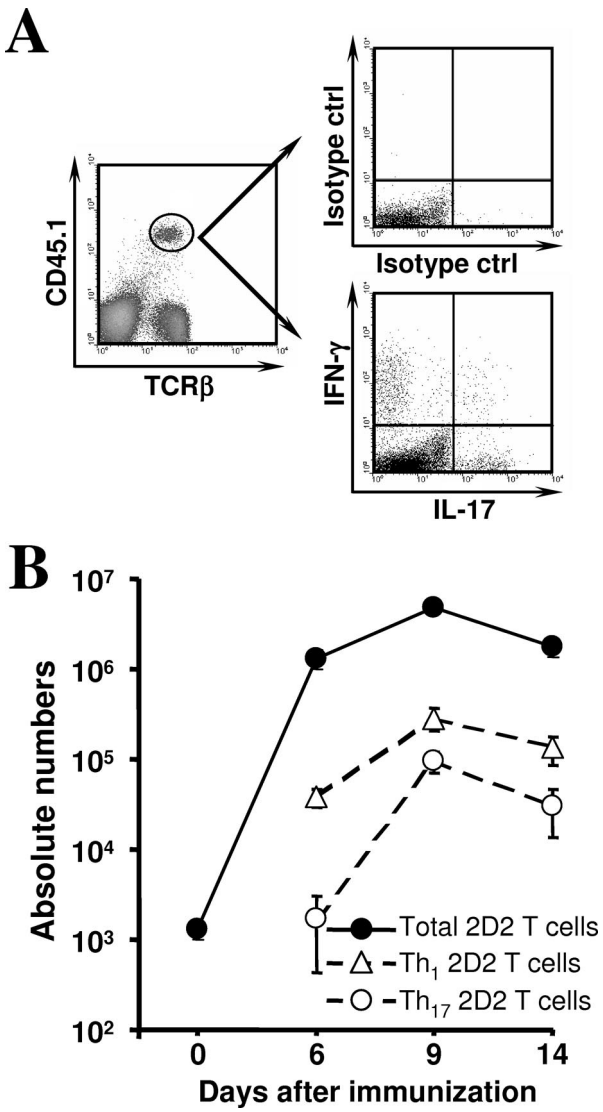

Fig. S1. Kinetics of MOG-specific Th_1 and Th_{17} cell development in the spleen. C57BL/6 mice received 10^5 congenic $CD62L^+CD25^-$ 2D2 $CD4^+$ T cells and were immunized 24 h later with $MOG_{35–55}$ in the presence of pertussis toxin. (*A*) The intracellular IFN-γ and IL-17 content was assessed on gated congenic 2D2 cells. (*B*) The number of congenic 2D2, Th_1 2D2, and Th_{17} 2D2 cells was determined on the day of immunization (day 0; $n = 6$), and 6 ($n = 4$), 9 ($n = 8$), and 14 ($n = 8$) days after immunization. The number of Th_{17} 2D2 cells was significantly inferior to Th_1 2D2 cells at days 6, 9, and 14 after immunization.

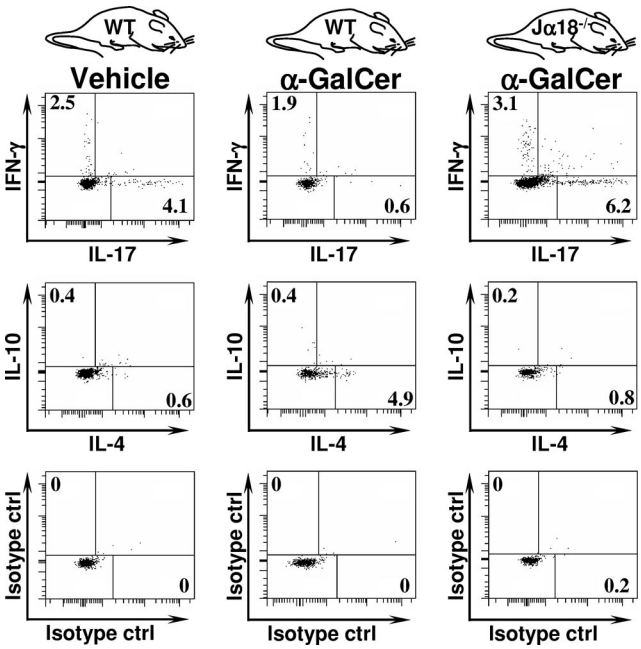

Fig. S2. Detection of MOG-specific Th_1, Th_{17}, Th_2, and Tr_1 2D2 cells after activation of iNKT cells with α-GalCer. MOG-specific T-cell differentiation was assessed in cells from the draining LNs of immunized C57BL/6 (WT) and $J\alpha18^{-/-}$ mice after transfer of 10^5 congenic $CD62L^+CD25^-$ 2D2 $CD4^+$ cells. Nine days after immunization, the intracellular expression of IFN-γ, IL-17 (*Top*) and IL-4, IL-10 (*Middle*) was assessed in $CD45.1^+TCRV\alpha3.2^+$ $CD4^+$ T cells after treatment with either α-GalCer (WT, $n = 11$; $J\alpha18^{-/-}$, $n = 4$) or vehicle (WT, $n = 11$; $J\alpha18^{-/-}$, $n = 4$). Isotype-matched mAbs with irrelevant specificity were used as controls (*Bottom*). All mice were analyzed individually in 3 separate experiments (WT) or a single experiment ($J\alpha18^{-/-}$).

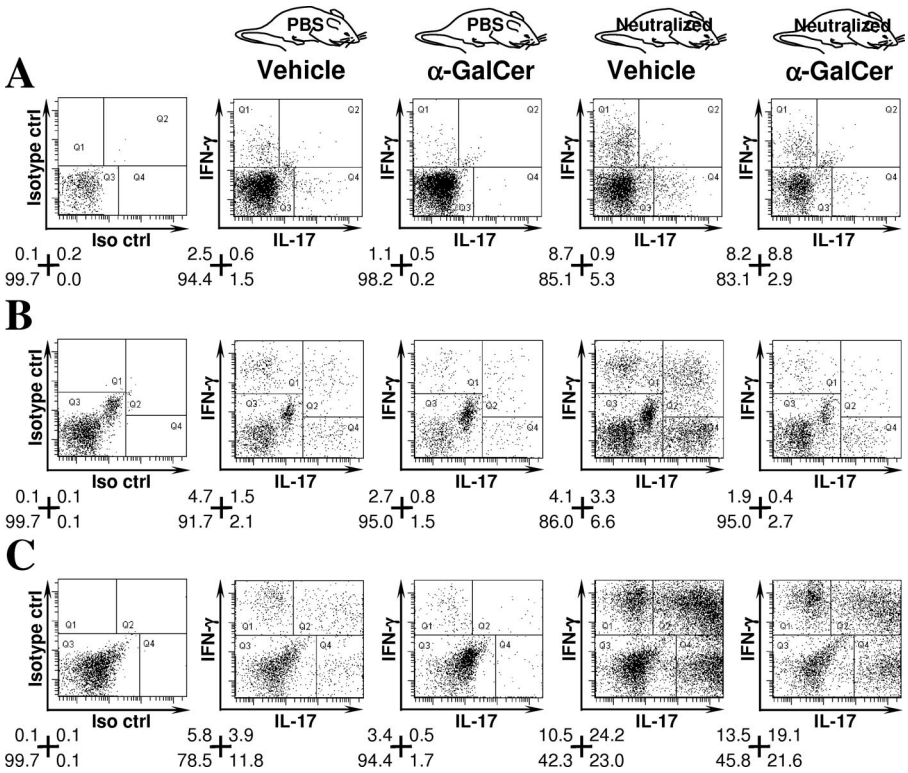

Fig. S3. α-GalCer-activated iNKT cells require IL-4, IL-10, and IFN-γ to block Th_{17} lineage commitment by naïve 2D2 T cells. The impact of (*A*) IL-4 + IL10R, (*B*) IFN-γ, and (*C*) IL-4 + IL-10R + IFN-γ neutralization on Th_{17} differentiation in MOG-immunized C57BL/6 (WT) recipients of 10^5 congenic $CD62L^+CD25^-$ 2D2 $CD4^+$ T cells was evaluated 9 days postimmunization. The intracellular expression of IFN-γ and IL-17 was assessed in $CD45.1^+TCRV\alpha3.2^+$ $CD4^+$ T cells after treatment with either α-GalCer ($n = 4$ per group, column 3,5) or vehicle ($n = 4$ per group, column 2,4 in cytokine-neutralized (column 4,5) and PBS-treated mice (column 2,3). Isotype-matched mAbs with irrelevant specificity were used as controls (column 1). All mice were analyzed individually. Quadrants placed below the dot plots represent the frequency of cytokine expression 2D2 cells in the representative example.

Table S1. Clinical severity of EAE

Treatment	Neutralization	*n*	Incidence, %	Mean day of onset (SEM)	Mean cumulative severity (SEM)	Mortality, %
Vehicle	None	4	100	13 (±0.5)	66 (±32)	25
Vehicle	IL4 + IL10R	4	100	14 (±1.0)	145 (±4)	100
Vehicle	IFN-g	4	75	12 (±1.2)	112 (±37)	75
Vehicle	IFN-g + IL4 + IL10R	3	100	15 (±0.3)*	142 (±4)	100
a-GalCer	None	4	0		0 (±0)	0
a-GalCer	IL4 + IL10R	4	100	14 (±1.6)**	147 (±7)*	100
a-GalCer	IFN-g	4	75	16 (±1.7)*	91 (±33)	50
a-GalCer	IFN-g + IL4 + IL10R	4	75	15 (±2.0)*	107 (±36)	75

Statistical significance was tested by comparing the cytokine-neutralized mice with their respective nonneutralized vehicle or α-GalCer-treated counterpart (*, $P < 0.05$; **, $P < 0.01$).