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Article

IFN-γ Induces PD-L1 Expression in Primed Human Basophils

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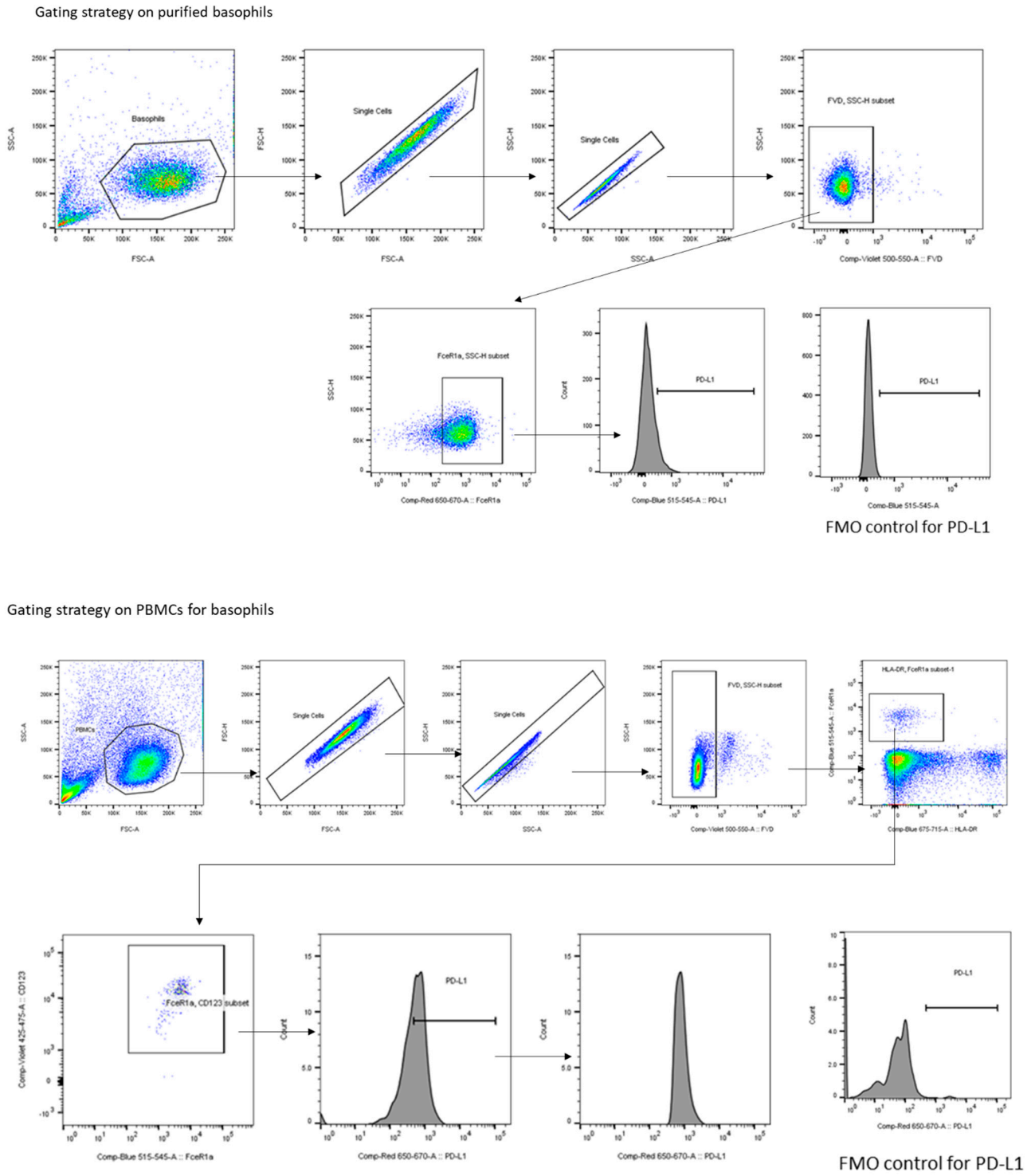

Figure S1. Flow cytometry gating strategy for the purified basophils (upper panels) and peripheral blood mononuclear cells containing basophils (lower panels).

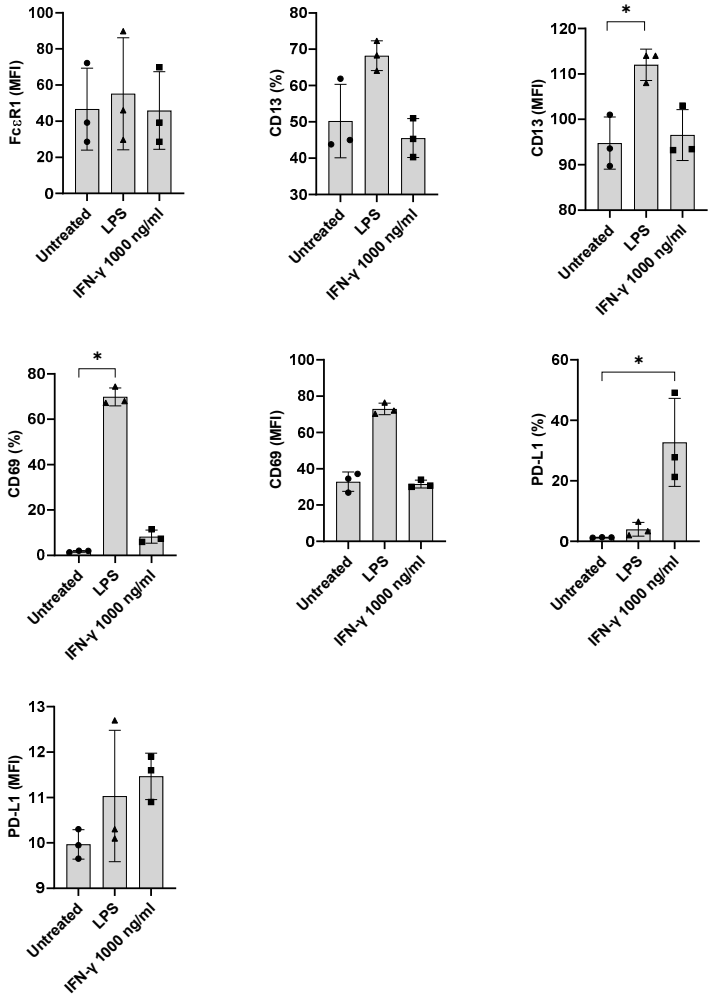

Figure S2. Prolonged stimulatory effect of IFN-γ on PD-L1 and other surface markers of untouched basophils. Basophil-containing PBMCs (1x10^6 cells/ml/24-well plate) of healthy donors were cultured with or without IFN-γ for measuring different parameters. PBMCs were cultured either without (untreated) or with LPS (100 ng/ml) or with IFN-γ (1000 ng/ml) for 48 h. After incubation, PD-L1 expression (% positive cells and median fluorescence intensities (MFI), mean ± SD; n = 3 independent donors from two independent experiments) was evaluated by flow cytometry. *P < 0.05, one-way ANOVA Friedman test with Dunn's multiple comparisons post-test.

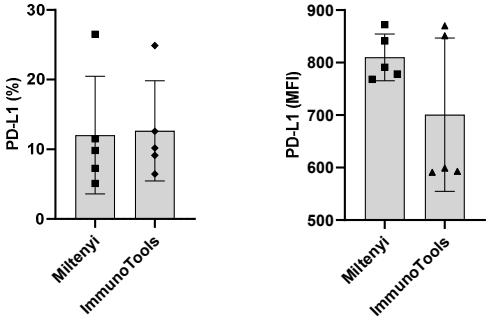

Figure S3. The effect of IFN-γ from different sources on PD-L1 expression in human basophils. Basophils (0.1x10^6 cells/200 µl/96-well plate) isolated from PBMCs of healthy donors were cultured with IFN-γ at 1000 ng/ml. Basophil phenotype was evaluated by flow cytometry after 24 h. Expression of PD-L1 on basophils (% positive cells and median fluorescence intensities (MFI), mean ± SD; n = 5 independent donors from three independent experiments) was represented.

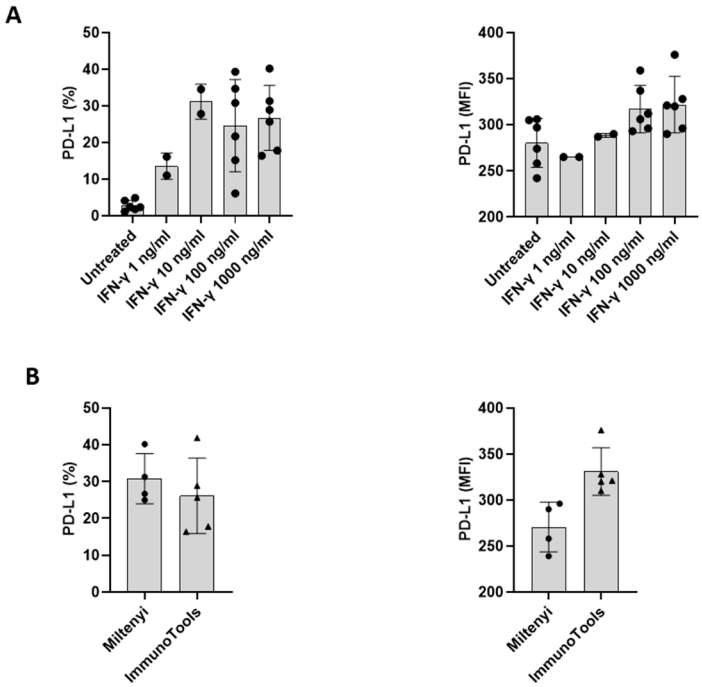

Figure S4. The influence of dose and source of IFN-γ towards PD-L1 expression in untouched basophils. Basophil-containing PBMCs ($1x10^6$ cells/ml/24-well plate) of healthy donors were cultured with or without IFN-γ for measuring different parameters. (A) PBMCs were cultured either without (untreated) or with IFN-γ at 1-1000 ng/ml for 24 h. After incubation, PD-L1 expression (% positive cells and median fluorescence intensities (MFI), mean ± SD; n = 2-6 independent donors from two or three independent experiments) was evaluated by flow cytometry. (B) PBMCs were treated with IFN-γ at 1000 ng/ml, from different sources, for 24 h. Expression of PD-L1 on basophils (% positive cells and median fluorescence intensities (MFI), mean ± SD; n = 4 independent donors from two independent experiments) was presented.

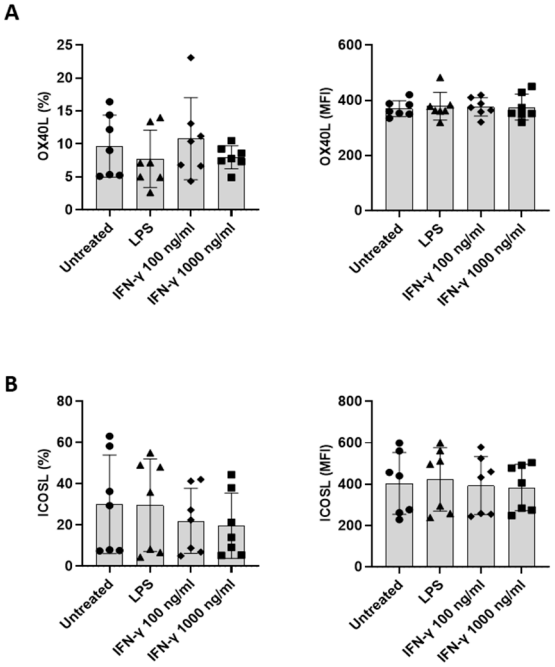

Figure S5. The effect of IFN-γ on OX40L and ICOSL expression in basophils from untouched PBMCs. Basophil-containing PBMCs ($1x10^6$ cells/ml/24-well plate) from the healthy donors were cultured with or without IFN-γ at 100 ng/ml and 1000 ng/ml or LPS at 100 ng/ml for 24 h. After incubation, cells phenotype was evaluated by flow cytometry. Expression of OX40L (A) and ICOSL (B) on the basophils (% positive cells and median fluorescence intensities (MFI), mean ± SD; n = 7 independent donors with three independent experiments) are presented.