Plexin B1 controls the size of the Treg pool *in vivo* and limits allergic airway inflammation

Svetlana P. Chapoval [1, 2], Hongjuan Gao [2], Rachel Fanaroff [3], Achsah D. Keegan [1, 4]

[1]Department of Microbiology and Immunology, [2]Center for Vascular and Inflammatory Diseases, [3]Department of Pathology, [4]Baltimore VA Medical Center, University of Maryland School of Medicine, Baltimore, MD, USA

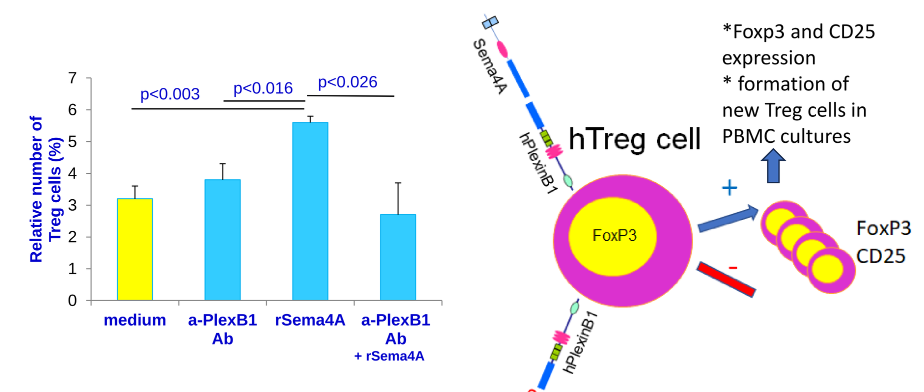
Introduction

Plexin B1

- Receptor for class IV semaphorin family members, **Sema4A** and **Sema4D**
- Its signaling has been implicated in a variety of human diseases including cancer, multiple sclerosis, diabetic retinopathy, atherosclerosis, rheumatoid arthritis, and osteoporosis
- Is involved in migration and proliferation of neuronal, epithelial, endothelial, and tumor cells

Cowan R et al. 2023. JBC. 299:104740

Plexin B1 regulates hTreg cells



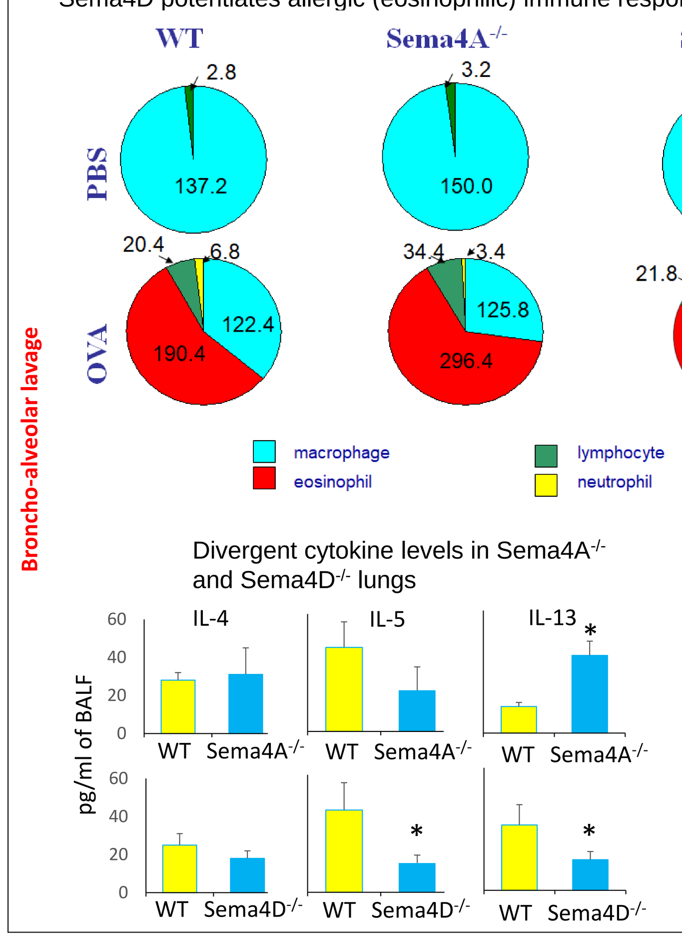
Blocking of Plexin B1 before rSema4A applications decreases the relative numbers of Treg cells in the *in vitro* PBMC cutlures

By engaging either **Sema4A** or **Sema4D**, Plexin B1 elicits distinct effects on Treg cells in the *in vitro* PBMC cultures

Chapoval SP….Keegan AD. 2019. Immunohorizons. 3:71-87

Sema4A and Sema4D in asthma

Sema4A and Sema4D display the opposite effects in allergic airway inflammation in OVA experimental model: Sema4A downregulates while Sema4D potentiates allergic (eosinophilic) immune response

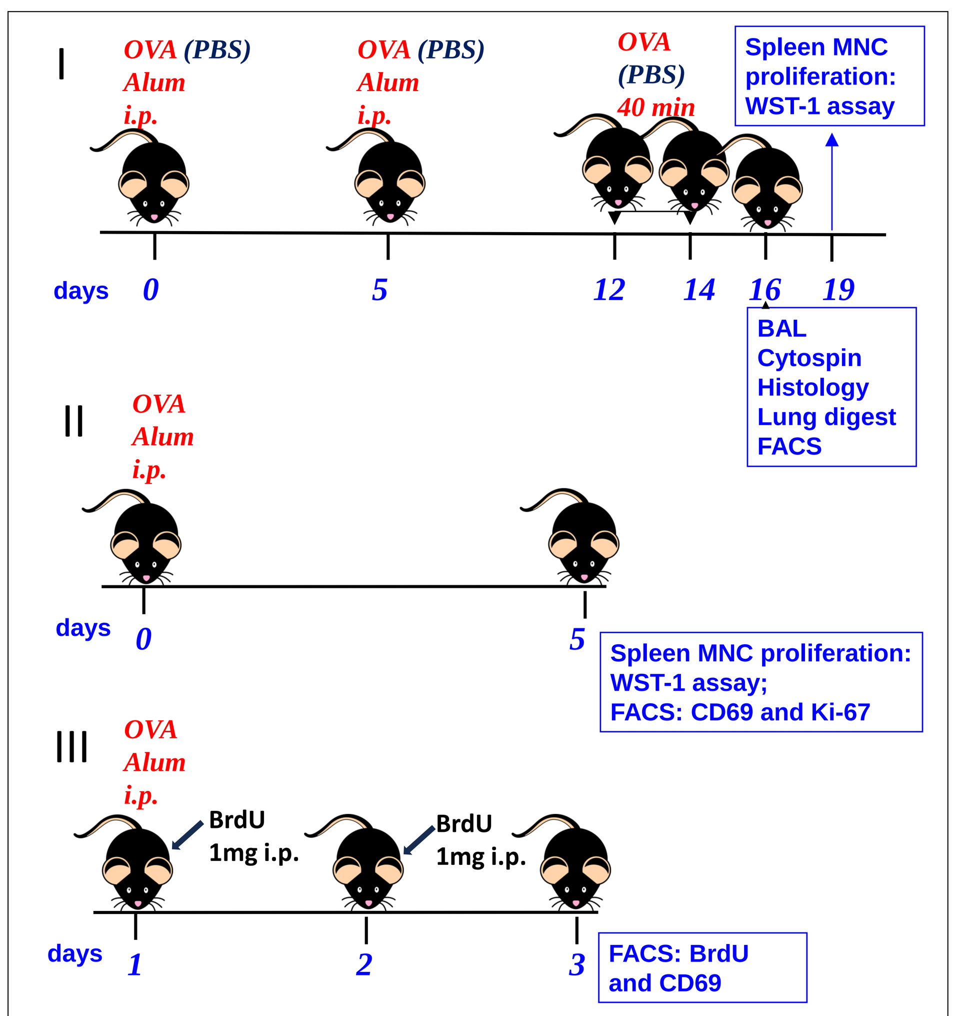


Nkyimbeng-Takwi EH ….Chapoval SP. 2012. Mucosal Immunol. 5:409-19

Shanks K…Chapoval SP. 2013. Molecular Immunol. 56:480-7

Chapoval SP. 2018. Molecular Medicine. 24:13

Experimental protocols



Results

Plexin B1 deficient mice

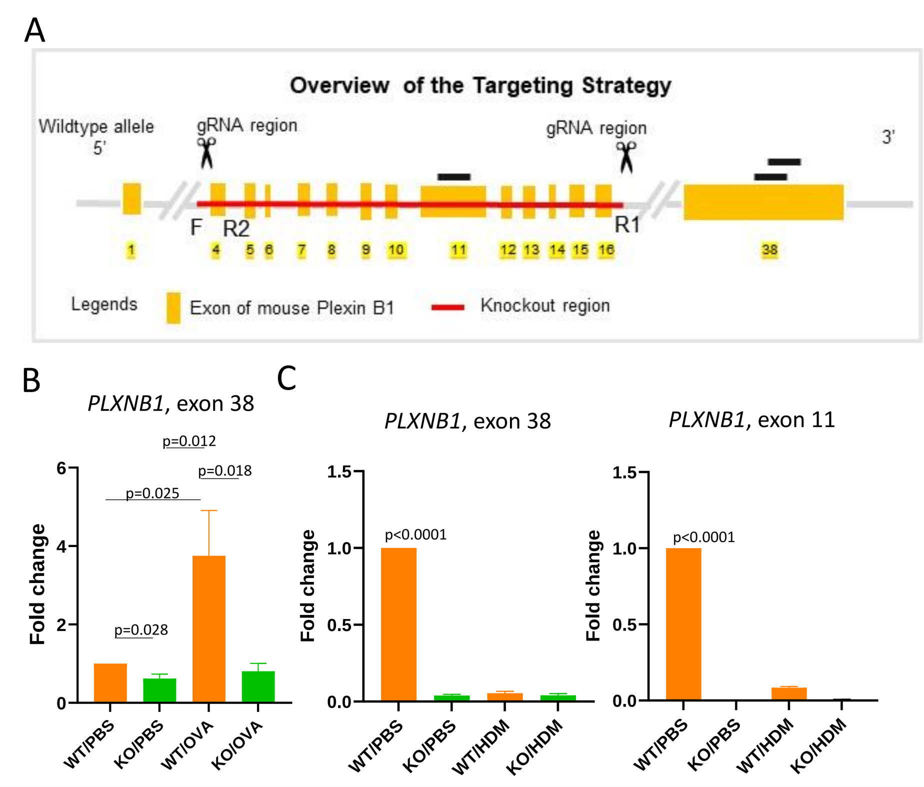


Figure 1. (A) The knockout region in *PLXNB1* gene includes Exons 4-16 of 201 transcripts. Mice were treated with either OVA (B) or HDM (C) (n=5/group). Lung RNA was obtained and evaluated for *PLXNB1* mRNA by qRT-PCR using primer pairs located in Exon 11 and Exon 38 (black lines on panel A). Data statistics were calculated using two-way ANOVA with Tukey's multiple comparison test.

Plexin B1 deficiency affects the numbers of Treg cells in lymphoid tissues

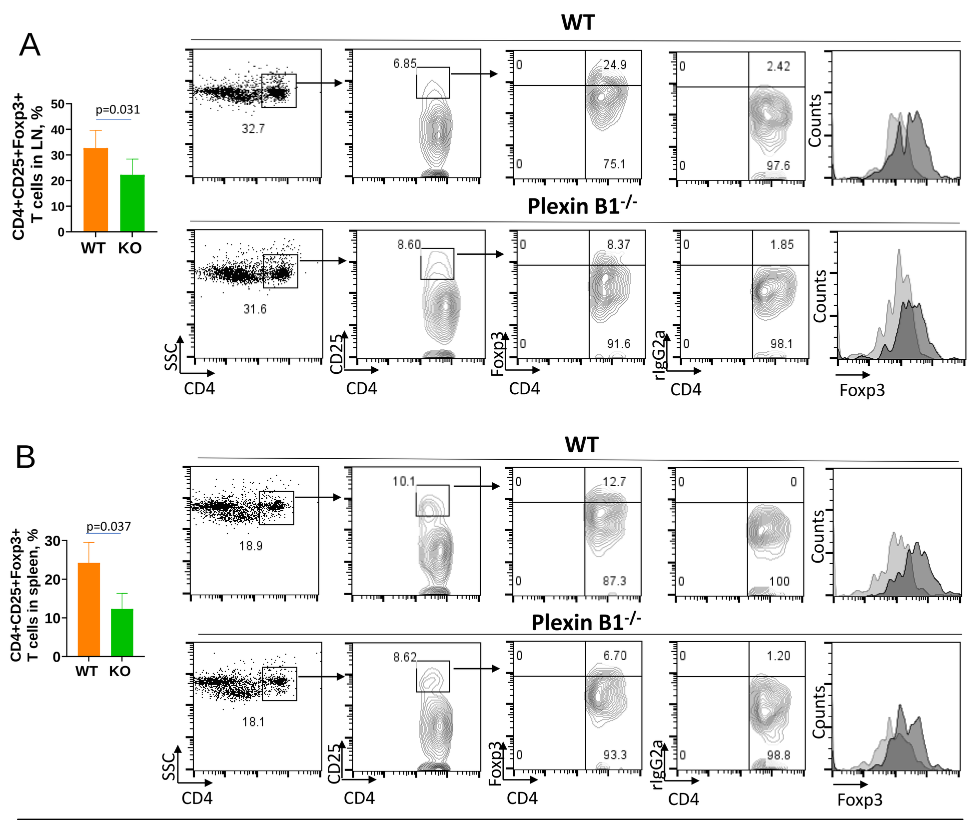


Figure 2. Spleen and lymph node (LN) cells were isolated from WT and Plexin B1$^{-/-}$ mice and evaluated by flow cytometry. The right panel shows the gating strategy for Treg cell determination where LN (A) or spleen (B) MNC were gated on CD4+ cells on dot plots and CD4+ CD25^{high} cells were selected on contour plots for the evaluation of Foxp3 expression. The histograms show the Foxp3 expression (dark grey) on CD4+CD25+ T cells over isotype control Ab (light grey) staining.

Plexin B1 deficiency affects allergic inflammatory lung response to OVA

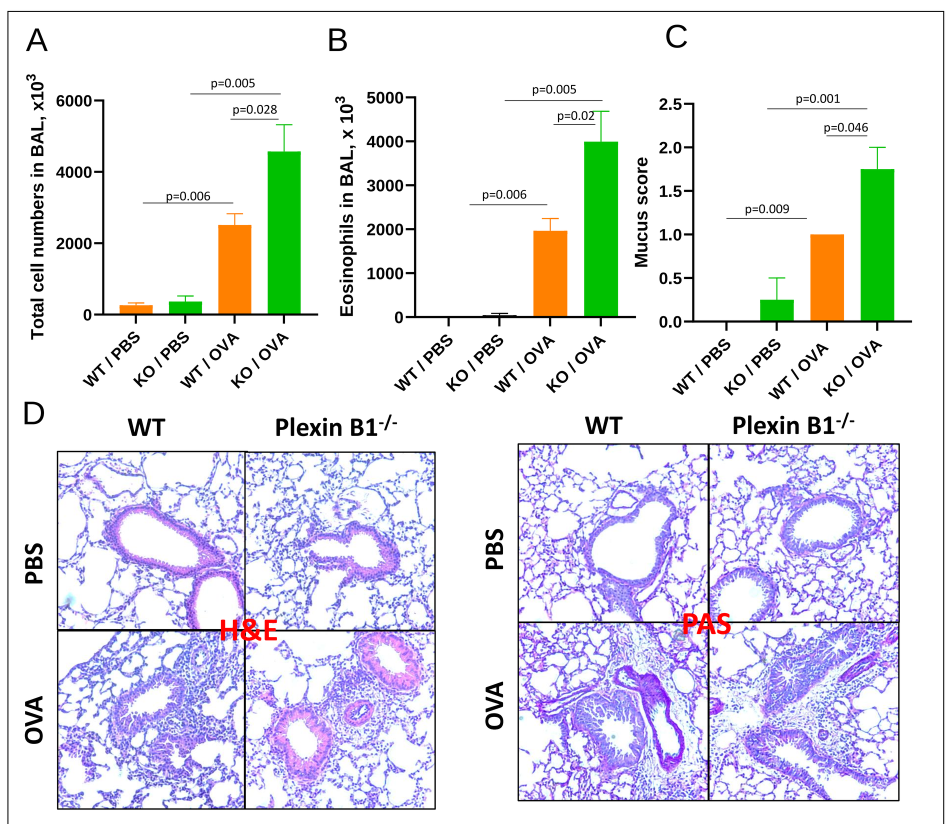


Figure 3. Bronchoalveolar lavage (BAL) was performed and evaluated for total number of cells in BAL (A) and eosinophil numbers (B). Lung sections were stained with PAS and scored for level of or mucus production (C-D). Representative photomicrographs for H&E and PAS are shown in panel D. Data statistics was calculated using two-way ANOVA with Tukey's multiple comparison test.

Increased CD25+CD4+ T cell and decreased Treg cell numbers are associated with a heightened allergic response in Plexin B1$^{-/-}$ mice

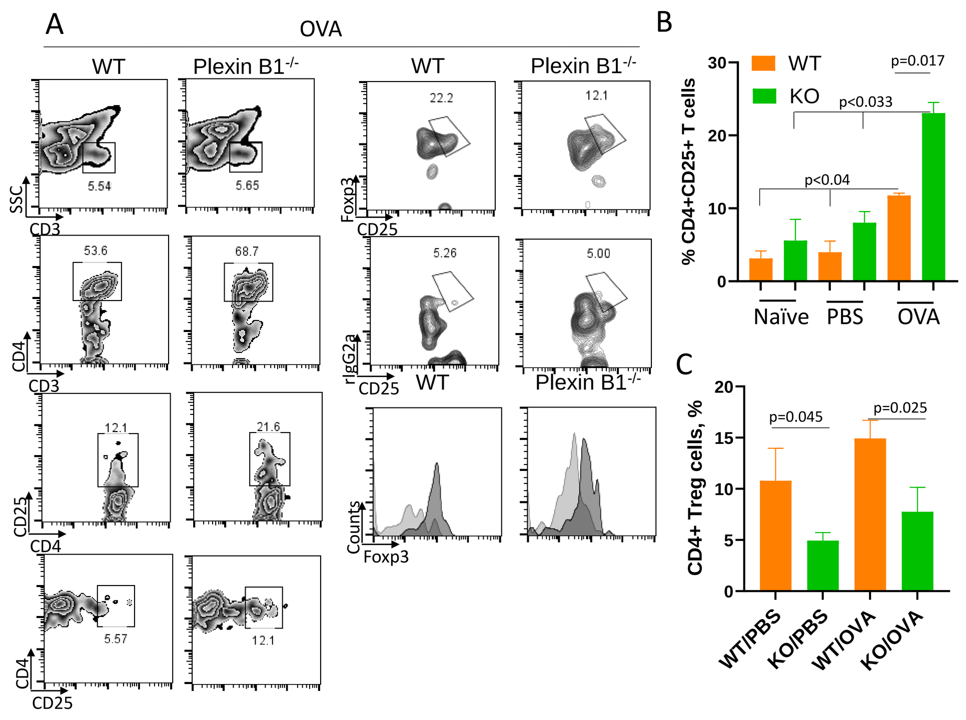


Figure 4. Lung tissues obtained from naïve and PBS- or OVA-treated mice. Single-cell suspensions were analyzed by flow cytometry. (A) Gated CD4 + CD25+ T cells were analyzed for CD25^{high} and Foxp3 expression. The histograms show the fluorescent intensity of Foxp3 expression (dark grey) on CD4+CD25+ T cells over isotype control Ab (light grey) staining. The percentage of CD25+ cells among CD4+ T cells (B) and CD4+ Treg cells (C) was calculated based on panel A.

Plexin B1 regulates *in vitro* and *in vivo* T cell proliferation

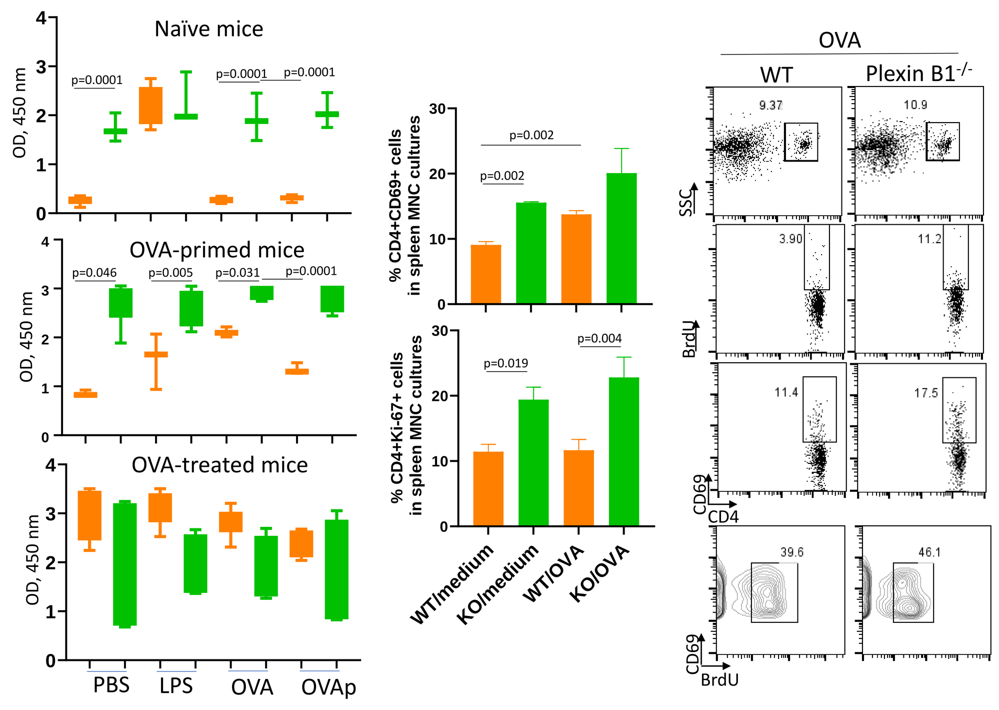
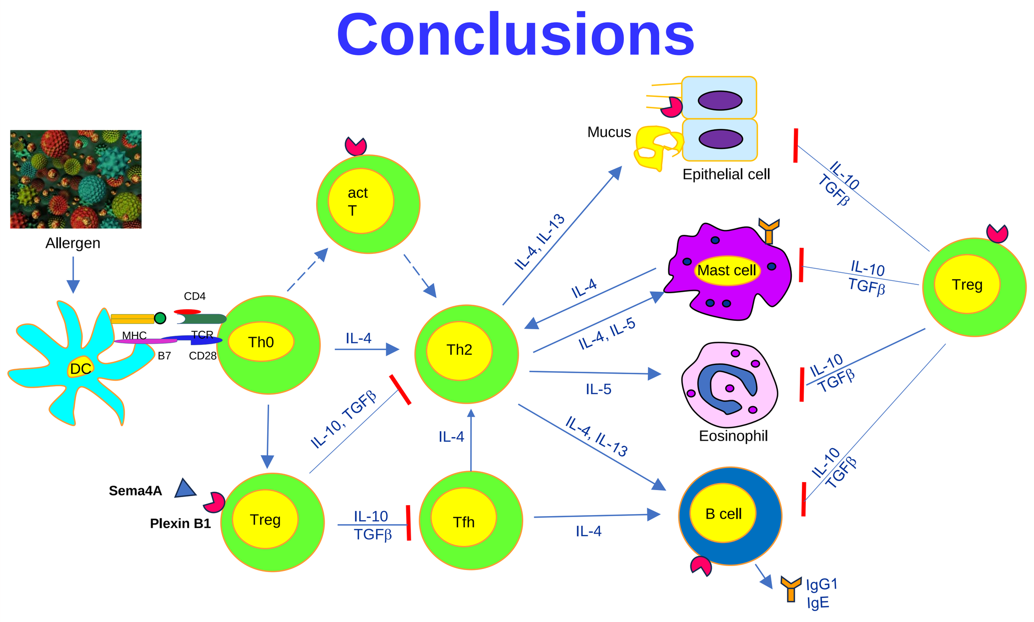


Figure 5. Spleens were harvested from naïve WT and Plexin B1$^{-/-}$ mice, from OVA-primed mice on day 5, and from OVA-treated mice on day 5 post-treatment . (A) Spleen MNC proliferation with or without LPS, OVA, and OVA peptide stimulation was measured by the WST-1 colorimetric assay. (B) Spleen cells were cultured for 36h and analyzed by gating on CD4+ cells and then on CD69 or Ki-67 expression. (C) Mice were injected i.p. with either PBS/Alum or OVA/Alum and BrdU as described. Spleens were harvested from mice 24h after last BrdU injection. Spleen MNC were stained with the indicated Abs to cell surface markers and for interchromatin BrdU and analyzed by flow cytometry. Data statistics was calculated using two-way ANOVA with Tukey's multiple comparison test.

Conclusions



Mechanism of PlexinB1-mediated regulation of allergic airway inflammation. Plexin B1 expression is induced on activated CD4+ T cells. Th2 cells secrete Th2 cytokines which regulate: a) mucin gene expression and stimulate mucus secretion by lung bronchial epithelial cells; b) mast cell activation and degranulation: c) eosinophil generation in bone marrow, recruitment into circulation, migration to lung tissue, and degranulation. B cells get activated by Th2 cells through allergen epitope presentation and by Th2 cytokines to switch to allergen-specific IgE (human and mouse) and IgG1 (mouse) secretion. Tfh cells play a Th2-independent stimulatory role in B cell activation and Ig switch by early secretion of IL-4. All these immunologic processes can be inhibited by Treg cells mainly through their production of anti-inflammatory cytokines IL-10 and TGFβ or/and by a cell-cell contact. Plexin B1 expression on Treg cells regulates their stability and function using Sema4A as a ligand. The role of inducible Plexin B1 on epithelial cells and B cells is unknown.

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schapoval@som.umaryland.edu