SUPPLEMENTARY MATERIAL

MATERIAL AND METHODS

Subjects

Venous blood was drawn from HDs at the Swiss Transfusion Center (Swiss Red Cross) of Geneva in accordance with the Declaration of Helsinki under local ethical approval. Volunteers were asked to read an information sheet for blood donation and to complete an online medical questionnaire on the day of donation. After the questionnaire was finalized, a PDF file was generated for printing and signed to give approval for blood donation. Mastocytosis patients' blood (**Table I** for clinical characteristics, EC Approval CCER 2017-00544) was obtained upon informed consent at the Division of Immunology and Allergology, University Hospitals of Geneva. The diagnosis of mastocytosis was established according to the criteria provided by the World Health Organization (E1). PBMCs were isolated and immediately used or cryopreserved in accordance with the ethical committee of Geneva Hospital (Switzerland).

Flow cytometry analysis and fluorescence assisted cell sorting

Human ILC were identified as lineage (Lin) negative and CD127 positive cells. Lineage markers, all FITC-conjugated, include: anti-human CD8 (MEM- 31, Immunotools, 1:50), anti-human CD14 (RMO52, BC, 2:50), anti-human CD15 (80H5, BC, 2:50), anti-human CD16 (3G8, BC, 1:100), anti-human CD19 (J3-119, BC, 1:100), anti-human CD20 (2H7, Biolegend, 1:100), anti-human CD33 (HIM3-4, Biolegend, 1:50), anti-human CD34 (561, Biolegend, 1:100), anti-human CD203c (E-NPP3, 1:50) (NP4D6, Biolegend, 2:50), anti-human FcεRIα (AER-37, Biolegend 2:50). Additional markers used include: BUV737 anti-human CD56 (NCAM16.2, BD Bioscience, 1:50), APC anti-human CD3 (SP34-2, BD Bioscience, 1:50), Brilliant Violet 605 anti-human CD4 (OKT4, Biolegend, 1:50), PE/Dazzle anti-human CD127 (IL-7Rα) (A019D5, Biolegend, 1:100), APC-Cy7 anti-human CD117 (cKit) (B290144, Biolegend, 1:50), PerCP/Cyanine5.5 anti-human CRTH2 (CD294) (BM16, Biolegend, 1:50), PE anti-human IL-9R (CD129) (AH9R7, Biolegend, 1:50). Dead cells were excluded using the viability dye Live/Dead Aqua or Vivid IR (Invitrogen). For the evaluation of cytokine production, cells were stimulated with 1 μg/mL PMA plus 0.5 μg/mL Ionomycin, in the

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presence of BrefeldinA (all from Sigma-Aldrich) for 3 h prior to intracellular staining. Intracellular staining was performed after fixation and permeabilization with 0.1% saponin (Sigma), using PE-Cy7 anti-human IL-13 (JES10-5A2, Biolegend, 1:50), PE anti-human IL-4 (11B11, Biolegend, 1:50), Brilliant Violet 421 anti-human IL-5 (TRFK5, Biolegend, 1:50) and Alexa Fluor 647 anti-human IL-9 (MH9A3, BD Bioscience, 1:50). Samples were acquired on a Fortessa flow cytometer (Becton-Dickinson, San Jose, CA) and data were analyzed using FlowJo software (TreeStar V.10). For ILC isolation, aliquots of cells were sorted to 98% purity using a FACSAria (Becton Dickinson). Human CD4 T cell subsets and Treg were identified using the following additional antibodies: Brilliant Violet 785 anti-human CD45RO (UCHL1, Biolegend, 1:50), Brilliant Violet 711 anti-human CXCR3 (1C6, Biolegend, 2:50), Brilliant Violet 650 anti-human CD196 (CCR6) (G034E3, Biolegend, 2:50), Brilliant Violet 510 anti-human CD25 (B261355, Biolegend, 1:50). Cells were gated on live $CD3^{+}CD4^{+}CD45RO^{+}$ cells and the Th subsets were identified as follow: Th1 as $CXCR3^{+}CRTH2^{-}CCR6^{-}$, Th* $CXCR3^{+}CRTH2^{-}CCR6^{+}$, Th2 as $CRTH2^{+}$, Th17 as $CXCR3^{-}CRTH2^{-}CCR6^{+}$ and Treg as $CD25^{+}CD127^{low}$.

Cell culture

For ILC2 expansion, freshly sorted ILC2 were cultured for 2 weeks in RPMI-1640 supplemented with 10% heat-inactivated FBS, IL-2 (100 U/mL), IL-7 (5 ng/ml) and by adding PHA (1 μg/ml) at day 0. Medium was replaced every 2–3 days and cell phenotype was verified by flow cytometry. When indicated, IL-9 (50 ng/ml, Adipogen), IL-33 (50 ng/ml, Adipogen), IL-1β (10 ng/ml, Adipogen), VEGF-A (10 ng/ml, Adipogen) and PGD2 (100 nM, Cayman Chemical) were added.

Source of mRNA sequencing data

The expression level of IL-9R gene was assessed using previously published mRNA sequencing data of sorted human ILC and Th subsets (deposited in the ArrayExpress under accession number E-MTAB-8494). The raw count data were processed and converted to log2 normalized counts per million as described in Ercolano et al.(6)

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Multiplex cytokine assay

The concentrations of various cytokines in ILC2 supernatants, HDs and patients' sera were determined using the multi-LEGENDplex™ analyte flow assay kit (human Cytokine Panel 2, Biolegend) according to manufacturer's instructions. Samples were acquired at a Gallios flow cytometer. The results were analysed by using the Legendplex software (version 8.0).

ELISA

PGD2 and VEGF-A plasma concentrations were evaluated using ELISA kit according to the manufacturer's instruction (Prostaglandin D2-MOX ELISA Kit, Cayman Chemicals and LEGEND MAX Human VEGF ELISA Kit, Biolegend).

Statistical analysis

Statistical analysis was performed using GraphPad Prism software version 6. According to the normality test, the non-parametric Mann–Whitney or the t test were used. Simple linear regression was used for the correlation analysis. The data are shown as mean ± SEM. A p value < 0.05 was considered statistically significant and labelled with *. p values < 0.01, 0.001 or 0.0001 were labelled with **, *** or ****, respectively.

REFERENCES

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