

IL-9R loss in innate lymphoid cell type 2 (ILC2) reflects Treg impairment in mastocytosis patients

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Mastocytosis is a heterogenous group of disorders characterized by the abnormal expansion and infiltration of mast cells (MC) in multiple organs. Although MC degranulation is the primary event related to the pathophysiology of mastocytosis, the release of several mediators allows the interaction of MC with other immune cells, such as innate lymphoid cell type 2 (ILC2). In that regard, a direct crosstalk between MC and ILC2 has been recently reported in helminth infection, lung transplantation and other lung-associated inflammatory disorders [1-3]. ILC2-derived IL-9 sustains MC proliferation and differentiation, and promotes ILC2 survival, amplifying their cytokine production. In mastocytosis, ILC2 are increased in the peripheral blood (PB), arguing for their contribution to disease pathogenesis [4]. Nonetheless, ILC2 activation and function in mastocytosis remain unexplored.

We evaluated by flow cytometry the frequency of total ILC and ILC subsets in PB of mastocytosis patients (Supplementary Table I for clinical characteristics) as compared to healthy donors (HDs). The frequency of total ILC was similar between the two groups (data not shown), whilst a significant increase of ILC2, both cKit^{high} and cKit^{low} [5], but not ILC1 and ILCP, was observed in mastocytosis patients, as previously reported [4] (Supplementary Figure 1A). Given that ILC are defined as the innate counterpart of CD4 T helper subsets, we analysed the frequency of Th1, Th2, Th17 and Th1/Th17 CCR6⁺CXCR3⁺ cells [6,7]. Th17 and Th1/17 frequencies were significantly reduced in mastocytosis patients compared to HDs, while Th1 and Th2 frequencies were similar between groups, indicating that the type-2 dysregulation was not present in the ILC2 adaptive counterpart (Supplementary Figure 1B).

Next, we assessed the functionality of ILC2 by evaluating their production of type-2 cytokines in patients and HDs, after *ex vivo* stimulation (Figure 1A, Supplementary Figure 1C). ILC2 from patients produced more IL-9, IL-13, IL-5 and IL-4 compared to HDs (Figure 1A). In line with these findings, we observed elevated type-2 cytokine levels in the serum of mastocytosis

patients (Supplementary Figure 1D). IL-9 is a key regulator of ILC2, acting in an autocrine manner through the binding to the IL-9 receptor (IL-9R). IL-9R is selectively expressed in ILC2, as compared to ILC1 and ILCP, as observed by mining our publicly available RNA-sequencing (RNA-seq) analysis on freshly sorted ILCs from the PB of HDs [8] (Supplementary Figure 1E). To better define the role of IL-9/IL-9R, we evaluated the expression of IL-9R in both cKit^{high} and cKit^{low} ILC2 in mastocytosis patients and HDs (Supplementary Figure 1F). As shown in Figure 1B, IL-9R was significantly reduced in ILC2 in patients and the frequency of IL-9R⁺ ILC2 inversely correlated with the levels of IgE in mastocytosis patients (Figure 1C). When exposed to recombinant IL-9, short-term expanded ILC2 lost the expression of IL-9R, indicating an autocrine regulation of the IL-9/IL-9R axis, as also supported by the inverse correlation between circulating IL-9 and IL-9R expression on ILC2 in patients (Figure 1D and E).

Next, we screened for different factors potentially implicated in ILC2 triggering and survival. We found that IL-33, IL-1 β , VEGF and PGD2 were highly expressed in the serum of mastocytosis patients (Figure 1F). Of note, most of these factors have been reported to be secreted by MC and as critical in supporting disease pathogenesis [9,10]. In fact, exposure of short-term expanded ILC2 from HDs to these ILC2-triggering factors increased the secretion of IL-13, IL-5 and IL-9, recapitulating our *ex-vivo* observations in patients (Supplementary Figure 1G).

IL-9-triggered ILC2 also drive Treg responses, rendering them more immunosuppressive, by inducing the upregulation of GITR and ICOS through a contact dependent mechanism [11]. Therefore, we speculated that the absence of IL-9R on ILC2 in patients resulted in decreased Treg frequency. Indeed, we observed reduced circulating Treg in mastocytosis patients compared to HDs (Figure 1G, Supplementary Figure 1H) and a direct correlation between IL-9R expression on ILC2 and Treg frequencies in mastocytosis patients (Figure 1H), but not in HDs (data not shown).

Our results suggest that, by becoming hypo-responsive to IL-9 through IL-9R downregulation, ILC2 impair Treg differentiation and activation. Thus, IL-9R⁺ MC would fuel circulating IL-9 to survive and proliferate [12], to the expenses of Th17 cells or Treg, that are known to also react to IL-9 [13,14]. Taken together these results suggest the existence of a MC-IL-9-ILC2-Treg axis that promotes MC survival at the expenses of Treg activation in mastocytosis patients and might contribute to explain the disease pathophysiology (Figure 1I).

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CONFLICT OF INTEREST

The authors do not have any conflict of interest.

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FIGURE LEGEND

Figure 1. Mast cell-IL-9R-ILC2-Treg axis in mastocytosis patients. A. Quantification of *ex vivo* ILC2 producing type-2 cytokines in HDs and mastocytosis patients (n = 6). B. IL-9R expression assessed by flow cytometry analysis in cKit^{high} and cKit^{low} ILC2. C. Correlation between frequency of IL-9R⁺ ILC2 and IgE levels. D. Expression of IL-9R in ILC2 treated or not with IL-9 (n = 3). E. Correlation between circulating IL-9 and frequency of IL-9R⁺ ILC2. F. Quantification of different ILC2-triggering factors in plasma. G. Frequencies of Treg among lymphocytes (HDs and mastocytosis patients, n = 6 each). H. Correlation between circulating IL-9R⁺ ILC2 and circulating Treg in mastocytosis patients. I. Schematic representation of mast cell-IL-9-ILC2-Treg axis. Data are shown as mean ± SEM and were analysed by Mann-Whitney (A, B, F), *t* test (D, G) or simple linear regression (correlation analysis in C, E and H) (* p < 0.05; ** p < 0.01, *** p < 0.001 **** p < 0.0001).

