IL-9R Loss in Type 2 Innate Lymphoid Cells Reflects Treg Impairment in Patients With Mastocytosis

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Mastocytosis is a heterogenous group of disorders characterized by the abnormal expansion and infiltration of mast cells (MCs) in multiple organs. Although MC degranulation is the primary event in the pathophysiology of mastocytosis, the release of several mediators enables the interaction between MCs and other immune cells, such as type 2 innate lymphoid cells (ILC2s). In that regard, direct cross-talk between MCs and ILC2s has been recently reported in helminth infection, lung transplantation, and other lungassociated inflammatory disorders [1-3]. ILC2-derived IL-9 sustains MC proliferation and differentiation and promotes ILC2 survival, thus amplifying cytokine production. In mastocytosis, ILC2s are increased in peripheral blood, arguing in favor of their contribution to pathogenesis [4]. Nonetheless, the activation and function of ILC2 in mastocytosis remain unexplored.

We used flow cytometry to evaluate the frequency of total ILCs and ILC subsets in the peripheral blood of mastocytosis patients (Supplementary Table I for clinical characteristics) and compared our findings with those of healthy donors. The frequency of total ILCs was similar in both groups (data not shown), whilst a significant increase in ILC2s (both cKithigh and cKitlow [5], but not ILC1 and ILCP) was observed in mastocytosis patients, as previously reported [4] (Supplementary Figure 1A). Given that ILCs are defined as the innate counterpart of CD4 T helper (T_H) subsets, we analyzed the frequency of T_H1 , T_H2 , T_H17 , and $T_{\rm H}1/T_{\rm H}17$ CCR6⁺CXCR3⁺ cells [6,7]. The frequency of $T_{\rm H}17$ and $T_{\rm H} 1/17$ was significantly lower in mastocytosis patients than in healthy donors, while that of $T_H 1$ and $T_H 2$ was similar between the groups, indicating that type 2 dysregulation was not present in the ILC2 adaptive counterpart (Supplementary Figure 1B).

Next, we assessed the functionality of ILC2s by evaluating their production of type 2 cytokines in patients and healthy donors after ex vivo stimulation (Figure, A; Supplementary Figure 1C). ILC2 from patients produced more IL-9, IL-13, IL-5, and IL-4 than those of healthy donors (Figure, A). 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 ILC2s, acting in an autocrine manner through binding to the IL-9 receptor (IL-9R). IL-9R is selectively expressed in ILC2s-compared with ILC1s and ILCPs—as we observed by mining data from our publicly available RNA sequencing (RNA-seq) analysis on freshly sorted ILCs from the peripheral blood of healthy donors [8] (Supplementary Figure 1E). To better define the role of IL-9/IL-9R, we evaluated the expression of IL-9R in both cKithigh and cKitlow ILC2s in mastocytosis patients and healthy donors (Supplementary Figure 1F). As shown in the Figure (Figure, B), IL-9R was significantly reduced in ILC2s in patients, and the frequency of IL-9R+ ILC2s was inversely correlated with IgE levels in patients (Figure, C). When exposed to recombinant IL-9, short-term expanded ILC2s lost their 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 ILC2s in patients (Figure, D and E).

Next, we screened for 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, F). Of note, most of these factors have been reported to be secreted by MCs and to play a critical role in supporting pathogenesis [9,10]. In fact, exposure of short-term expanded ILC2s from healthy donors to these ILC2-triggering factors increased the secretion of IL-13, IL-5, and IL-9, thus reinforcing our ex vivo observations in patients (Supplementary Figure 1G).

IL-9–triggered ILC2s also drive Treg responses, rendering them more immunosuppressive by inducing upregulation of GITR and ICOS through a contact-dependent mechanism [11]. Therefore, we speculated that the absence of IL-9R on ILC2s in patients reduced the frequency of Tregs. Indeed, we observed lower values for circulating Treg in mastocytosis patients than in healthy donors (Figure, G; Supplementary Figure 1H) and a direct correlation between IL-9R expression on ILC2s and Treg frequencies in mastocytosis patients (Figure, H), but not in healthy donors (data not shown).

Our results suggest that, by becoming hyporesponsive to IL-9 through IL-9R downregulation, ILC2s impair Treg differentiation and activation. Thus, IL-9R⁺ MCs would fuel circulating IL-9 to survive and proliferate [12] at the expense of T_H17 cells or Tregs, which are also known to react to IL-9 [13,14]. Taken together, these results suggest the existence of an MC–IL-9–ILC2–Treg axis that promotes MC survival at the expense of Treg activation in mastocytosis patients and might elucidate pathophysiology (Figure, I).

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Figure. Figure. Mast cell–IL-9R–ILC2–Treg axis in mastocytosis patients. A, Quantification of ex vivo ILC2s producing type 2 cytokines in healthy donors and mastocytosis patients (n = 6). B, Expression of IL-9R assessed using flow cytometry analysis in cKit^{high} and cKit^{high} and cKit^{high} ILC2s. C, Correlation between frequency of IL-9R⁺ ILC2 and IgE levels. D, Expression of IL-9R in ILC2s treated and not treated with IL-9 (n=3). E, Correlation between circulating IL-9 and frequency of IL-9R⁺ ILC2s. F, Quantification of ILC2-triggering factors in plasma. G, Frequencies of Tregs among lymphocytes (healthy donors and mastocytosis patients, n=6 each). H, Correlation between circulating IL-9R⁺ ILC2s and circulating Tregs in mastocytosis patients. I, Schematic representation of the mast cell–IL-9–ILC2–Treg axis. Data are shown as mean (SEM) and were analyzed using the Mann-Whitney test (A, B, F), *t* test (D, G), or simple linear regression (correlation analysis in C, E, and H) (**P*<.05; ***P*<.001 *****P*<.0001).

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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