Letter to the Editor

Thymic stromal lymphopoietin does not activate human basophils

To the Editor:

Basophils exhibit nonredundant effector functions that contribute to the development of type 2 inflammation.[1] Even if IL-3 is the major basophil-activating cytokine, recent data indicated that mouse basophils can also be activated by thymic stromal lymphopoietin (TSLP).[2] TSLP is a member of the IL-2 family of cytokines that is thought to play important roles in human allergic diseases by instructing dendritic cells (DCs) to induce T_H2 responses.[3] TLSP binds to a heterodimeric receptor composed of the thymic stromal lymphopoietin receptor (TLSPR) and IL-7Rα (CD127) chains, and mainly signals through STAT5 phosphorylation (P-STAT5).[3] TSLP has been shown to promote the expansion of phenotypically and functionally distinct basophil subsets in mice.[2] Indeed, TSLP-elicited basophils differ from classical IL-3–elicited basophils by preferentially responding to IL-33 rather than IgE-cross-linking.[2,4] In humans, TSLP has been shown to promote the differentiation of human $CD34^+$ cells into eosinophils and basophils,[5] and a recent report has suggested that blood basophils from allergic patients respond to TLSP.[6] However, whether normal basophils from healthy donors (HDs) also respond to TSLP is unknown.

To address this question, we compared the effect of IL-3 and TSLP on purified blood basophils from HDs. An induction of CD203c, CD69, and CD63 was observed when basophils were cultured with IL-3 but not when basophils were cultured with TSLP (Fig 1, *A*; see Fig E1, *A*, in this article's Online Repository at www.jacionline.org). In addition, basophils produced IL-4, IL-6, IL-13, CCL2/monocyte chemoattractant protein-1, CCL3/macrophage inflammatory protein-1α, and TNF-α on IL-3 stimulation, whereas no significant secretion of these mediators nor of IL-3 was induced by TSLP (Fig 1, *B*). As a positive control of TSLP-mediated activation, we used purified blood myeloid DCs (mDCs) from the same donor and confirmed that TSLP induced their expression of CD83 and CD86 (Fig 1, *C*, and Fig E1, *B*) as well as secretion of IL-3, TNF-α, macrophage inflammatory protein-1α, and monocyte chemoattractant protein-1 (Fig E1, *C*). We then thought to assess whether TSLP could readily signal through TSLPR/IL7Rα in basophils and analyzed P-STAT5 expression by flow cytometry (FCM) (see Fig E2, *A*, in this article's Online Repository at www.jacionline.org). Although IL-3 induced an early and persistent P-STAT5 expression in basophils, no P-STAT5 was detected on TSLP stimulation (Fig 1, *D*). In contrast, both IL-3 and TSLP induced P-STAT5 in mDCs (Fig 1, *D*). We then assessed whether basophils expressed a functional TSLP receptor. As far as we know, this question has never been addressed. We analyzed the expression of TSLPR and IL-7Ra by FCM on basophils and mDCs from HDs (Fig 1, *E*). Unstimulated basophils and mDCs did not express IL-7Rα or TSLPR. Culture of mDCs induced the expression of both TLSPR and IL-7Rα on 40% of cells, which was further increased to 65% by IL-3 (Fig 1, *E*). Although TSLPR was induced in basophils on culture with IL-3, we could not detect any IL-7Rα expression (Fig 1, *E*). Similar results were obtained after intracellular staining (data not shown). Because TSLPR in basophils was induced by IL-3, we assessed whether a 24-hour priming by IL-3 could render them responsive to TSLP. Even in these conditions, however, no significant induction of P-STAT5 (Fig E2, *B*) or surface activation markers (data not shown) could be detected in response to TSLP.

We then sought to determine whether basophils could respond to TSLP in the context of allergy as shown recently by others.[6] For this purpose, we developed a whole blood assay that allowed simultaneous assessment of mDC and basophil responses in the same sample. Blood samples obtained from 8 patients with demonstrated allergy to a pneumallergen (see Table E1 in this article's Online Repository at www.jacionline.org) and HDs were stimulated with IL-3, TSLP, or an anti-FcεRI antibody for 6 hours and analyzed by FCM. Basophils and mDCs were identified in lineage-negative $CD45^+$ cells as HLA-DR^- $CCR3^{high}$ $CD11c^{low}$ and HLA-DR^{high} $CCR3^-$ $CD11c^{high}$ cells, respectively (Fig 2, *A*). IL-3 but not TSLP induced P-STAT5 (Fig 2, *B*; see Fig E3, *A*, in this article's Online Repository at www.jacionline.org), CD203c expression, and CCR3 downregulation (Fig 2, *C*, and Fig E3, *B*) on blood basophils from both HDs and allergic patients. We also observed a tendency to increased responsiveness of basophils from allergic patients to FcεRI cross-linking (Fig 2, *C*). In contrast, both IL-3 and TSLP induced P-STAT5 in mDCs with no differences between HDs and allergic patients (Fig 2, *B*, and Fig E3, *A*). We finally assessed the expression of TSLPR and IL-7Rα chains on basophils and mDCs using the same assay. We observed an upregulation of TSLPR and IL-7Rα on DCs on culture of blood with medium, IL-3, or TSLP with no difference between HDs and allergic patients (Fig 2, *D*, and Fig E3, *C*). IL-3 and FcεRI cross-linking but not TSLP induced the expression of TSLPR on a small fraction of basophils with no difference between HDs and allergic patients (Fig 2, *D*, and Fig E3, *D*). Nevertheless, we did not observe any IL-7Rα expression on basophils from either HDs or allergic patients in any of these conditions (Fig 2, *D*). We further confirmed these data by RT-PCR on purified basophils from allergic patients (see Fig E4, *A*, in this article's Online Repository at www.jacionline.org). We concluded that blood basophils from both HDs and our allergic patients do not express IL-7Rα and do not respond to TSLP.

Our results contrast with those published recently by another group that showed a responsiveness of isolated blood basophils from allergic patients to TSLP.[6] Of note, we also isolated blood basophils from allergic patients according to this study, and confirmed their lack of response to TSLP and of IL-7Rα expression (Fig E4, *B* and *C*). In the report from Salter et al,[6] TSLP was shown to activate purified basophils after 1-hour stimulation, contrasting with the rather late kinetics of TSLPR expression we observed on basophils. In addition, Salter et al[6] reported increased CCR3 expression on basophils following IL-3, TSLP, or anti-IgE stimulation, whereas previous literature indicates that basophil activation is associated with a downregulation of CCR3 expression,[7] a finding we confirmed here. Whether these discrepancies are related to differences in patients included in both studies remains to be explored.

In conclusion, our results suggest that, in contrast to murine basophils, human basophils do not respond to TSLP, likely

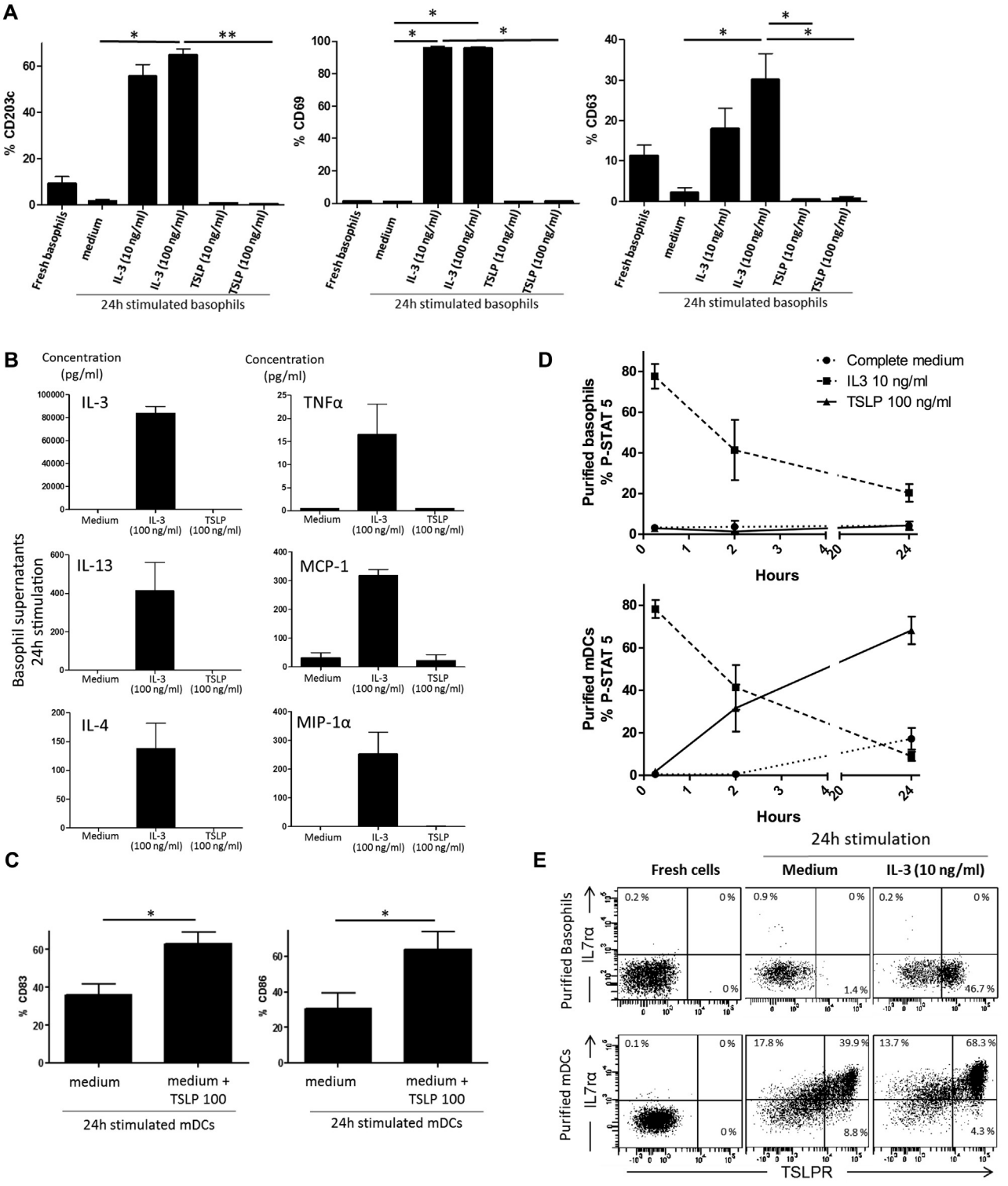

FIG 1. TSLP does not activate purified blood basophils from HDs. Blood basophils and mDCs were isolated from HDs and cultured with medium, IL-3, or TSLP for expression of CD63, CD69, and CD203c on basophils (n = 4-7) **(A)**. Cytokine and chemokine concentration in basophil supernatants (n = 4) **(B)**. Expression of CD83 and CD86 on mDCs (n = 6) **(C)**. Kinetics of P-STAT5 expression in basophils (top) and mDCs (bottom) (n = 3) **(D)**. TSLPR and IL-7Rα expression on basophils (top) and mDCs (bottom) **(E)**. *MCP-1*, Monocyte chemoattractant protein; *MIP-1α*, macrophage inflammatory protein-1α. Data are shown as mean ± SEM. $*P < .05$ and $**P < .01$.