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ORIGINAL ARTICLE **Basic Mechanisms in Allergic Disease**

Human basophils are a source[+] of – and are differentially activated by - IL-31

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Summary

Background Basophils are important effector cells involved in the pathogenesis of inflammatory skin diseases including chronic urticaria which is associated by increased IL-31 serum levels. So far the effects of IL-31 on human basophils are unknown.

Objective To analyse the functional role of IL-31 in basophil biology.

Methods IL-31 expression was evaluated in skin samples derived from chronic spontaneous urticaria patients. Oncostatin M receptor (OSMR), IL-31 receptor A (RA) and IL-31 protein expressions were analysed on human basophils from healthy donors. Basophil responses to IL-31 were assessed for chemotaxis, externalization of CD63 and CD203c as well as the release of histamine, IL-4 and IL-13.

Results IL-31RA and OSMR were expressed on human basophils. IL-31 was strongly expressed in the skin of patients with chronic spontaneous urticaria and was released from isolated basophils following either anti-IgE, IL-3 or fMLP stimulation. IL-31 induced chemotaxis and the release of IL-4 and IL-13 which was specifically inhibited by anti-IL-31RA and anti-OSMR. Conversely, IL-31 had no effect on CD63 and CD203c externalization or histamine release.

Conclusions and Clinical Relevance Human basophils are a source of –and are activated by – IL-31 with the release of pro-inflammatory cytokines and the induction of chemotaxis indicating an important novel function of IL-31 in basophil biology.

Keywords basophils, chemotaxis, chronic urticaria, histamine, IL-31

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Introduction

IL-31 plays an important role in inflammation and itch. Increased levels of this cytokine have been shown in inflammatory skin diseases including chronic spontaneous urticaria (CsU), atopic dermatitis, contact eczema and in a subset of patients with mastocytosis [1–7]. In atopic dermatitis, IL-31 levels correlate with disease severity and increased Th2 cytokines, such as IL-4 and IL-13, in serum and skin [2, 4, 5]. In a mouse model of atopic dermatitis, anti-IL-31 treatment led to a significant inhibition of scratching [8]. Furthermore, it has been shown that the successful therapy of 15 patients with CsU, which were excellent responders to omalizumab, was associated with reduced IL-31 serum levels [9].

IL-31 signals *via* a heterodimeric receptor composed of the IL-31 receptor A (IL-31RA) and the oncostatin M receptor (OSMR). These IL-31 receptors are expressed on several different cell types including T cells, keratinocytes, dendritic cells, eosinophils, macrophages and dorsal root ganglia [3, 10–14]. Expression of IL-31 has been described in mast cells of patients with psoriasis [15] and mastocytosis [6], in CD45RO$^+$ CLA$^+$ T cells and in PBMCs of patients with atopic dermatitis [3, 16, 17] as well as eosinophils [13]. Functionally, IL-31 leads to the release of pro-inflammatory cytokines in human monocytes, macrophages and keratinocytes [12, 14]. This cytokine also regulates the differentiation and expression of filaggrin in human organotypic skin models [18].

Basophils have been recognized as important effector cells in allergic reactions and in immune responses against helminths [19]. These cells are highly mobile in comparison with their mast cell counterparts and can invade various tissues including the skin, lung and nose

[+]Correction added on 10 March 2017, after first online publication: a typographical error in 'source' has been corrected.

([19, 20]. Inflammatory skin of chronic idiopathic urticaria patients is also associated with increased numbers of basophils [21]. Basophils are capable of releasing histamine, eicosanoids and several immunomodulatory cytokines including IL-6 and IL-13. Moreover, basophils are the prime early producers of IL-4 which is rapidly released in large quantities from these cells and is important for the early activation of T cells. The effect of IL-31 on human basophils has not yet been elucidated, and their ability to generate this cytokine themselves has not been determined. As basophils contribute to inflammatory skin diseases, our aim was to investigate the role of IL-31 in these cells and to discover whether basophils are a source of IL-31.

Methods

Isolation of basophils from peripheral blood

Human basophils were obtained from leucocyte concentrates (obtained by thrombopheresis) derived from healthy blood donors and purified by magnetic cell sorting (Stem Cell Technologies, Grenoble, France) as previously described by us [22, 23]. The purity of isolated basophils was 99–100% as assessed by alcian blue staining and FACS analysis with antibodies against CD123 (clone: 6H6, eBioscience, Frankfurt, Germany) and $Fc_{\varepsilon}R1\alpha$ (clone: AER-37, eBioscience). All procedures were approved by the local ethical committees of the Hannover Medical School (approval number 5807) and the University of Kent (NHS REC 12/WM/0319). Basophils were cultivated in RPMI 1640 with 10% heat-inactivated fetal calf serum (FCS) including 2 mM L-glutamine, 10 000 U/mL penicillin and 10 mg/mL streptomycin (all Seromed; Biochrom AG, Berlin, Germany) at 37°C and 5% CO_2.

Isolation of $CD4^+$ T cells

$CD4^+$ T cells were isolated from the same leucocytes that were also used for basophil isolation. $CD4^+$ T cells were enriched using the $CD4^+$ T cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany). The purity of isolated $CD4^+$ T cells was 99–100% as assessed by FACS. In 96-well plates, $CD4^+$ T cells (1×10^5 per 100 μL) were incubated in the same culture medium as described above for basophils. $CD4^+$ T cells were stimulated with purified NA/LE mouse anti-human CD3 antibodies (1 μg/mL, clone: HIT3a; BD Bioscience, Heidelberg, Germany) and purified mouse anti-human CD28 antibodies (0.2 μg/mL, clone: CD28.2, BD Bioscience).

IL-31 expression in skin samples

Paraffin-embedded lesional skin biopsies from patients with CsU were analysed by double immunofluorescence using the Vectastain kit (Vector Laboratories Inc., Burlingame, CA, USA) as outlined before [6, 24]. Sections (6 μm) were pre-treated for antigen retrieval and permeabilization (Vector Laboratories). Slides were then incubated with either polyclonal rabbit anti-human IL-31 (1 : 200; Abcam, Cambridge, UK) or rabbit IgG isotype control and subsequently stained with goat anti-rabbit FITC (Jackson ImmunoResearch, West Grove, PA, USA). Afterwards, monoclonal mouse anti-human 2D7 antibodies (2 μg/mL; Abcam) or respective mouse IgG1 isotype control (Jackson ImmunoResearch) was applied with subsequent incubation with PE-conjugated goat anti-mouse antibodies (Jackson ImmunoResearch). Sections were then incubated overnight at 4°C after which all slides were examined using a Zeiss Axiocam with Axiovision software (Carl Zeiss, MicroImaging GmbH, Göttingen, Germany). Nuclei were stained with DAPI (Thermo Fisher, Waltham, MA USA). The number of IL-31-positive cells and IL-31-positive basophils in addition to IL-31 negative basophils was determined in nine different slides of four different patients with CsU. Furthermore, we performed phase contrasts of representative skin samples (Zeiss Axiocam).

Western blot analysis

For IL-31 protein detection, a total of 1×10^6 basophils were stimulated for 4 h and homogenized in M-Per mammalian extraction reagent (Thermo Fisher). SDS-PAGE using the extracted proteins was performed on 4–20% gradient precise protein gels (Pierce, Thermo Scientific). Proteins were then transferred to nitrocellulose membranes and blocked with 5% non-fat dry milk. Membranes were then sequentially incubated with polyclonal rabbit anti-human IL-31 (1 : 200; Abcam) followed by a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1 : 2000, Cell Signaling Technology, Danvers, MA, USA). The blots were visualized using a chemiluminescence kit according to the manufacturer's instructions (Pierce, Thermo Fisher) and documented with Chemilmager 4400 (Biozym, Hess. Oldendorf, Germany). Equal loading was assessed by comparing with GAPDH expression using rabbit monoclonal anti-human GAPDH (14C10) antibodies (Cell Signaling Technology). As a positive control, we used 100 ng/mL rhIL-31 (Peprotech, Rocky Hill, NJ, USA).

IL-31RA and OSMR expression

IL-31RA and OSMR expressions were analysed using flow cytometry. Purified basophils (2×10^5) were incubated with either APC-conjugated polyclonal goat anti-human IL-31RA (0.5 μg/mL; R & D Systems, Wiesbaden, Germany) or goat anti-human IgG

isotype control (R&D Systems). For the detection of OSMR, we used PE-conjugated monoclonal mouse anti-human OSMR (0.5 μg/mL, clone: An-V2, eBioscience) or monoclonal mouse anti-human IgG1 isotype control (R & D Systems). Staining was performed for 1 h at 4°C, and basophils were analysed using a FACS Calibur platform (Becton Dickinson, San Jose, CA, USA).

Cytokine release

After 16 h in culture, purified basophils (2×10^5 per 200 μL) were stimulated for 30, 60 and 240 min with anti-IgE (100 ng/mL, Sigma-Aldrich, Munich, Germany), fMLP (1 μM, Sigma-Aldrich) or IL-31 (1 and 10 ng/mL). For blocking experiments, basophils were stimulated with 10 μg/mL goat anti-human IL-31RA (unconjugated; R & D Systems), 10 μg/mL mouse anti-human OSMR (unconjugated; R & D System) or both antibodies, or with a respective isotype control (normal goat IgG and mouse IgG1, 10 μg/mL; R & D Systems) before stimulation with IL-31 (10 ng/mL). Cytokine release was determined for IL-4 (eBioscience, detection limit: 2 pg/mL) and IL-13 (eBioscience, detection limit: 7.8 pg/mL) according to the manufacturer's instructions as previously described by us [22]. IL-31 release was analysed from basophils stimulated with either anti-IgE, fMLP, IL-3 (10 ng/mL, ImmunoTools, Friesoythe, Germany) or buffer alone after 4 h incubation with a commercial IL-31 ELISA (Blue Gene, detection limit: 2.5 pg/mL) as previously described [6]. All experiments were conducted in duplicate for each basophil donor employed; analysis was performed using the FLUOStar Optima plate reader (BMG Labtechnologies, Offenburg, Germany).

Externalization of CD63 and CD203c

Heparinized human whole blood was incubated for 20 min with or without IL-31 (10 ng/mL) and subsequent stimulation with anti-IgE (100 ng/mL) and fMLP (1 μM) for a further 20 min. Externalization of the basophil activation marker CD63 (FITC-conjugated monoclonal mouse anti-human CD63 antibodies, clone: H5C6) was measured using a commercially available basophil activation test (BAT, Orpegen Pharma, Heidelberg, Germany) as outlined before [25].

Expression of CD203c was analysed using PE-conjugated monoclonal mouse anti-human antibodies (clone: NP4D6; eBioscience). The samples were prepared and stained using the aforementioned basophil activation kit under the same conditions.

Histamine release

Purified basophils (2×10^5 cells) were resuspended in HEPES-buffered Tyrode's solution (400 μL per tube) containing 1 mM $CaCl_2$, placed in a water bath warmed to 37°C and incubated with either IL-3, IL-31, IL-33 (each at 10 ng/mL) or buffer alone. After 15 min, basophils were stimulated with anti-IgE (100 ng/mL, Sigma-Aldrich, goat affinity-isolated antibody) or buffer alone. Reactions were allowed to proceed for 30 min for the analysis of histamine release. Reactions in the latter case were terminated by adding ice-cold calcium-free HEPES buffer followed by centrifugation and immediate transfer of supernatants into new vials. Histamine contents in the supernatants, together with the cell pellets, which were diluted accordingly and lysed with perchloric acid (4%) were measured spectrofluorometrically. Histamine release was determined from the total histamine content in the sum of pellet and supernatant as outlined before [26].

Chemotaxis

Chemotactic activity was assessed using modified Boyden chambers, which contained either IL-31 (10 ng/mL) or fMLP (1 μM, used as positive control) or medium alone (baseline) and were covered with polycarbonate filters (pore size 3 μm). Basophil suspensions (5×10^5 per 100 μL) were then added on top of these filters for each chamber. After incubation for 3 h at 37°C, basophils, which migrated to the lower part of the Boyden chambers, were lysed by adding 0.1% Triton X-100. ß-Glucuronidase activity in the lysates was determined photometrically using p-nitrophenyl ß-glucuronide as a substrate. Values were calculated by a computer-assisted technique from a standard curve using known numbers of unchallenged basophils. Chemotactic activity was presented as a chemotactic index with the ratio of the number of migrated cells in the presence of stimulus to the number of migrated cells in the presence of medium alone.

Statistical analysis

All data are presented as box-and-whisker plot with minimum and maximum, if not otherwise stated. Statistical analysis was performed with GraphPad Prism 5. Data, which passed the normality test, were analysed with either a Student's t-test or by a one-way ANOVA followed by a Tukey post-test. If the data did not follow a Gaussian bell-shaped distribution for more than two groups of data, the Kruskal–Wallis test or the Friedmann test followed by Dunn's post-test was used. Additionally, a two-way ANOVA followed by a Bonferroni post-test was performed. A P-value of < 0.05 was considered to be statistically significant.

Results

Basophils in chronic urticaria skin lesions are IL-31-positive

CsU is characterized by increased IL-31 serum levels [7]. Further, CsU lesions display an infiltration of basophils [27]. Thus, we analysed whether basophils could also be a source of IL-31 in skin samples of patients with CsU using immunofluorescence. We were able to detect IL-31-expressing basophils (2D7/IL-31, yellow) alongside other IL-31-producing cells (green) (Fig. 1a-c). To show the anatomical location of IL-31-positive basophils, we performed phase contrast imaging (Fig. 1d) together with respective double staining by immunofluorescence (Fig. 1e). Finally, we analysed the number of IL-31-expressing basophils using nine slides from four different patients with CsU (Fig. 1f). We observed that almost all basophils in the skin were also positive for IL-31 and that the majority of IL-31-expressing cells were basophils. In contrast, healthy donor skin samples showed no IL-31-positive basophils (isotype controls were also negative; data not shown).

Basophils express and release IL-31

The *in vivo* findings led us to investigate the role of IL-31 in basophils in more detail. Hence, we incubated purified basophils from healthy donors for 4 h with either anti-IgE, fMLP, IL-3 or IL-3 in combination with anti-IgE and performed Western blotting for IL-31 expression. Stimulation with anti-IgE and fMLP increased IL-31 expression compared to unstimulated basophils (Fig. 2a). IL-3 alone had no significant effect and did not increase IgE-dependent IL-31 protein expression (Fig. 2a). Using cell lysates of stimulated basophils, we could see a significant increase in IL-31 expression by ELISA after stimulation with fMLP compared to unstimulated basophils (Fig. 2b). Next, we analysed basophil supernatants by ELISA and again observed a significant increase in IL-31 release from basophils stimulated with fMLP compared to unstimulated basophils (Fig. 2c).

T cells – especially of the CLA^+ subtype – release increased levels of IL-31 after 24 h [3]. Thus, we wanted to assess whether basophils express similar levels of IL-31 compared to T cells *in vitro*. For this purpose, we isolated basophils and $CD4^+$ T cells from the same donors. The cells were cultivated for 24 h and then stimulated for 4 h with either fMLP or the T cell-specific stimulus CD3/CD28. We could confirm our previous finding with respect to IL-31 release after stimulation with fMLP in basophils (Fig. 2d). The T cell-specific stimulus CD3/CD28 also led to a significant release of IL-31 in $CD4^+$ T cells (Fig. 2d). Comparing both cell types, basophils displayed a significantly higher release of IL-31 than $CD4^+$ T cells (Fig. 2d).

Basophils express IL-31 receptors

IL-31 activates the signal cascade through its interaction with a heterodimeric receptor composed of IL-31RA and OSMR [1]. Therefore, we assessed IL-31RA and OSMR

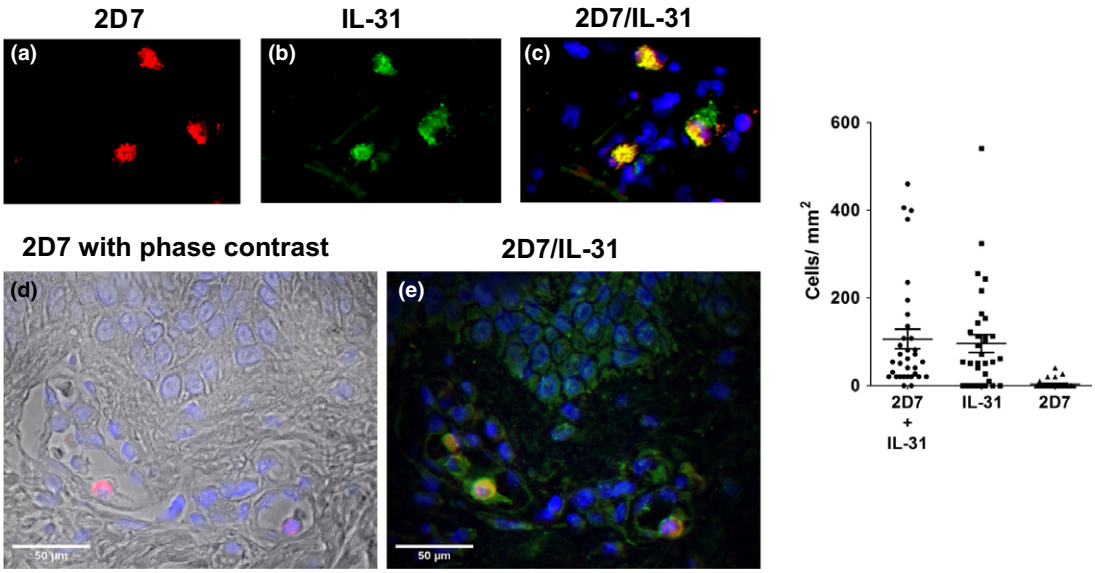

Fig. 1. IL-31 expression in basophils from patients with CsU. Double immunofluorescence staining for IL-31 and basophils in paraffin sections from CsU skin lesions. Staining was performed with antibodies against human basophils (a, c, d, e, 2D7, red) and IL-31 (b, c, e, green). Arrows point to IL-31-positive basophils displayed as yellow in the overlay. Asterisks mark IL-31-positive cells other than basophils. Nuclei were stained with DAPI (blue). (f) Quantification of 2D7/IL-31-positive and single-stained cells from sections of patients with CsU (n = 4 patients with nine slides each displayed as scatter dot blot with mean ± SEM).

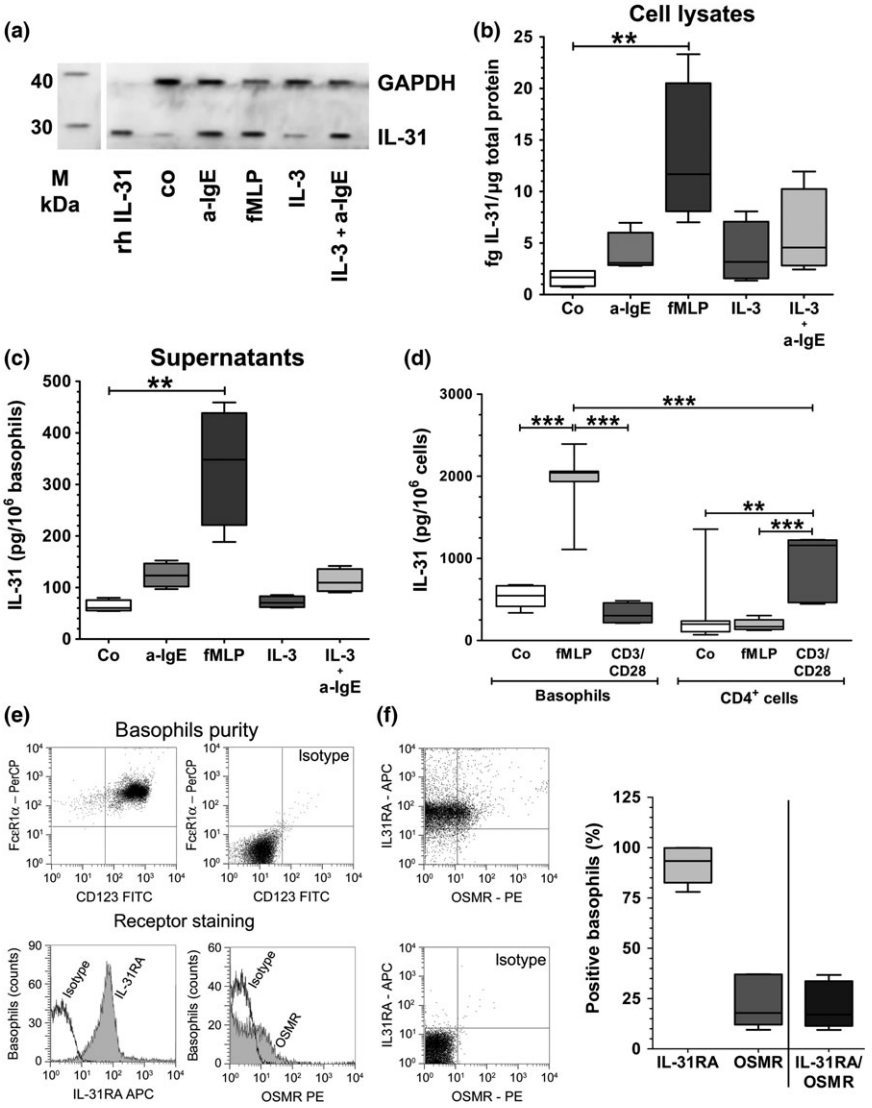

Fig. 2. Expression of IL-31, IL-31RA and OSMR on human basophils. Human basophils from healthy donors were isolated as described in material and methods (Co: unstimulated basophils, $^{**}P < 0.01$, $^{***}P < 0.001$). (a) Detection of IL-31 was performed from protein lysates by Western blot (WB). Basophils were stimulated for 4 h with anti-IgE (a-IgE, 100 ng/mL), fMLP (1 µM) and IL-3 (10 ng/mL) alone or combined with a-IgE. (One representative WB is shown out of four; M: ladder; Co: unstimulated basophils.) GAPDH was used as a reference protein. As control, rh IL-31 was used. (b) Determination of IL-31 protein in cell lysates employed for WB using ELISA ($n = 4$). (c) IL-31 release of the corresponding supernatants from the same basophils employed for WB using ELISA ($n = 4$). (d) Determination of IL-31 in the supernatants of isolated basophils and $CD4^+$ T cells (1×10^5 cells per 100 µL from the same donors; $n = 7$ different donors) cultivated for 24 h and then stimulated with either fMLP or CD3/CD28 (CD3: 1 µg/mL; CD28: 0.2 µg/mL) for 4 h. (e) Purity of freshly isolated basophils determined by FACS analysis using antibodies against FcεR1α and CD123 (one representative scatter dot blot out of five); receptor staining of IL-31RA and OSMR on freshly isolated basophils from healthy donors analysed by FACS displayed as a histogram (one representative shown out of five). (f) One representative scatter dot plot of human basophils stained for IL-31RA and OSMR ($n = 5$) and quantification of IL-31RA and OSMR double-positive basophils ($n = 5$).

expression in isolated basophils from healthy donors by flow cytometry. Initially, to avoid possible contamination, the isolated cell population was analysed for FcεR1α and CD123 expressions to verify the purity of our basophil population (Fig. 2e, upper panel). Only $FcεR1α^+$/$CD123^+$-expressing cells were then analysed for IL-31RA and OSMR expressions. While nearly all basophils were IL-31RA-positive, only a small subpopulation of basophils were positive for OSMR (Fig. 2e, lower panels). OSMR/IL-31RA double staining of basophils showed that almost all OSMR-positive basophils were also positive for IL-31RA (Fig. 2f).

IL-31 induces the release of cytokines

IL-31 is known for its functional effects on the secretion of pro-inflammatory cytokines of several immune cells. Thus, we stimulated purified basophils with IL-

31 (10 ng/mL) for 4 h to assess cytokine release. The secretion of IL-4 and IL-13 was significantly increased after stimulation with IL-31 in comparison with unstimulated basophils (Fig. 3a, c), although levels were not as high as IL-4 and IL-13 release following IgE-dependent stimulation (Fig. 3a, c). In addition, we also analysed the kinetics of IL-4 and IL-13 secretions from basophils stimulated with two concentrations of IL-31 (1 and 10 ng/mL). IL-4 release and IL-13 release were rapidly and significantly increased in IL-31-stimulated basophils compared to those with no stimulation (Fig. 3b, d). Interestingly, we observed significant release of IL-4 and IL-13 with a concentration of 1 ng/mL IL-31 after 4 h of stimulation (Fig. 3b, d). With a concentration of 10 ng/mL IL-31, IL-4 secretion was significantly increased after 240 min (Fig. 3b) and IL-13 secretion was significantly increased after 60 and 240 min of stimulation (Fig. 3d). To verify the specificity of these responses, we compared stimulated basophils with those co-incubated with IL-31 antibodies which block IL-31RA and OSMR. These blocking antibodies significantly inhibited IL-13 release in IL-31-stimulated

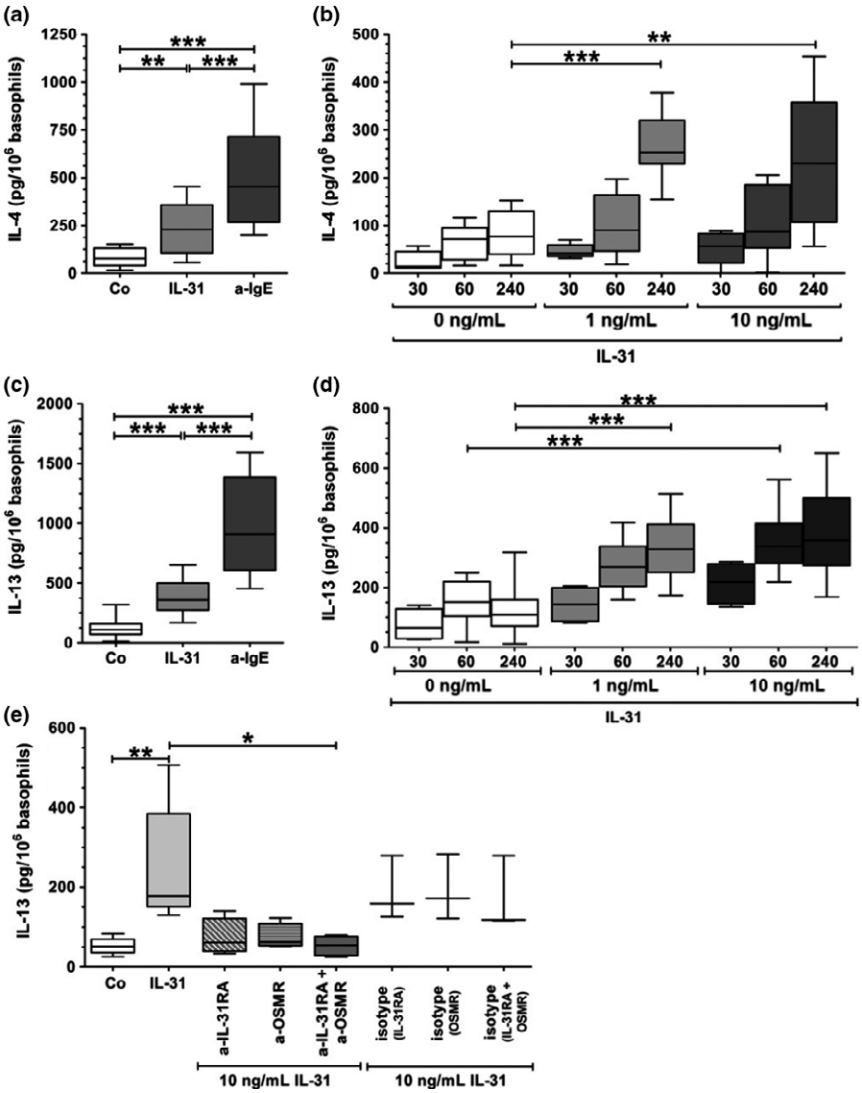

Fig. 3. IL-31 stimulation induces IL-4 and IL-13 secretion. Human basophils from healthy donors were stimulated with IL-31 (10 ng/mL) or anti-IgE (a-IgE, 100 ng/mL). (Co: unstimulated basophils, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). (a) Secretion of IL-4 from purified basophils was measured by ELISA after 4 h of stimulation (Co and IL-31: $n = 21$; a-IgE: $n = 10$). (b) Kinetics of IL-4 secretion. Purified human basophils from healthy donors were measured by ELISA after 30, 60 and 240 min stimulation with 1 and 10 ng/mL IL-31 (Co: unstimulated basophils; (30 min: $n = 6$; 60 min: $n = 10$; 240 min: $n = 21$;). (c) Secretion of IL-13 from purified basophils as measured by ELISA after 4 h of stimulation (Co and IL-31: $n = 16$; a-IgE: $n = 5$). (d) Kinetics of IL-13 secretion. Purified human basophils from healthy donors were measured by ELISA after 30, 60 and 240 min stimulation with 1 and 10 ng/mL IL-31 (Co: unstimulated basophils; (30 min: $n = 4$; 60 min: $n = 10$; 240 min: $n = 16$). (e) Determination of IL-13 release after stimulation with IL-31 for 4 h and co-incubation with either anti-IL-31 RA (a-IL-31RA) or anti-OSMR (a-OSMR) or a combination of both antibodies. As controls, the respective isotype antibodies were used ($n = 4$).

basophils (Fig. 3e). Similar observations were made regarding IL-4 release (data not shown). The isotype controls for the IL-31RA and OSMR had no effect on IL-31-stimulated basophils and led to IL-13 release as seen with IL-31 stimulation alone (Fig. 3e).

IL-31 does not affect CD63 and CD203c externalization or histamine release

As cytokines or hormones can affect the degranulation of basophils [28, 29], we wondered whether IL-31 has an impact on the externalization of CD203c and CD63. For this, basophils were stimulated with either anti-IgE, fMLP or IL-31, or costimulated with IL-31 and anti-IgE. Surprisingly, IL-31 stimulation did not increase the externalization of CD63 (Fig. 4a) and CD203c (Fig. 4b) in comparison with unstimulated basophils. Additionally, incubation with IL-31 did not affect histamine release and did not enhance it in conjunction with anti-IgE stimulation (Fig. 4c). In contrast, IL-3 and IL-33, which are known primers for enhanced IgE-dependent histamine release, caused substantial potentiation of anti-IgE-induced histamine release in the same settings (Fig. 4c).

IL-31 induces basophil migration

To investigate whether IL-31 induces chemotaxis, a mechanism playing an important role in the accumulation of basophils at sites of inflammation, we performed a modified Boyden chamber assay. We observed a clear induction of chemotaxis after stimulation with 10 ng/mL IL-31 (Fig. 4d). FMLP was used as positive control and induced chemotaxis as expected (Fig. 4d).

Discussion

Our study demonstrates for the first time that human basophils express IL-31 receptors and are a source of IL-31 *in vitro* and *in vivo*. Furthermore, we show novel data regarding the functional effects of IL-31 on human basophils including the induction of chemotaxis and release of pro-inflammatory cytokines (IL-4 and IL-13). Surprisingly, however, basophil histamine release and externalization of CD63 as well as CD203c were not affected by IL-31 exposure.

Mast cells, which share many common phenotypic features with basophils, express IL-31 when stimulated with antimicrobial peptides or in diseases such as

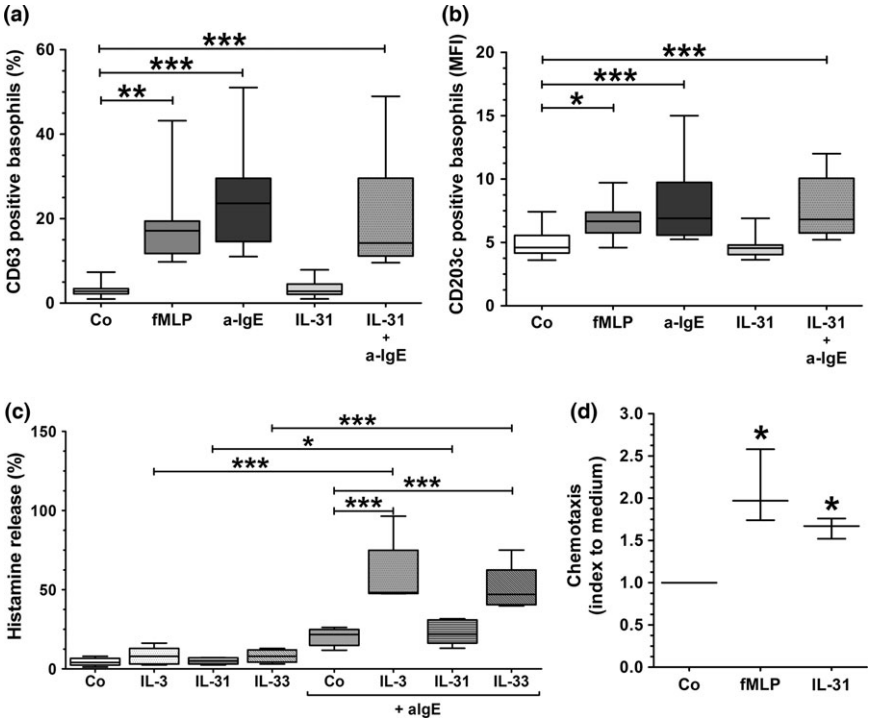

Fig. 4. IL-31 induces chemotaxis but not CD63/CD203c externalization or histamine release. (a–c) Basophil activation test (BAT): Externalization of CD63 (a) and CD203c (b) on basophils stimulated with IL-31 (10 ng/mL), fMLP and anti-IgE (a-IgE, 100 ng/mL) or a combination of IL-31/anti-IgE (Co: control; $n = 11$; MFI: mean fluorescence intensity; $*P < 0.05$; $***P < 0.001$). (c) Effect of IL-31 (10 ng/mL) on histamine release from purified basophils in comparison with IL-3 (10 ng/mL) and IL-33 (10 ng/mL) (used as positive control) either alone or in conjunction with anti-IgE (a-IgE, 100 ng/mL). (Co: control; $n = 5$ separate donors; $*P < 0.05$, $***P < 0.001$). (d) Migration of freshly isolated basophils in the presence of IL-31 (10 ng/mL) was investigated in a modified Boyden chamber. fMLP (1 μM) was used as a positive control. Chemotactic activity is presented as the ratio of the number of migrated basophils in the presence of stimulus/migrated basophils in the presence of the medium ($*P < 0.05$; $n = 5$ separate donors).

myeloproliferative disorders [15, 30]. Additionally, it was shown that mast cells express the IL-31 receptor [31]. In this context, it is interesting that atopic dermatitis mouse models show high IL-31 concentrations in addition to an increased number of mast cells in the skin [1, 32]. Until now, the effect of IL-31 on basophils and mast cells and the function of IL-31-expressing basophils and mast cells *in vivo* have not been investigated. In our study, we could show that IL-31 induced basophil chemotaxis and induced the secretion of IL-4 and IL-13 from these cells. This suggests that IL-31 participates in orchestrating and enhancing pro-allergic immune responses, because the induction of IL-4 release may favour the subsequent differentiation of Th0-CD4$^+$ T cells to a Th2 phenotype. Indeed, it has been shown that CsU is associated with increased IL-4 serum levels [33]. In this context, it is interesting to note that IL-31 serum levels are higher in CsU patients compared to healthy donors [7]. It is thus tempting to speculate that increased serum IL-31 is the key mechanism for increased IL-4 serum levels in these patients. However, further experiments are needed to clarify this. It was shown that IL-31 expression by Th1 cell clones depends on the presence of IL-4, suggesting the regulation of IL-31 tissue levels to be an additional immunomodulatory function of basophils supplemental to their regulation of T cell differentiation [34].

The functional interactions are likely to be mediated *via* expression of the IL-31 receptor complex consisting of IL-31RA and OSMR in basophils, which was shown for other types of granulocytes including eosinophils, which are derived from similar stem cells as basophils [13, 35]. Interestingly, surface expression of IL-31RA was significantly higher than OSMR but all basophils positive for OSMR were also positive for IL-31RA. The functional activity seems to depend on the expression of both the OSMR and IL-31RA because we show here that blocking of one or the other receptor decreases IL-4 and IL-13 release in IL-31-stimulated basophils.

Stimulation of basophils with IL-31 clearly led to the release of IL-4 and IL-13. This is interesting, given the fact that children with atopic dermatitis display increased IL-31 serum levels and a positive correlation between IL-31 and IL-4 and IL-13 levels with disease severity [4, 5]. Another interesting function of IL-31 is its involvement in filaggrin expression [18]. Atopic dermatitis is mostly characterized by filaggrin deficiency, whereas in chronic urticaria, filaggrin expression has been reported to be enhanced [36]. As IL-31 serum levels are higher in atopic dermatitis compared to CsU [7], it may be possible that regulation of filaggrin expression is dependent on IL-31 serum levels.

The surface marker CD203c, an ecto-nucleotide pyrophosphatase/phosphodiesterase, is specifically expressed on basophils and mast cells. We did not observe an externalization of CD203c in basophils stimulated with IL-31. Additionally, IL-31 failed to increase the externalization of CD63 and did not affect basophil histamine release. Histamine release from anaphylactic degranulation is accompanied by increased externalization of CD63, whereas an upregulation of CD203c alone could indicate piecemeal degranulation in basophils [37]. However, as IL-31 caused the secretion of IL-4 and IL-13 from basophils without affecting both CD63 or CD203c externalization (and subsequent histamine release), this demonstrates a unique mechanism of action compared to other known basophil activators.

Our results clearly demonstrate that basophils are a source of IL-31 themselves. We detected IL-31 in the supernatants of basophil cultures using ELISA, Western blot analysis and cell lysates where IL-31 concentrations were increased after stimulation. Given the fact that IL-31 orchestrates several pro-inflammatory functions in various other immune cells, including T cells, this further underlines the potential immunomodulatory function on basophils.

In CsU skin lesions, increased numbers of basophils have been detected [27]. Furthermore, IL-31 serum levels are increased in patients with CsU [7]. Also, in a study of 15 excellent responders to omalizumab, successful treatment of these patients not only improved clinical symptoms but was associated with reduced IL-31 serum levels [9]. In this regard, the striking IL-31 release *in vitro* suggests that basophils are an important source of IL-31 in the inflammatory response. Furthermore, our *in vitro* findings of increased chemotaxis induced by IL-31 in basophils indicate a role in the orchestration and accumulation of these cells in inflamed skin such as in urticaria. Indeed, we could show here that basophils are positive for IL-31 in skin lesions of CsU patients. In other chronic inflammatory skin diseases, IL-31-positive mast cells have been described in psoriasis and in mastocytosis and for CLA$^+$ T cells in atopic dermatitis and infiltrating T cells [3, 6, 14, 15]. Thus, it seems that local expression of IL-31 is predominantly regulated by immune cells including basophils, underlining a novel modality of basophils in orchestrating the inflammatory response.

In conclusion, IL-31 stimulates a pro-inflammatory activation pattern in human basophils which are a source of IL-31 themselves. Thus, IL-31 therefore is a potential target for novel therapeutic treatment strategies in basophil-associated inflammatory diseases.

Author contributions

Designed research: URa, MG, URü, HH, BG. Performed research: URa, MG, SK, URü, HH, BG. Contributed new analytical tools: URa, MG, URü, BEV, HH, BG. Collected data: URa, MG, URü, BG. Analysed and interpreted

data: URa, MG, SK, URü, BEV, HH, AK, BG. Performed statistical analysis: URa, MG, URü, BG. Wrote/edited the manuscript: URa, MG, SK, URü, BEV, HH, AK, BG.

Conflict of interest

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