Interleukin 33 and interleukin 4 regulate interleukin 31 gene expression and secretion from human laboratory of allergic diseases 2 mast cells stimulated by substance P and/or immunoglobulin E

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ABSTRACT

Background: Cytokine interleukin (IL) 31 has emerged as an important component of allergic and inflammatory diseases associated with pruritus, such as atopic dermatitis (AD) and mastocytosis. Mast cells (MC) are stimulated by allergic and nonallergic triggers, and play a critical role in such diseases by secreting histamine and tryptase as well as cytokines and chemokines. IL-33 has been reported to augment MC responses, but its effect on secretion of IL-31 is not known.

Objectives: To investigate whether IL-33 can stimulate the secretion of IL-31 from cultured human MCs and whether this response is augmented by either the neuropeptide substance P (SP) or immunoglobulin E (IgE) and anti-IgE in the absence or presence of IL-4.

Methods: Laboratory of Allergic Diseases (LAD2) human MCs were cultured in StemProH-34 SFM medium supplemented by stem cell factor and were stimulated either with IL-33 (10 ng/mL) or SP (2 μ M), or preincubated with IgE (1 μ g/mL) overnight, and then stimulated with anti-IgE (1 μ g/mL) for 24 hours. IL-31 gene expression was measured by quantitative polymerase chain reaction, and protein was measured by enzyme-linked immunosorbent assay.

Results: IL-33 (10 ng/mL) induces IL-31 gene expression, synthesis, and secretion from LAD2 cells in the absence of degranulation, whereas SP and IgE on their own have no effect. However, the effect of IL-33 is augmented by SP (2 μ M) and/or IgE and anti-IgE (1 μ g/mL both) and especially their combination. Moreover, this response is significantly further increased when LAD2 cells are cultured in the presence of IL-4.

Conclusion: These findings provide evidence that IL-33 induced secretion of IL-31 from LAD2 MC, an action augmented by novel neuroimmune interactions that may help in the development of new treatments of allergic and inflammatory diseases, especially AD and mastocytosis.

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Mast cells (MC) are critical for allergic,¹ immune, and inflammatory responses.^{2,3} They are characterized by a panel of receptors⁴ through which they are stimulated by many triggers that lead to secretion of multiple mediators.⁵ MCs are known to be involved in the allergic airway⁶ and skin⁷ inflammatory diseases

such as atopic dermatitis (AD)⁸ and mastocytosis.¹ In addition to activation by antigen cross-linking immunoglobulin E (IgE) molecules bound to their surface receptors (high affinity IgE receptor [Fc ϵ RI]),^{9–11} neuropeptides, e.g., substance P (SP), also stimulate MC either through the high affinity Neurokinin-1 (NK-1) receptor¹² or the low affinity Mas-related G-protein coupled receptor member X2 receptor.¹³ We showed that stimulation of human MCs by SP was augmented by the “alarmin” interleukin (IL) 33 and resulted in secretion of either vascular endothelial growth factor¹⁴ or tumor necrosis factor (TNF),¹⁵ without degranulation. IL-33 belongs to the IL-1 family of cytokines¹⁶ and is involved in autoimmune and inflammatory processes.^{17–19}

IL-31 is a helical molecule, which belongs to the cytokine family that includes IL-6, IL-11, IL-27, leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, cardiotrophin-1, cardiotrophic-like cytokine, and neuropoietin.²⁰ IL-31 was initially described as being synthesized by activated CD4⁺ T-

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helper cells and less so by activated CD8⁺ cells.²¹ Monocytes, macrophages, dendritic cells, human epidermal keratinocytes, and dermal fibroblast synthesize IL-31.²² Basophils stimulated by IgE/anti-IgE²³ and MCs stimulated by β -defensins²⁴ were reported to secrete IL-31.

However, the regulation of IL-31 production from human MCs has not been adequately studied. IL-31 is expressed in the skin of patients with AD,²⁶ and IL-1-producing T cells are increased in the skin of chronic AD.²¹ Moreover, serum levels of IL-31 were increased and correlated with disease severity in AD.^{27,28} In fact, IL-31 was shown to induce dermatitis in mice.²⁹ IL-31 was also elevated in adult patients with mastocytosis and was correlated with severity of symptoms.³⁰ The role of IL-31 in skin diseases was recently reviewed.^{28,31,32} Here we showed that human-cultured LAD2 MCs secreted IL-31 in response to IL-33 and that this effect was augmented by SP, IgE/anti-IgE, or their combination, and was further increased by IL-4.

METHODS

Reagents

Recombinant human stem cell factor (rhSCF) was donated by Orphan Biovitrum AB, Stockholm, Sweden. SP was purchased from Sigma-Aldrich, St. Louis, Missouri, and was diluted in distilled water. Recombinant human IL-33 (rhIL-33) and IL-4 (rhIL-4) were purchased from R&D Systems, Minneapolis, Minnesota, and were reconstituted in sterile phosphate-buffered saline solution that contained 0.1% of human serum albumin. Human myeloma IgE was purchased from EMD Millipore, San Diego, California. IgE goat-antihuman polyclonal antibody (anti-IgE) was purchased from Life Technologies, Carlsbad, California; both IgE and anti-IgE were both diluted in sterile water.

Human LAD2 Cell Culture

LAD2 human MC (provided by A.S. Kirshenbaum, M.D., National Institutes of Health, Bethesda, MD) were derived from a single patient with human MC leukemia.³³ LAD2 cells were cultured in StemProH-34 SFM medium (Life Technologies), supplemented with 100 U/mL of penicillin-streptomycin and 100 ng/mL of rhSCF. LAD2 cells were maintained at 37°C in a humidified incubator at 5% CO₂. In certain experiments, rhIL-4 (100 ng/mL) was added to the culture medium for 2 weeks before stimulation. IgE (1 μ g/mL) was added overnight to sensitize MC and was washed before stimulation with anti-IgE (1 μ g/mL). LAD2 cells were stimulated by the different triggers for 24 hours. Working dilutions for all compounds were prepared in culture medium immediately before use.

Degranulation Assay

β -Hexosaminidase (β -hex) secretion was assayed as an index of MC degranulation. LAD2 cells (0.5×10^5) were stimulated with SP (2 μ M), IL-33 (10 ng/mL), or SP plus IL-33 for 30 minutes. Supernatant fluids were collected, and cell pellets were lysed with 1% Triton X-100. Supernatant fluids and cell lysates were incubated in the reaction buffer (p-nitrophenyl-N-acetyl- β -D glucosaminidase; Sigma) for 1.5 hours and then 0.2 mol/L of glycine was added to stop the reaction. Absorbance was measured at 405 nm. The results are expressed as the percentage of β -hex secreted over the total amount (pellet plus supernatant) present in LAD2 cells.

LAD2 Stimulation for Gene Expression and Secretion

LAD2 cells (6×10^5 cells per 500 μ L) were distributed in 48-well microtiter assay plates in triplicates and stimulated with the indicated concentrations of SP and/or IL-33. In the case of IgE, LAD2 cells were incubated overnight with IgE (1 μ g/mL), washed, and then stimulated with either anti-IgE (1 μ g/mL) or the other triggers for 24 hours. In the experiments that used IL-4, LAD2 cells were cultured with media to which IL-4 (100 ng/mL) was added for 2 weeks, and then washed and resuspended with cultured media to which the same amount of IL-4 (100 ng/mL) was added. So that the LAD2 cells be similarly stimulated for 24 hours with all triggers, IgE was added overnight before the wash. Unstimulated LAD2 cells were used as negative controls. After 24 hours, the LAD2 cells were centrifuged and IL-31 was assayed in the supernatant fluids with a commercial enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN) according to the manufacturer's directions. IL-31 protein production and secretion is expressed as pg/mL/ 10^6 cells.

RNA Isolation and Quantitative Real-Time Polymerase Chain Reaction

LAD2 cells (1×10^6 /mL) were incubated with IL-4 (100 ng/mL) for 2 weeks and/or were sensitized with 1 μ g/mL of IgE overnight before stimulation with SP, IL-33, or anti-IgE for 24 hours. The cell pellets were used for total RNA extraction. Total RNA was extracted with an RNeasy Mini kit (Qiagen, Valencia, CA). An iScript complementary DNA synthesis kit (Bio-Rad Laboratories, Hercules, CA) was used for reverse transcription of each sample. Quantitative real-time polymerase chain reaction (PCR) was performed with TaqMan gene expression assays (Life Technologies) for IL-31 (Hs01098710 m1). Samples were run at 45 cycles by using a real-time PCR system (Model 7300; Applied Biosystems). The messenger RNA (mRNA)

gene expression values were normalized to human Glyceraldehyde 3-phosphate dehydrogenase used as the endogenous control (4310884E; Applied Biosystems).

Cell Viability

Cell viability was assayed by means of Trypan blue exclusion. LAD2 cell viability was >98% viable after 24 hours of incubation with SP, IL-33, or anti-IgE.

Statistical Analysis

All the experiments were performed in triplicate ($n = 3$) and repeated at least three times. Results are presented as fold over control for gene expression and as mean $\pm$ standard deviation (SD) for protein synthesis and secretion. Data between different treatment groups were analyzed by using the unpaired 2-tailed Student's *t*-test.

RESULTS

IL-33 Did Not Affect MC Degranulation Stimulated by SP

IL-33 (10 ng/mL) did not trigger degranulation of human LAD2 cells on its own as measured by β -hex secretion at 30 minutes. As expected, SP (2 μ M), used as "positive" control, triggered statistically significant degranulation ($p < 0.001$). The addition of IL-33 (10 ng/mL) to SP (2 μ M) did not increase this effect (Online Supplemental Fig. 1 A). Moreover, the addition of IL-4 (100 ng/mL) to the cultured media for 2 weeks also did not have any additional effect on MC degranulation (Online Supplemental Fig. 1 B).

IL-33 Induced IL-31 Gene Expression, An Effect Augmented by SP or IgE/anti-IgE and increased by IL-4

Preliminary experiments tested various concentrations of IL-33 and SP (1, 10, and 50 ng/mL) for different time periods (3, 6, 9, 12, and 24 hours) to determine the optimal conditions for stimulation of MCs and secretion of IL-31. IL-33 (10 ng/mL) alone induced a statistically significant increase (115.1 ± 14.24 fold over control) ($p < 0.01$) in IL-31 mRNA expression 6 hours after stimulation (Fig. 1 A), and this effect was further increased (313.6 ± 21.38 fold over control) ($p < 0.01$) when IL-33 was combined with SP. SP alone had no effect.

We then investigated whether IL-4 could further increase IL-31 mRNA expression triggered by IL-33 or IL-33 plus SP. LAD2 cells were incubated with IL-4 (100 ng/mL) for 2 weeks and then triggered with SP, IL-33, or IL-33 plus SP for 24 hours. The addition of IL-4 had no effect on SP alone but significantly ($p < 0.0001$) increased the effect of IL-33 alone (156.5 ± 1.73

fold over control) or together with SP (501.8 ± 20.4 fold over control) ($p < 0.0001$) (Fig. 1 B). When LAD2 cells were sensitized with IgE in the absence of IL-4 and then were triggered with anti-IgE, SP, IL-33, or IL-33 plus SP, IL-31 mRNA expression increased significantly with anti-IgE plus IL-33 (155 ± 5.7 fold over control) and anti-IgE plus IL-33 plus SP (390.1 ± 12.2 fold over control) (Fig. 1 C). In the presence of IL-4, the effect of anti-IgE plus IL-33 and anti-IgE plus SP plus IL-33 was further increased ($p < 0.0001$) to 731.0 ± 79.4 fold and 2186.0 ± 223.8 fold over control, respectively (Fig. 1 D).

IL-33 Induced IL-31 Synthesis, An Effect Augmented by SP or IgE/anti-IgE and increased by IL-4

We then investigated whether the increased IL-31 gene expression under the different conditions used resulted in increased IL-31 synthesis. Stimulating LAD2 cells with SP and/or IL-33 increased IL-31 synthesis from 826 ± 45.53 pg/mL/ 10^6 cells in unstimulated control cells to 1006 ± 23.97 pg/mL/ 10^6 cells ($p < 0.001$) when stimulated with IL-33 to 1172 ± 53.35 pg/mL/ 10^6 cells ($p < 0.0001$) when stimulated with IL-33 plus SP (Fig. 2 A). When LAD2 cells were cultured in IL-4 (100 ng/mL) for 2 weeks, IL-31 synthesis increased from 848.9 ± 42.83 pg/mL/ 10^6 cells in unstimulated control cells to 1420 ± 24.19 pg/mL/ 10^6 cells ($p < 0.0001$) when stimulated by using IL-33 plus SP (Fig. 2 B). In LAD2 cells sensitized with IgE (1 μ g/mL) overnight and then triggered with anti-IgE (1 μ g/mL), SP, and/or IL-33 for 24 hours, IL-31 synthesis was increased from 850 ± 9.89 pg/mL/ 10^6 cells in unstimulated control cells to 1316 ± 18.71 pg/mL/ 10^6 cells ($p < 0.0001$) when stimulated with SP plus IL-33 (Fig. 2 C). Finally, when LAD2 cells were cultured in IL-4 (100 ng/mL) for 2 weeks and then sensitized with IgE (1 μ g/mL) overnight and triggered with anti-IgE (1 μ g/mL), SP, and/or IL-33, IL-31 synthesis was further increased, from 940.2 ± 7.85 pg/mL/ 10^6 in unstimulated control cells to 1922 ± 43.64 pg/mL/ 10^6 cells ($p < 0.0001$) when stimulated with SP plus IL-33 (Fig. 2 D).

IL-33 Induced IL-31 Secretion, An Effect Augmented by SP or IgE/anti-IgE and Increased by IL-4

We then investigated the effects of the conditions previously studied on the secretion of IL-31. IL-33 stimulated secretion of IL-31 from 47.1 ± 1.1 pg/mL/ 10^6 cells ($p < 0.001$) in unstimulated control cells to 91.8 ± 7.2 pg/mL/ 10^6 cells when stimulated with IL-33, and the combination of IL-33 plus SP further increased secretion to 203.7 ± 7.1 pg/mL/ 10^6 cells ($p < 0.0001$), (Fig. 3 A). Preincubation with IL-4 before stimulation with IL-33 or IL-33 plus SP significantly further in-

Figure 1. IL 33 induces IL-31 gene expression, an effect augmented by substance P (SP) and/or immunoglobulin E (IgE), and increased by IL-4. LAD2 cells were stimulated with (A) IL-33 (10 ng/mL), SP (2 μ M), or their combination for 24 hours; (B) incubated with IL-4 (100 ng/mL) for 2 weeks and then stimulated as in (A); (C) sensitized overnight with IgE (1 μ g/mL), followed by anti-IgE and/or IL-33 or SP alone, or all three triggers together, as indicated, for 24 hours; (D) incubated with IL-4 (100 ng/mL) for 2 weeks and then stimulated as in (C). Gene expression was normalized to the endogenous control GAPDH. Results are expressed as gene expression relative to GAPDH and are discussed as fold increase over unstimulated control cells ($n = 3$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; and ### $p < 0.01$; #### $p < 0.001$; ##### $p < 0.0001$ when values were compared between each combination and the relative trigger used alone. IL = Interleukin; LAD2 = Laboratory of allergic diseases 2; GAPDH = Glyceraldehyde 3-phosphate dehydrogenase.

creased ($p < 0.0001$) IL-31 secretion from 47.1 ± 1.1 pg/mL/ 10^6 in unstimulated control cells to 322.3 ± 18.5 pg/mL/ 10^6 cells when stimulated with IL-33 to 440.5 ± 14.2 pg/mL/ 10^6 cells when stimulated with SP plus IL-33 (Fig. 3 B).

LAD2 cells sensitized with IgE (1 μ g/mL) overnight and triggered with anti-IgE, SP, IL-33, SP plus IL-33 for 24 hours, increased IL-31 secretion from 27.06 ± 1.06 pg/mL/ 10^6 in unstimulated control cells to 54.4 ± 3.9 pg/mL/ 10^6 cells when stimulated with anti-IgE (1 μ g/mL) and to 53.8 ± 4.0 pg/mL/ 10^6 cells ($p < 0.001$) when stimulated with anti-IgE plus SP. IL-31 secretion by anti-IgE plus IL-33 was 99.5 ± 5.0 pg/mL/ 10^6 cells and increased to 257.4 ± 12.1 pg/mL/ 10^6 cells ($p < 0.0001$) (Fig. 3 C) with anti-IgE plus IL-33 plus SP. IL-31 protein secretion was significantly increased when LAD2 cells, in addition, were cultured in IL-4 (100 ng/mL) for 2 weeks, from 78.5 ± 7.2 pg/mL/ 10^6 cells in unstimulated control cells to 1227 ± 48.9 pg/mL/ 10^6

cells ($p < 0.0001$) when stimulated with anti-IgE plus SP plus IL-33 (Fig. 3 D).

DISCUSSION

Here we showed that cultured human LAD2 MCs could synthesize and secrete IL-31. We also reported that IL-33 on its own, but mainly when combined with SP and IgE/anti-IgE, triggered a significant increase in IL-31 gene expression and secretion from LAD2 cells. It was reported that human basophils can secrete IL-31 when stimulated by IgE/anti-IgE.²³ One article reported that human LAD2 MCs can produce and secrete IL-31 but only after being triggered by human β -defensins and cathelicidin LL-37.²⁴ MCs from patients with myeloproliferative disorders were also shown to secrete IL-31.²⁵ The possible secretion of IL-31 from human MCs is also supported by the finding that the anti-IgE biologic omalizumab lowered serum IL-31 levels and improved chronic spontaneous urticaria in humans.³⁴

Figure 2. IL-33 induces IL-31 synthesis, an effect augmented by substance P (SP) and/or immunoglobulin E (IgE), and increased by IL-4. LAD2 cells were stimulated with (A) IL-33 (10 ng/mL), SP (2 μ M), or their combination for 24 hours; (B) incubated with IL-4 (100 ng/mL) for 2 weeks and then stimulated as in (A); (C) sensitized overnight with IgE (1 μ g/mL), followed by anti-IgE and/or IL-33 or SP alone or all three triggers together as indicated for 24 hours; (D) incubated with IL-4, sensitized overnight with IgE, and stimulated as in (C) ($n = 3$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; and ### $p < 0.01$; #### $p < 0.001$; ##### $p < 0.0001$ when values were compared between each combination and the relative trigger used alone. Stimulated LAD2 cell lysates were prepared by lysing LAD2 cells in lysis buffer that contained a protease inhibitor mixture. IL = Interleukin; LAD2 = Laboratory of allergic diseases 2.

IL-33 belongs to the IL-1 family of cytokines,³⁵ is mostly secreted through tissue damage, and is implicated as an “alarmin” in many inflammatory diseases, including AD.^{17–19,36} IL-33 augments the secretion of proinflammatory mediators from MCs and acts to orchestrate the allergic and/or inflammatory response.³⁷ When including targeting basophils and eosinophils involved in allergic diseases,³⁸ IL-33 augments allergic MCs and basophil stimulation³⁹ and can also be produced by MCs after allergic stimulation.⁴⁰ In addition, IL-33 can promote maturation of human MCs.⁴¹ It is important that MC-derived tryptase cleaves IL-33 and liberates the (107–270 fragment) “mature” fragment, which is 30-fold more active than the full-length form.⁴²

We also showed that IL-33 augmented the effect of SP, a peptide originally described by Ulf von Euler and then isolated from the rat brain,⁴³ which is implicated in inflammatory processes.^{44–49} SP can stimulate MC secretion of histamine⁵⁰ and TNF^{51–54} from rat peritoneal MCs^{51,55} and cultured human skin MCs.^{52,53} We reported that IL-33 augments SP-stimulated synthesis and secretion of vascular endothelial growth factor¹⁴ and TNF¹⁵ from human cultured MCs without degranulation. It is interesting that mouse bone marrow-derived MCs secrete the SP structural analog hemo-kinin-1, which augments allergic stimulation of TNF, which implies an autocrine action.⁵⁶ SP-positive nerve

fibers are increased close to MC in lesional areas of patients with psoriasis.⁵⁷ Furthermore, SP is increased in the skin of patients with AD.⁵⁸ Increased plasma levels of SP correlated with the disease severity in patients with AD^{59,60} and mastocytosis.³⁰

We also showed that IL-4 further increased IL-31 gene expression, synthesis, and secretion from LAD2 MC. The mechanism *via* which IL-33, SP, and IL-4 induce IL-31 secretion in LAD2 MC is presently not known. We hypothesized that IL-4 and IL-33 increased NK-1 expression and led to higher IL-31 secretion after stimulation with SP. It was previously reported that IL-4 together with stem cell factor induce NK-1 expression in mouse bone marrow-derived MC.⁶¹

We recently reported that IL-33 increases the expression of NK-1 receptor in human cultured MC.¹⁵ These findings might explain at least how IL-4 and IL-33, together with SP, result in the higher IL-31 synthesis and secretion described here. Another possible mechanism might be through IL-4 and/or IL-33 induction of IL-31 gene expression. For instance, one study reported that IL-31 is induced by IL-4,⁶² whereas another found that the IL-31 promoter contains a positive regulatory region that mediates IL-4-dependent induction of the IL-31 gene in rat basophilic leukemia (RBL-2H3) MC through Signal transducer and activator of transcription 6 binding sites.⁶³

Figure 3. IL-33 induces IL-31 secretion, an effect augmented by substance P (SP) and/or immunoglobulin E (IgE), and increased by IL-4. LAD2 cells were stimulated with (A) IL-33 (10 ng/mL) and/or SP (2 μ M) for 24 hours; (B) incubated with IL-4 (100 ng/mL) for 2 weeks and then stimulated as in (A); (C) sensitized overnight with IgE (1 μ g/mL), followed by anti-IgE and/or IL-33 or SP alone, or all three triggers together, as indicated, for 24 hours; (D) incubated with IL-4, sensitized overnight with IgE and stimulated as in (C) ($n = 3$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; and ### $p < 0.01$; #### $p < 0.001$; ##### $p < 0.0001$ when values were compared between each combination and the relative trigger used alone. IL = Interleukin; LAD2 = Laboratory of allergic diseases 2.

A recent article reported that IL-33 pretreatment increased both the number of degranulated MCs and the magnitude of individual MC degranulation in response to Fc ϵ RI triggering.⁶⁴ However, this finding was not applicable to the ability of IL-33 reported here to induce IL-31 synthesis and secretion without degranulation. Another study reported that IL-4 augments histamine secretion from purified human skin MCs stimulated by an allergic trigger but not by SP.⁶⁵ Interestingly, SP was reported to decrease expression of Fc ϵ RI expression,⁶⁶ yet we observed an augmentation of IL-31 secretion when SP was added after MC stimulation by IgE/anti-IgE. These findings indicated that the effects of IL-4, IL-33, and SP are different in the presence or absence of an allergic trigger.

Our results further indicated that the amount of IL-31 synthesized is more than the amount secreted

irrespective of the trigger. This finding implied that (a) longer stimulation with the triggers may be required to secrete the remaining amount of IL-31, or (b) that some other triggers may be able to stimulate additional IL-31 secretion, or (c) that some IL-31 may be sequestered in some cytoplasmic compartment. MCs may also contribute to IL-31 production indirectly. For instance, type 2 T-helper cells express one of the four isotypes of histamine receptors, the H4 receptor, stimulation of which resulted in significant upregulation of IL-31.⁶⁷

Increased IL-31 is associated with many diseases,^{22,28,31,32} especially AD^{21,54,68} and mastocytosis.³⁷ It is interesting that IL-31 used together with IL-33 was reported to increase cell surface expression of intercellular adhesion molecule-1 on eosinophils and fibroblasts cultured together.⁵⁴ IL-31 may constitute the link between T cells and pruritus in atopic skin inflamma-

tion.³¹ In this article, we used LAD2 MCs derived from a patient with MC leukemia³³ but that were repeatedly shown to behave like primary human MCs.^{15,69–71} It is desirable to use primary human MCs whenever possible. However, even isolated primary human skin MCs were shown to have great variability in their responsiveness to an allergic trigger.^{7,72}

CONCLUSION

Our findings provided new insights into how IL-33 by itself, as well as through interactions with SP, IgE/anti-IgE, and IL-4, regulated IL-31 expression and secretion from human LAD2 MC. These findings could help, in addition to the anti-IL-31A receptor antibody used for AD,⁷³ in the development of effective treatments of allergic and inflammatory disorders, especially AD⁷⁴ and mastocytosis.⁷⁵

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REFERENCES

- Theoharides TC, Valent P, Akin C. Mast cells, mastocytosis, and related disorders. *N Engl J Med*. 2015; 373:163–172.
- Theoharides TC, Alysandratos KD, Angelidou A, et al. Mast cells and inflammation. *Biochim Biophys Acta*. 2012; 1822:21–33.
- Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. *Nature*. 2008; 454:445–454.
- Gilfillan AM, Tkaczuk C. Integrated signalling pathways for mast-cell activation. *Nat Rev Immunol*. 2006; 6:218–230.
- Wernersson S, Pejler G. Mast cell secretory granules: Armed for battle. *Nat Rev Immunol*. 2014; 14:478–494.
- Bradding P, Arthur G. Mast cells in asthma—State of the art. *Clin Exp Allergy*. 2016; 46:194–263.
- Babina M, Guhl S, Artuc M, Trivedi NN, Zuberbier T. Phenotypic variability in human skin mast cells. *Exp Dermatol*. 2016; 25:434–439.
- Otsuka A, Kabashima K. Mast cells and basophils in cutaneous immune responses. *Allergy*. 2015; 70:131–140.
- Siraganian RP. Mast cell signal transduction from the high-affinity IgE receptor. *Curr Opin Immunol*. 2003; 15:639–646.
- Kinet JP. The high-affinity IgE receptor (Fc epsilon RI): From physiology to pathology. *Annu Rev Immunol*. 1999; 17:931–972.
- Dema B, Suzuki R, Rivera J. Rethinking the role of immunoglobulin E and its high-affinity receptor: New insights into allergy and beyond. *Int Arch Allergy Immunol*. 2014; 164:271–279.
- Okada T, Hirayama Y, Kishi S, Miyayasu K, Hiroi J, Fujii T. Functional neurokinin NK-1 receptor expression in rat peritoneal mast cells. *Inflamm Res*. 1999; 48:274–279.
- McNeil BD, Pundir P, Meeker S, et al. Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions. *Nature*. 2015; 519:237–241.
- Theoharides TC, Zhang B, Kempuraj D, et al. IL-33 augments substance P-induced VEGF secretion from human mast cells and is increased in psoriatic skin. *Proc Natl Acad Sci U S A*. 2010; 107:4448–4453.
- Tarcanova A, Alevizos M, Karagkouni A, et al. SP and IL-33 together markedly enhance TNF synthesis and secretion from human mast cells mediated by the interaction of their receptors. *Proc Natl Acad Sci U S A*. 2017; 114:E4002–E4009.
- Martin NT, Martin MU. Interleukin 33 is a guardian of barriers and a local alarmin. *Nat Immunol*. 2016; 17:122–131.
- Theoharides TC, Petra AI, Tarcanova A, Panagiotidou S, Conti P. Targeting IL-33 in autoimmunity and inflammation. *J Pharmacol Exp Ther*. 2015; 354:24–31.
- Saluja R, Ketelaar ME, Hawro T, Church MK, Maurer M, Nawijn MC. The role of the IL-33/IL-1RL1 axis in mast cell and basophil activation in allergic disorders. *Mol Immunol*. 2015; 63:80–85.
- Liew FY, Pitman NI, McInnes IB. Disease-associated functions of IL-33: The new kid in the IL-1 family. *Nat Rev Immunol*. 2010; 10:103–110.
- Zhang Q, Putheti P, Zhou Q, Liu Q, Gaob W. Structures and biological functions of IL-31 and IL-31 receptors. *Cytokine Growth Factor Rev*. 2008; 19:347–356.
- Szegedi K, Kremer AE, Kezic S, et al. Increased frequencies of IL-31-producing T cells are found in chronic atopic dermatitis skin. *Exp Dermatol*. 2012; 21:431–436.
- Cornelissen C, Luscher-Firzlauff J, Baron JM, Luscher B. Signaling by IL-31 and functional consequences. *Eur J Cell Biol*. 2012; 91:552–566.
- Raap U, Gehring M, Kleiner S, et al. Human basophils are a source of – and are differentially activated by – IL-31. *Clin Exp Allergy*. 2017; 47:499–508.
- Niyonsaba F, Ushio H, Hara M, et al. Antimicrobial peptides human beta-defensins and cathelicidin LL-37 induce the secretion of a pruritogenic cytokine IL-31 by human mast cells. *J Immunol*. 2010; 184:3526–3534.
- Ishii T, Wang J, Zhang W, et al. Pivotal role of mast cells in pruritogenesis in patients with myeloproliferative disorders. *Blood*. 2009; 113:5942–5950.
- Kato A, Fujii E, Watanabe T, et al. Distribution of IL-31 and its receptor expressing cells in skin of atopic dermatitis. *J Dermatol Sci*. 2014; 74:229–235.
- Raap U, Wichmann K, Bruder M, Ständer S, Wedi B, Kapp A, et al. Correlation of IL-31 serum levels with severity of atopic dermatitis. *J Allergy Clin Immunol*. 2008; 122:421–423.
- Saleem MD, Oussedik E, D’Amber V, Feldman SR. Interleukin-31 pathway and its role in atopic dermatitis: A systematic review. *J Dermatolog Treat Mar*. 2017; 2:1–9.
- Dillon SR, Sprecher C, Hammond A, et al. Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. *Nat Immunol*. 2004; 5:752–760.
- Hartmann K, Wagner N, Rabenhorst A, et al. Serum IL-31 levels are increased in a subset of patients with mastocytosis and correlate with disease severity in adult patients. *J Allergy Clin Immunol*. 2013; 132:232–235.
- Sonkoly E, Muller A, Lauerma AI, et al. IL-31: A new link between T cells and pruritus in atopic skin inflammation. *J Allergy Clin Immunol*. 2006; 117:411–417.
- Gangemi S, Quartuccio S, Casciaro M, Trapani G, Minciullo PL, Imbalzano E. Interleukin 31 and skin diseases: A systematic review. *Allergy Asthma Proc*. 2017; 38:401–408.
- Kirshenbaum AS, Akin C, Wu Y, et al. Characterization of novel stem cell factor responsive human mast cell lines LAD 1 and 2 established from a patient with mast cell sarcoma/leukemia; activation following aggregation of Fc epsilon RI or Fc gamma RI. *Leuk Res*. 2003; 27:677–682.
- Altrichter S, Hawro T, Hanel K, et al. Successful omalizumab treatment in chronic spontaneous urticaria is associated with lowering of serum IL-31 levels. *J Eur Acad Dermatol Venereol*. 2016; 30:454–455.
- Schmitz J, Owyang A, Oldham E et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity*. 2005; 23:479–490.

36. Vocca L, Di Sano C, Uasuf CG, et al. IL-33/ST2 axis controls Th2/IL-31 and Th17 immune response in allergic airway diseases. *Immunobiology*. 2015; 220:954–963.
37. Enoksson M, Lyberg K, Moller-Westerberg C, Fallon PG, Nilsson G, Lunderius-Andersson C. Mast cells as sensors of cell injury through IL-33 recognition. *J Immunol*. 2011; 186:2523–2528.
38. Pecaric-Petkovic T, Didichenko SA, Kaempfer S, Spiegl N, Dahinden CA. Human basophils and eosinophils are the direct target leukocytes of the novel IL-1 family member IL-33. *Blood*. 2009; 113:1526–1534.
39. Silver MR, Margulis A, Wood N, Goldman SJ, Kasaian M, Chaudhary D. IL-33 synergizes with IgE-dependent and IgE-independent agents to promote mast cell and basophil activation. *Inflamm Res*. 2010; 59:207–218.
40. Hsu CL, Neilsen CV, Bryce PJ. IL-33 is produced by mast cells and regulates IgE-dependent inflammation. *PloS One*. 2010; 5:e11944.
41. Allakhverdi Z, Smith DE, Comeau MR, Delespesse G. Cutting edge: The ST2 ligand IL-33 potently activates and drives maturation of human mast cells. *J Immunol*. 2007; 179:2051–2054.
42. Morita H, Nakae S, Saito H, Matsumoto K. IL-33 in clinical practice: Size matters? *J Allergy Clin Immunol*. 2017; 140:381–383.
43. Chang MM, Leeman SE. Isolation of a sialogic peptide from bovine hypothalamic tissue and its characterization as substance P. *J Biol Chem*. 1970; 245:4784–4790.
44. Saraceno R, Kleyn CE, Terenghi G, Griffiths CE. The role of neuropeptides in psoriasis. *Br J Dermatol*. 2006; 155:876–882.
45. Douglas SD, Leeman SE. Neurokinin-1 receptor: Functional significance in the immune system in reference to selected infections and inflammation. *Ann N Y Acad Sci*. 2011; 1217: 83–95.
46. O'Connor TM, O'Connell J, O'Brien DI, Goode T, Bredin CP, Shanahan F. The role of substance P in inflammatory disease. *J Cell Physiol*. 2004; 201:167–180.
47. Steinhoff MS, von Mentzer B, Geppetti P, Pothoulakis C, Bunnett NW. Tachykinins and their receptors: Contributions to physiological control and the mechanisms of disease. *Physiol Rev*. 2014; 94:265–301.
48. Richard AC, Ferdinand JR, Meylan F, Hayes ET, Gabay O, Siegel RM. The TNF-family cytokine TL1A: From lymphocyte costimulator to disease co-conspirator. *J Leukoc Biol*. 2015; 98: 333–345.
49. Munoz M, Covenas R. Involvement of substance P and the NK-1 receptor in human pathology. *Amino Acids*. 2014; 46: 1727–1750.
50. Fewtrell CM, Foreman JC, Jordan CC, Oehme P, Renner H, Stewart JM. The effects of substance P on histamine and 5-hydroxytryptamine release in the rat. *J Physiol*. 1982; 330:393–411.
51. Ansel JC, Brown JR, Payan DG, Brown MA. Substance P selectively activates TNF-alpha gene expression in murine mast cells. *J Immunol*. 1993; 150:4478–4485.
52. Okayama Y, Ono Y, Nakazawa T, Church MK, Mori M. Human skin mast cells produce TNF-alpha by substance P. *Int Arch Allergy Immunol*. 1998; 117(suppl. 1):48–51.
53. Okabe T, Hide M, Koro O, Yamamoto S. Substance P induces tumor necrosis factor-alpha release from human skin via mitogen-activated protein kinase. *Eur J Pharmacol*. 2000; 398:309–315.
54. Bilsborough J, Leung DY, Maurer M, et al. IL-31 is associated with cutaneous lymphocyte antigen-positive skin homing T cells in patients with atopic dermatitis. *J Allergy Clin Immunol*. 2006; 117:418–425.
55. Azzolina A, Bongiovanni A, Lampiasi N. Substance P induces TNF-alpha and IL-6 production through NF kappa B in peritoneal mast cells. *Biochim Biophys Acta*. 2003; 1643:75–83.
56. Sumpter TL, Ho CH, Pleet AR et al. Autocrine hemokinin-1 functions as an endogenous adjuvant for IgE-mediated mast cell inflammatory responses. *J Allergy Clin Immunol*. 2015; 135:1019–1030.e8.
57. Al'Abadie MS, Kent GG, Gawkrödger DJ. The relationship between stress and the onset and exacerbation of psoriasis and other skin conditions. *Br J Dermatol*. 1994; 130:199–203.
58. Kim HO, Lee CH, Ahn HK, Park CW. Effects of tacrolimus ointment on the expression of substance P, nerve growth factor, and neurotrophin-3 in atopic dermatitis. *Int J Dermatol*. 2009; 48:431–438.
59. Toyoda M, Nakamura M, Makino T, Hino T, Kagoura M, Morohashi M. Nerve growth factor and substance P are useful plasma markers of disease activity in atopic dermatitis. *Br J Dermatol*. 2002; 147:71–79.
60. Hon KL, Lam MC, Wong KY, Leung TF, Ng PC. Pathophysiology of nocturnal scratching in childhood atopic dermatitis: The role of brain-derived neurotrophic factor and substance P. *Br J Dermatol*. 2007; 157:922–925.
61. van der Kleij HP, Ma D, Redegeld FA, Kraneveld AD, Nijkamp FP, Bienenstock J. Functional expression of neurokinin 1 receptors on mast cells induced by IL-4 and stem cell factor. *J Immunol*. 2013; 171:2074–2079.
62. Stott B, Lavender P, Lehmann S, Pennino D, Durham S, Schmidt-Weber CB. Human IL-31 is induced by IL-4 and promotes TH2-driven inflammation. *J Allergy Clin Immunol*. 2013; 132:446–454.e5.
63. Park K, Park JH, Yang WJ, Lee JJ, Song MJ, Kim HP. Transcriptional activation of the IL31 gene by NFAT and STAT6. *J Leukoc Biol*. 2012; 91:245–257.
64. Joulia R, L'Faqihi FE, Valitutti S, Espinosa E. IL-33 fine tunes mast cell degranulation and chemokine production at the single-cell level. *J Allergy Clin Immunol*. 2017; 140:497–509.e10.
65. Babina M, Guhl S, Artuc M, Zuberbier T. IL-4 and human skin mast cells revisited: reinforcement of a pro-allergic phenotype upon prolonged exposure. *Arch Dermatol Res*. 2016; 308:665–670.
66. McCary C, Tancowny BP, Catalli A, et al. Substance P down-regulates expression of the high affinity IgE receptor (FcεpsilonRI) by human mast cells. *J Neuroimmunol*. 2010; 220:17–24.
67. Gutzmer R, Mommert S, Gschwandtner M, Zwingmann K, Stark H, Werfel T. The histamine H4 receptor is functionally expressed on T(H)2 cells. *J Allergy Clin Immunol*. 2009; 123: 619–625.
68. Nygaard U, Hvid M, Johansen C, Buchner M, Fölster-Holst R, Deleuran M, et al. TSLP, IL-31, IL-33 and sST2 are new biomarkers in endophenotypic profiling of adult and childhood atopic dermatitis. *J Eur Acad Dermatol Venereol*. 2016; 30:1930–1938.
69. Guhl S, Babina M, Neou A, Zuberbier T, Artuc M. Mast cell lines HMC-1 and LAD2 in comparison with mature human skin mast cells—Drastically reduced levels of tryptase and chymase in mast cell lines. *Exp Dermatol*. 2010; 19:845–847.
70. Zhang B, Asadi S, Weng Z, Sismanopoulos N, Theoharides TC. Stimulated human mast cells secrete mitochondrial components that have autocrine and paracrine inflammatory actions. *PloS One*. 2012; 7:e49767.
71. Weng Z, Patel AB, Panagiotidou S, Theoharides TC. The novel flavone tetramethoxyluteolin is a potent inhibitor of human mast cells. *J Allergy Clin Immunol*. 2015; 135:1044–1052.e5.
72. Theoharides TC. Skin mast cells: Are we missing the forest for the trees? *Exp Dermatol*. 2016; 25:422–423.
73. Ruzicka T, Mihara R. Anti-interleukin-31 receptor antibody for atopic dermatitis. *N Engl J Med*. 2017; 376:2093.
74. Silverberg JI. Atopic dermatitis treatment: Current state of the art and emerging therapies. *Allergy Asthma Proc*. 2017; 38:243–249.
75. Siebenhaar F, Akin C, Bindslev-Jensen C, Maurer M, Broesby-Olsen S. Treatment strategies in mastocytosis. *Immunol Allergy Clin North Am*. 2014; 34:433–447. □