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ORIGINAL ARTICLE

Basic Mechanisms in Allergic Disease

IL-33 and IgE stimulate mast cell production of IL-2 and regulatory T cell expansion in allergic dermatitis

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Summary

Background: We have previously shown that mast cells (MCs) suppress chronic allergic dermatitis in mice. The underlying mechanism involves MC-derived IL-2, which supports regulatory T cell (Treg) response at the site of inflammation. However, it is not clear what are the factors that drive MCs to produce IL-2.

Objective: To understand the mechanisms that lead to IL-2 production from MCs in chronic allergic dermatitis.

Methods: Isolated murine bone marrow-derived MCs (BMMCs) were incubated with various stimulators, and IL-2 production was assessed by RT-PCR and ELISA. The response of signalling pathways was evaluated by MAPK inhibitors and Western blot analysis. The effect of MC-IL-2 on Tregs was studied by incubation of splenic T cells with conditioned media obtained from activated BMMCs. Dermatitis was elicited by repeated exposures of mouse ears to oxazolone. MCs in mouse and human skin samples were evaluated by immunostaining.

Results: BMMCs released IL-2 in response to IL-33, and IL-2 production was further enhanced by concomitant FcεRI activation. The effect of IL-33 was mediated by activation of the MAPK family members. IL-2 in conditioned media from IL-33 and IgE-stimulated BMMCs led to considerable expansion of Tregs in vitro. IL-33 levels were elevated in oxazolone-challenged ears along with increased numbers of IL-2-expressing MCs. Human skin with chronic inflammation also contained IL-2-expressing MCs that colocalized with IL-33 staining in the dermis.

Conclusions: IL-33, in collaboration with IgE, is critical for MC-IL-2 production in allergic skin disease, thus leading to Treg stimulation and suppression of allergic dermatitis.

KEYWORDS
IL-2, IL-33, mast cells, T cells

1 | INTRODUCTION

Chronic allergic dermatitis is a common condition in human subjects and presents mostly as atopic dermatitis (AD). It usually occurs in individuals with an allergic (atopic) background, belonging to families with a predisposition to produce IgE in response to common allergens.[1,2] AD is associated with cutaneous hyper-reactivity, a disturbance of epidermal-barrier function, dry skin and susceptibility to cutaneous infections. Inflammation has been attributed to the activation of a myriad of immune cells. Mast cells (MCs) play an important aetiologic role, and regulatory T cells (Tregs) are pivotal in limiting the inflammatory response. AD is characterized by T-helper

type 2 (Th2) cytokines in the acute phase, and increased levels of T-helper type 1 (Th1) cytokines in the chronic phase.[2,3] In addition, tissue-derived cytokines such as IL-33 have recently been suggested to take part in AD.[3]

Animal models have proven to be of great value in investigating the underlying mechanisms of AD. One of the known models is oxazolone-induced chronic allergic dermatitis. Oxazolone has been shown to induce allergic contact dermatitis, but in the course of repeated challenges, it evolves into an inflammatory response similar to human AD.[4-6] Oxazolone dermatitis causes an itchy skin disease, with activation and proliferation of MCs, eosinophils and lymphocytes as well as robust production of IgE and cytokines typical of human AD.

We have previously used this disease model to unravel the contribution of MCs in chronic allergic dermatitis. Our findings demonstrated that bone marrow-derived cultured MCs (BMMCs) are protective in late stages of oxazolone-induced chronic allergic dermatitis. Protection occurs by a previously unrecognized regulatory mechanism in which MC-derived IL-2 is required to diminish the ratio of activated T cells to Tregs at the site of inflammation.[7] This effect was associated with a gradual increase in the total numbers of MCs producing IL-2 during the course of disease. We, therefore, hypothesized that the microenvironment created by the inflamed skin tissue provides the stimuli required for MCs to produce IL-2, which is not a common product of these cells.[8] It is not clear however, what are the factors responsible for the stimulation of IL-2 production in BMMCs. In the present study, we show a distinct role for IL-33 and IgE in chronic dermatitis, which cooperate for the induction of MC-derived IL-2 production and subsequent proliferation of Tregs.

2 | MATERIALS AND METHODS

2.1 | Mice

For in vivo experiments, 8- to 12–week-old C57BL/6 mice were used. All mice were purchased from Envigo^{+} (Rehovot, Israel). In all experiments, mice were housed and used under specific pathogen-free conditions according to protocols approved by the Tel-Aviv University Institutional Animal Care Unit.

2.2 | Antibodies and reagents

The following antibodies were used for this study: anti-phospho-p38 MAPK and anti-phospho-JNK (Cell Signaling Technology, Danvers, MA, USA); anti-α-Tubulin (Sigma-Aldrich, St Louis, MO, USA); horseradish peroxidase (HRP)-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA, USA). Recombinant murine IL-33 and all cytokines used were purchased from PeproTech, Inc. (Rocky Hill, NJ, USA); anti-dinitrophenyl (DNP)-specific IgE, DNP-BSA (Ag), LPS, PD98059 and curcumin were purchased from Sigma-Aldrich; SB202190 was purchased from Calbiochem (San Diego, CA, USA). All cell culture reagents were purchased from Biological Industries (Beit Haemek, Israel).

2.3 | Generation of bone marrow MCs

Bone marrow-derived cultured MCs from male C57BL/6 mice were generated and cultured for 4 weeks in the presence of recombinant murine SCF and IL-3 (20 ng/mL) (PeproTech), as previously described.[7] MC populations were monitored for metachromatic granule formation by toluidine blue staining. Cells were used when cultures were more than 99% positive.

2.4 | Generation of fetal skin-derived cultured MCs

Fetal skin-derived cultured MCs (FSMC) were generated by a method based on a previously described protocol.[9] Briefly, 14 days after initial culture of skin single-cell suspensions, nonadherent and loosely adherent cells were collected. MC enrichment was done by density gradient centrifugation. Percoll density medium (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) was rendered isotonic by diluting concentrated RPMI1640 (Sigma-Aldrich, St Louis, MO, USA). Cells in culture medium were layered on diluted Percoll (30% isotonic Percoll+70% culture medium) and centrifuged at 500 $\times$ g for 20 minutes at room temperature. The cell pellet at the bottom was used as FSMC, with purity of >95%, as assessed by toluidine blue staining.

2.5 | MC activation

Activation of BMMCs or purified FSMCs (2×10^6/mL) was induced with anti-DNP-specific IgE (10 μg/mL, 2 hours, 37°C) and DNP-HSA (1 μg/mL), IL-33 (100 ng/mL or as indicated) or both for the indicated time periods. Other stimulants were used as indicated (100 ng/mL). Supernatants were collected for further analysis. In some experiments, MAPK inhibitors were introduced to the MC cultures 30 minutes before the addition of stimulation.

2.6 | Isolation of enriched splenic T cells and stimulation

Spleens were homogenized and red blood cells lysed in ACK lysing buffer (Gibco® by Life Technologies, Grand Island, NY, USA). T cells were enriched by passing splenocytes ($1\text{-}2 \times 10^8$/mL) over nylon wool columns (Uni-Sorb; Novamed, Jerusalem, Israel) for 60 minutes at 37°C, 5% CO_2 in a humidified incubator. Unbound cells were eluted by extensive washing with PBS, and the enriched T cells were resuspended in conditioned media from naïve-, IL-33-, IgE-antigen- or IL-33-IgE-antigen-stimulated BMMCs and incubated (37°C, 5% CO_2 in a humidified incubator) for overnight. Neutralizing antibody against mouse IL-2, isotype control (both from R&D Systems, Minneapolis, MN, USA) or recombinant IL-2 (50 ng/mL; PeproTech) was added 30 minutes prior to incubation, as specified.

2.7 | Cytokine assays

IL-2 in supernatants of activated MCs as indicated above or IL-33 in ear homogenate samples were determined using a commercial ELISA

development kit (PeproTech; DuoSet, R&D systems, respectively) as per the manufacturer's instructions.

2.8 | RNA isolation

Total RNA was extracted from BMMCs activated for 4 hours with IL-33, anti-DNP-specific IgE and DNP-HSA, or both by using TRIzol reagent (Sigma-Aldrich) according to the manufacturer's protocol.

2.9 | Real-time PCR

cDNA was synthesized using the high-capacity cDNA reverse transcriptase kit (Applied Biosystems, Carlsbad, CA, USA). Gene expression was determined by Fast Real-Time PCR using an ABI 7500 Thermal Cycler (Applied Biosystems). The expression of *Il2* gene was measured by specific TaqMan probes (Applied Biosystems; Mm00434256_m1). Expression of glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH; Applied Biosystems; Mm99999915_g1) was used as a housekeeping gene for analysis of changes in the cycle threshold values.

2.10 | SDS-PAGE and immunoblotting

Cellular extracts were separated by SDS-PAGE using 10% polyacrylamide gels, transferred to PVDF filters and processed for immunoblotting, as previously described.[10] Immunoreactive bands were visualized using the LAS-3000 imaging system (Fujifilm Corp., Tokyo, Japan).

2.11 | Oxazolone-induced dermatitis

Mice were sensitized on day 0 by the application of 15 microliters of 1% oxazolone (Sigma-Aldrich) in acetone on both aspects of 1 ear. For control, treatment was performed with acetone only on the second ear. Starting from day 7, mice were challenged in the same manner with 0.5% oxazolone, 3 times per week, for up to a total of 10 challenges. Ear thickness was measured by a dial thickness gauge (Mitutoyo, Aurora, IL, USA). Ear tissue samples were collected at the end of each experiment and placed in formalin solution, 10% neutral buffered (Sigma-Aldrich) for further embedding and sectioning or were homogenized in 700 μL PBS supplemented with complete protease inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany). After 10-min centrifugation at 14 000 × *g* (4°C), the supernatants were collected and kept at −80°C.

2.12 | Immunofluorescence staining

Embedding and sectioning of ear tissue samples were done in Meir Medical Center Institute of Pathology. Paraffin-embedded tissues were deparaffinized, immersed in citrate buffer (pH 6.0) and heated in a microwave oven. Ear sections were stained with H&E and were observed under Olympus BX41 microscope (Olympus America Inc., Center Valley, PA, USA). Cross sections were double-stained with anti-IL-33 antibody (R&D Systems) and Alexa Fluor® 488 conjugated Avidin (Molecular Probes, Inc., Eugene, OR, USA) in order to detect MCs.[11] Nuclei were stained with Hoechst 33342 (Sigma-Aldrich). Cross sections were also double-stained with Alexa Fluor® 488-conjugated Avidin and anti-IL-2 (Novus Biologicals, Littleton, CO, USA). Goat anti-rabbit Alexa Fluor® 594 (Jackson ImmunoResearch Laboratories) was used as secondary antibody. Sections were analysed using a Leica TCS STED confocal microscope (Leica Microsystems GmbH, Wetzlar, Germany). Non-specific signals were subtracted during the confocal analysis.

2.13 | Histological staining

Paraffin blocks of human skin biopsy specimens were retrieved from the archive of the Meir Medical Center Institute of Pathology. The keywords used for sample retrieval were the histological diagnose of "normal skin" or "chronic inflammation" (a predominantly lymphocytic infiltration, occasional eosinophils, collagen deposition and signs of epidermal thickening with tissue damage). Human tissues were used with the approval of the Institutional Review Board (Helsinki Committee; Ethics approval number: 0040-14-MMC). Paraffin-embedded sections were deparaffinized, immersed in citrate buffer (pH 6.0) and heated in a microwave oven. Endogenous peroxide activity was quenched in 3% H_2O_2. Sections were double-stained with antibodies against IL-2 (Santa Cruz Biotechnology, Inc., Heidelberg, Germany) and rabbit anti-human c-kit, CD117 (DAKO, Glostrup, Denmark). Detection of anti-IL-2 was performed using ZytoChem Plus AP polymer anti-rabbit (Zytomed Systems GmbH, Berlin, Germany) and developed with BCIP/NBT-chromogen (ScyTek Laboratories, Inc., Logan, UT, USA). Next, the slides were denatured with H_2O for 10 minutes at 95°C, followed by incubation with the second primary anti-c-kit antibody. Detection was performed using ZytoChem Plus HRP One-Step Polymer anti-mouse/rabbit/rat (Zytomed Systems GmbH) and developed with AEC Chromogen System (Cavendish Scott Ltd). Specimens were also stained with IL-33 mAb (Nessy-1) (Enzo, Lausen, Switzerland). Skin specimens were observed under Olympus BX41 microscope (Olympus America Inc.). In all specimens, irrelevant isotype-matched antibodies were used to exclude non-specific staining. No staining was observed in these procedures.

2.14 | Flow cytometry

For the detection of $CD4^+CD25^+Foxp3^+$ T cells, splenic-enriched stimulated T cells were collected, washed once with PBS and stained by using mouse regulatory T cell staining kit (eBioscience, San Diego, CA, USA) as per the manufacturer's instructions. Briefly, cells were first incubated with Abs directed against CD4 and CD25 for 30 minutes at 4°C before cell permeabilization and incubation with anti-Foxp3 (FJK-16s). All experiments also included staining with the appropriate isotype control (IgG2a PE). Fluorescent staining was analysed using the MACSQuant® Analyzer (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany).

2.15 | Statistical analysis

Results are expressed as mean ± SE of at least 3 experiments done in duplicate. Student's paired *t*-tests were used to analyse the differences between cohorts. An effect was considered statistically significant when a *P*-value was equal to or less than .05.

3 | RESULTS

3.1 | The release of IL-2 from MCs is induced by IL-33 and is further augmented by IgE

A number of stimulators were examined for their ability to induce IL-2 release from BMMCs. As shown in Figure 1A, IL-33 induced robust production of IL-2, whereas stimulation of the high-affinity IgE receptor (FcεRI) by cross-linking with IgE and antigen led to only modest IL-2 secretion, as previously reported.[7] We then evaluated the kinetics of IL-2 release induced by IL-33 over a period of 48 hours (Figure 1B). Incubation of BMMCs with IgE and antigen did not cause noticeable IL-2 release over time in spite of successful MC activation (demonstrated by TNF-α release; not shown). On the other hand, IL-2 concentration steadily increased following incubation of BMMCs with IL-33. Even though IgE did not appear to considerably induce MC-IL2 production, its markedly elevated levels in oxazolone dermatitis and in AD,[5] suggested that it may modulate the effect of IL-33 on BMMCs. Consequently, we observed that IL-2 mRNA expression (Figure 1C) and release (Figure 1D) were significantly augmented by IL-33 and FcεRI costimulation in comparison to IL-33 alone, thus proving the modulatory effect of IgE on the action of IL-33. In order to verify that BMMCs represent their in vivo

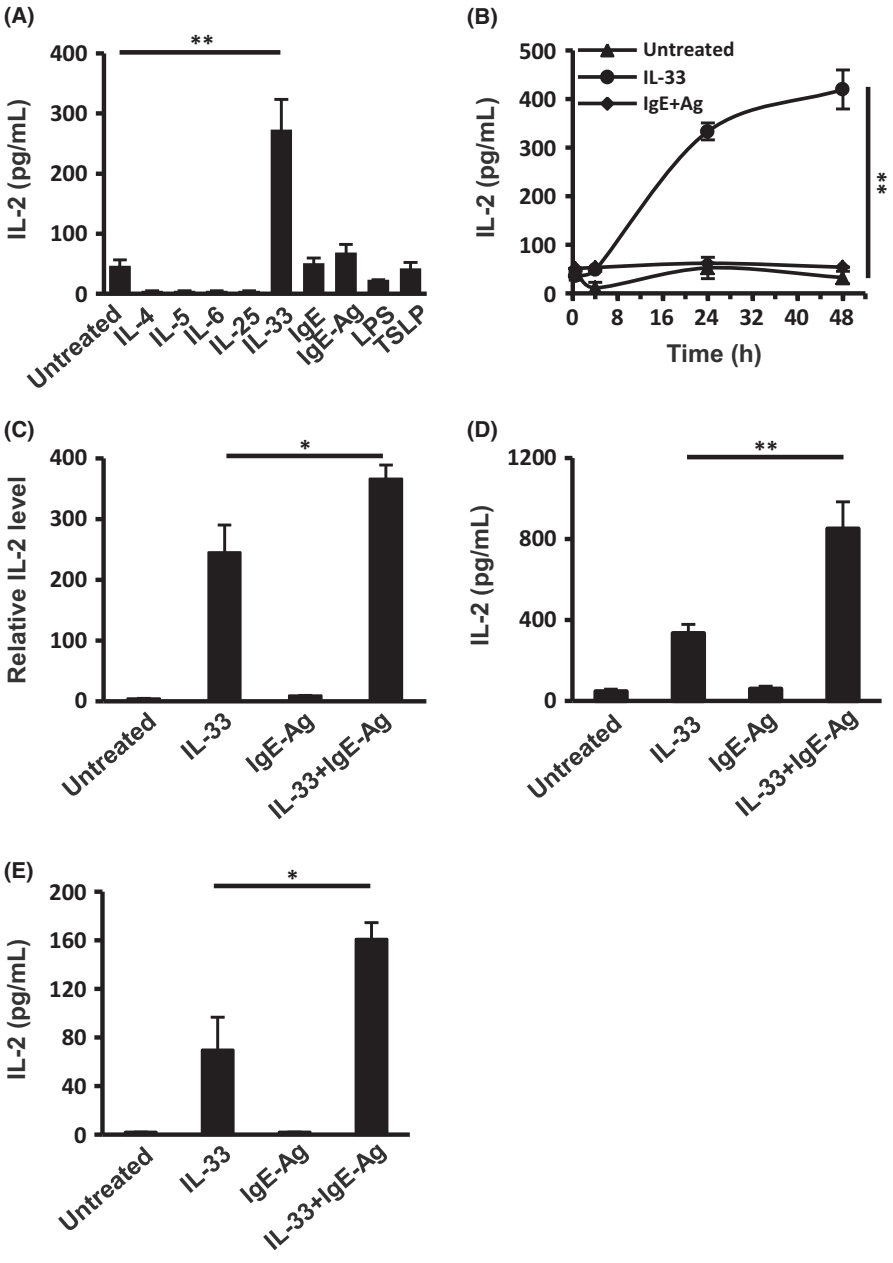

FIGURE 1 IL-2 release by MCs is induced by IL-33 and further enhanced by IgE antigen. A, BMMCs were incubated with indicated stimuli for 24 h. IL-2 release was measured by ELISA. B, Time course of IL-2 release from untreated or stimulated BMMCs as indicated (IL-33, 100 ng/mL). C, BMMCs were stimulated with IL-33 (100 ng/mL), IgE antigen, or both for 4 h and IL2 mRNA levels were analysed by RT-PCR (normalized to GAPDH levels). IL-2 release from BMMCs (D) or FSMCs (E) at 24 h was determined by ELISA. Results are shown as mean ± SE of 5 independent experiments done in duplicates (*$P < .05$; **$P < .01$)

counterparts, we purified primary FSMCs and analysed their response to stimulation. A marked increase in IL-2 release was found following incubation of FSMCs with IL-33 (Figure 1E), which was considerably enhanced by IgE and antigen costimulation, similar to the response observed in BMMCs (Figure 1D).

3.2 | Involvement of MAPKs in IL-2 production by MCs

It has recently been reported that the mitogen-activated protein kinases (MAPKs) extracellular signal-regulated kinase (ERK), p38 and Jun N-terminal kinase (JNK) are implicated in IL-13 and IL-6 release upon activation of murine MCs by IL-33.[12] Based on this observation, we investigated the possible involvement of the MAPK signalling pathway in IL-2 production by BMMCs. Inhibitors for ERK, JNK and p38 (PD98059, curcumin and SB202190, respectively) were introduced to cell cultures 30 minutes prior to the addition of the stimulants, and IL-2 release was measured after 18 hours of incubation. Consequently, JNK and p38 inhibitors significantly suppressed IL-2 release in IL-33 stimulated cells with or without FcεRI costimulation (Figure 2A). Conversely, ERK inhibition caused only negligible suppression. These results indicate that JNK and p38, but not ERK, are crucial for IL-33-induced MC production of IL-2.

Having found that JNK and p38 are pivotal players, we further investigated their response in activated MCs. IL-33 induced substantial phosphorylation of p38 and JNK within 5 minutes of stimulation, still enhanced after 30 minutes (Figure 2B). However, stimulation with IgE and antigen induced robust phosphorylation within 5 minutes, which declined at 30 minutes. Finally, IL-33 and IgE-antigen costimulation appears to have an additive effect on phosphorylation patterns, more evident in JNK than in p38 (Figure 2B).

3.3 | MC-derived IL-2 following IL-33 and IgE-antigen stimulation supports Treg expansion

IL-2 has been reported as an essential factor for the development, homeostasis and function of Tregs.[13] We therefore investigated whether IL-2 released by combined IL-33 and FcεRI cross-linking stimulation of BMMCs could support Treg expansion in vitro. Enriched splenic T cells were incubated overnight in conditioned media from naïve or stimulated BMMCs. Conditioned media obtained from IL-33-stimulated BMMCs led to an increase in the population of $CD4^+CD25^+Foxp3^+$ Tregs as compared with conditioned media from untreated or IgE-antigen-stimulated BMMCs (Figure 3). Costimulation of BMMCs by both IL-33 and IgE-antigen further enhanced this proportion, comparable to stimulation of T cells with recombinant IL-2. Addition of neutralizing anti-IL-2 mAb to conditioned media significantly abrogated Treg expansion. Taken together, these findings demonstrate that IL-2 produced by MCs stimulated with IL-33 and FcεRI cross-linking supports Treg expansion.

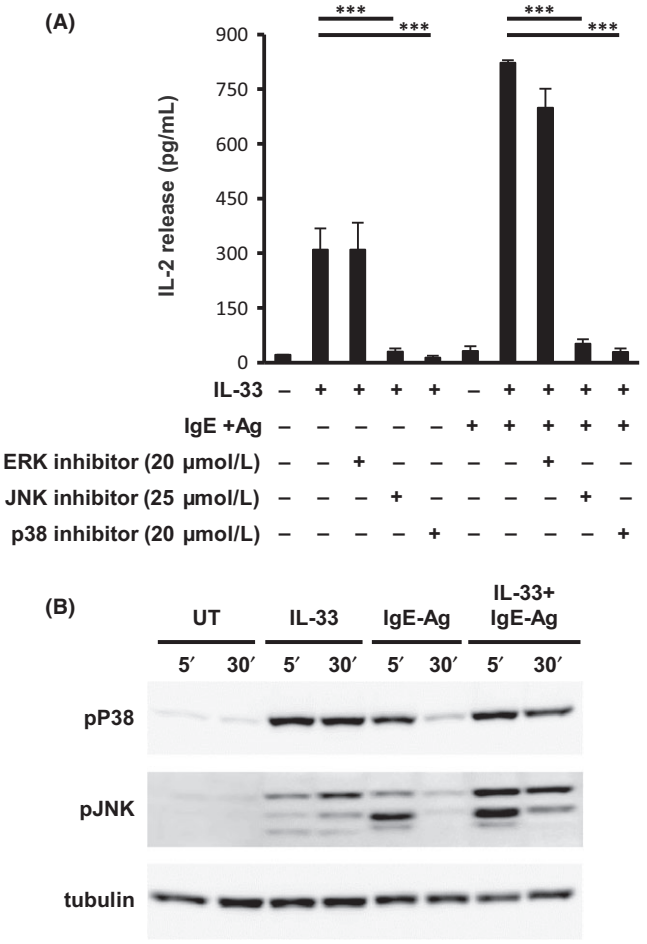

FIGURE 2 The involvement of MAPK in IL-2 release. A, BMMCs were pre-incubated for 30 min with inhibitors of ERK1/2 (PD98059), JNK (curcumin) and P38 (SB202190), prior to stimulation. After 24 h, IL-2 release was measured by ELISA. Results are shown as mean ± SE of 3 independent experiments done in duplicates (***$P < .001$). B, BMMCs were treated as shown for 5 or 30 min, and levels of phosphorylated p38 and JNK were analysed by immunoblotting. This is a representative of 3 different experiments

3.4 | IL-33 expression and IL-2-positive MCs in mouse and human skin with dermatitis

We investigated whether IL-33 expression is increased in oxazolone-induced chronic allergic dermatitis. For this purpose, ear homogenates were prepared, and a twofold increase in IL-33 was detected in mice with dermatitis as compared to control animals (Figure 4A). Furthermore, histological evaluation of skin sections showed an inflammatory response along with ear thickening and increased IL-33 expression in both the epidermis and dermis of oxazolone-treated mice (Figure 4B, lower panels). In untreated ears, however, only low IL-33 expression could be observed in the dermis (Figure 4B, upper panels). MC density in the dermis of mice with dermatitis was increased as well, thus colocalizing with the territory of increased IL-33 staining. Immunofluorescence staining confirmed that MCs in the dermis were IL-2-positive (Figure 4C). In human subjects, it is well known that MC numbers increase in skin affected

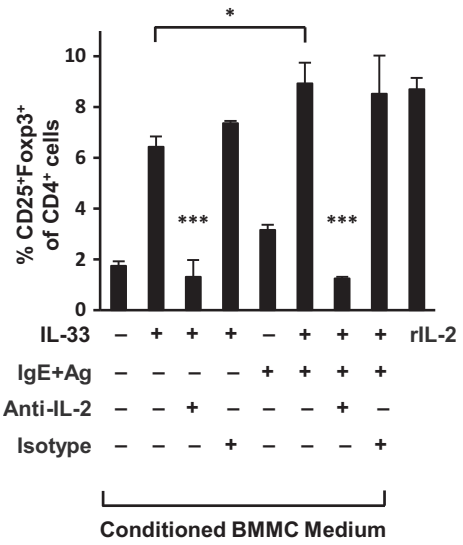

FIGURE 3 Treg expansion in response to MC-derived IL-2 induced by IL-33 and IgE-antigen. Splenic murine T cells were cultured for 24 h with conditioned media from BMMCs stimulated as indicated. Neutralizing anti-murine IL-2 or isotype control antibodies were added to the conditioned media 30 min prior to incubation, as specified. The percentage of $CD25^+Foxp3^+$ Tregs from $CD4^+$ T cells were determined by flow cytometry. Results are shown as mean ± SE of 5 independent experiments (*$P < .05$; ***$P < .001$)

with chronic dermatitis.[2] However, there are no data indicating whether these MCs express IL-2 as demonstrated in the murine model. To address this question, human skin sections, which had been previously given the histological diagnosis of "chronic inflammation," were histochemically analysed. Both intracellular and extracellular IL-33 expression was increased in the dermis of the chronically inflamed skin, in comparison with normal skin (upper panels of Figure 4D). Furthermore, c-Kit^+IL2^+MCs were observed in the dermis of dermatitis sections more than in normal skin (lower panels of Figure 4D). These results indicate that, in the setting of chronic inflammation, the human skin demonstrates increased IL-33 staining as well as IL-2-positive MCs.

4 | DISCUSSION

We have previously reported that MC-derived IL-2 is critical for Treg stimulation and suppression of chronic skin inflammation. This finding was described in a model of oxazolone-induced dermatitis, which involves typical allergic characteristics that parallel those of AD in human subjects. These features include, among others, increased production of IgE, proliferation and stimulation of MCs, and Th2-type cytokines. In search for conditions that favour MC-IL2 production, we screened several mediators implicated in the progression of AD.[14] Of these, IL-33 was found to be the most potent stimulator. Furthermore, even though the effect of IgE per se is minor, it considerably augments the action of IL-33. IL-33 is a cytokine that belongs to the IL-1 family and induces primarily Th2-type immune responses. It is expressed by stromal cells such as epithelial and endothelial cells present in barrier tissues such as the skin, gut and lungs. There is evidence that IL-33 is released during cell injury and necrosis, serving as an "alarmin," with a distinct impact on immune cells, including MCs.[15,16] Murine MCs were previously shown to respond to IL-33 stimulation by increased cytokine production.[12,17] In human subjects, IL-33 is increased in skin lesions of patients with AD, and thus has been thought to take part in the pathogenesis of this disease.[3,16]

Costimulation of BMMCs by IL-33 and FcεRI cross-linking, as shown herein, was previously reported to induce cytokines other than IL-2. This collaboration was shown to increase IL-6 and IL-13 release from BMMCs,[17] as well as up-regulation of IL-8 (CXCL8) and leucotrienes in primary human MCs.[18] Although FcεRI cross-linking can potentially up-regulate IL-33 production,[12,19] such an autocrine mechanism is unlikely in our study since stimulation of BMMCs via FcεRI resulted only in negligible IL-2 release. Of note, BMMCs are produced by in vitro differentiation of progenitor cells and may exhibit functional differences from mature tissue MCs. This issue was addressed in the present study by verifying that the response of BMMCs to IL-33 and IgE/antigen is identical to that of primary FSMCs (Figure 1E). Further, our previous report showed that reconstitution of mast cell-deficient mice with BMMCs dampened dermatitis and restored the immunological response observed in wild-type mice.[7]

IL-33 induces intracellular signalling by binding to a heterodimeric receptor consisting of membrane-bound ST2L and IL-1 receptor accessory protein (IL-1RAcP). This interaction, in turn, leads to NF-κB and MAPK activation.[20,21] Here, we demonstrate the involvement of the MAPK signalling pathway in MC-IL-2 production following IL-33 stimulation. This conclusion is drawn from the profound suppression of IL-2 release obtained with p38 and JNK inhibitors. The involvement of this pathway was further supported by the evaluation of phosphorylation patterns. Although IL-33 and FcεRI cross-linking both lead to MAPK phosphorylation, they appear to do so in a different temporal manner. IL-33 induces an early and sustained phosphorylation of p38 and JNK. Conversely, IgE-antigen leads to a transient early p38 and JNK phosphorylation, peaking at 5 minutes and declining at 30 minutes. Based on these observations, we hypothesize that the 2 distinct patterns of intracellular phosphorylation caused by IL-33 and FcεRI complement each other to enhance IL-2 production.

Our data also demonstrate that FcεRI cross-linking enhances the capacity of IL-33-stimulated BMMCs to support Treg expansion in an IL-2-dependent manner (Figure 3). Interestingly, a recent report demonstrated that lung MCs express IL-2 and stimulate Tregs following papain- or IL-33-induced airway inflammation.[11] However, the airway disease model described involved non-antigen-specific acute inflammation, lacking allergic features such as IgE production.[11] Our results indicate that this effect is relevant to allergic inflammation, which is typically an antigen-specific response characterized by excessive IgE production. We conclude that elevated IgE titres contribute to IL-2 production and thereby to the increase in Tregs.

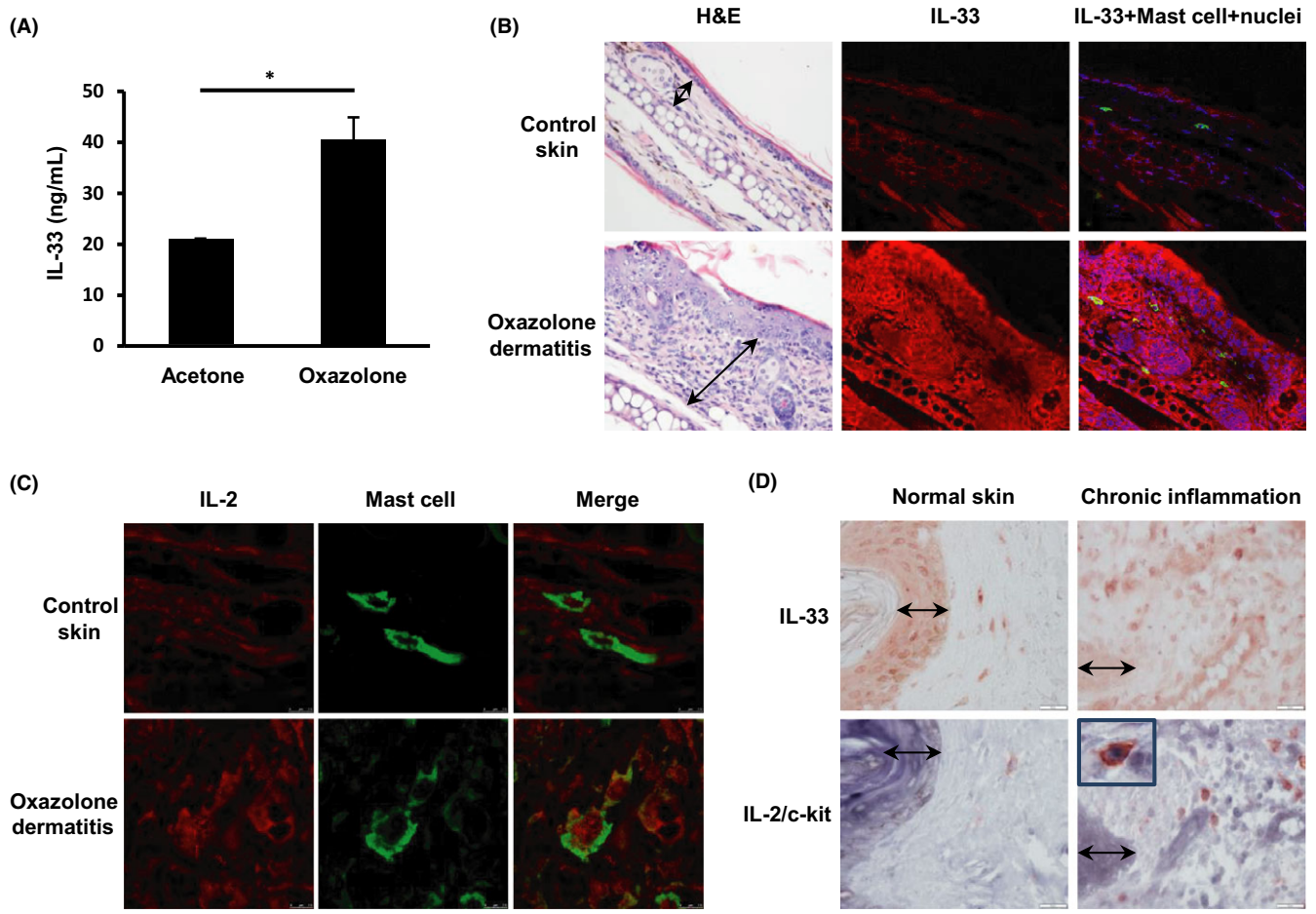

FIGURE 4 IL-33 and IL-2$^+$ MCs in tissue samples of chronic dermatitis. Oxazolone dermatitis was induced in C57BL/6 mice as previously described.[7] A, Ear homogenates of oxazolone-induced dermatitis and control mice were examined for their IL-33 content by ELISA. Results are shown as mean ± SE of 3 independent experiments (*$P < .05$). B, Tissue sections of dermatitis and control ears were stained with H&E, anti-murine IL-33 antibody (red), Avidin-Alexa Fluor® 488 (MCs, green) and Hoechst 33342 (blue). Representative ear sections are shown; bidirectional arrows, dermis layer. C, Sections were stained with Avidin-Alexa Fluor® 488 (MCs, green) and anti-murine IL-2 antibody (red). Scale bar = 7.5 μm. D, Paraffin-embedded cross sections of normal human skin tissue or skin samples with a pathologist-given diagnosis of chronic inflammation were stained as indicated (anti-c-kit, brown; anti-IL-2, purple). Bidirectional arrow, epidermis layer; inset, a single representative IL-2$^+$MC. This is a representation of 5 patients. Scale bar = 20 μm

Based on this study and previous reports, we propose that IL-33 has a dual pro- and anti-inflammatory effect, the latter mediated by MCs. Several observations support this hypothesis. Administration of IL-33 to murine ear skin by intradermal injections was found to induce marked ear swelling, with inflammatory cell infiltrates, which contained higher MC numbers in comparison with control ears.[22] In addition, transgenic mice expressing higher IL-33 levels in their ear skin developed spontaneous itchy dermatitis, with MC abundance in lesions and elevated blood levels of histamine and total IgE.[23] Here, we show that in oxazolone-induced chronic allergic dermatitis, ear swelling is associated with increased IL-33 content and IL-2-positive MC numbers within the dermis layer (Figure 4). The presence of MCs in an IL-33-rich milieu appears to underlie their ability to produce IL-2, as we have previously shown.[7]

We propose that the findings presented herein from the murine dermatitis model and in vitro studies apply to human subjects as well. Previous studies have shown that IL-33 mRNA and protein are substantially higher in skin lesions of patients with AD or with psoriasis compared with non-inflamed skin samples.[16] Furthermore, increased expression of IL-33 was found in both OVA- and staphylococcal enterotoxin B (SEB)-sensitized human AD skin.[3] Here, we present increased IL-33 in chronically inflamed human skin (Figure 4), with colocalization of IL-2-positive MCs, implying that this regulatory mechanism takes place in humans.

In summary, the data presented in this work offers a mechanism for the skewing of MC function towards disease suppression in the setting of allergic skin inflammation. The abundance of IL-33 and its infiltration within the dermis, along with copious amounts of IgE found during chronic disease, are robust stimulators of IL-2 production by MCs. This outcome, in turn, supports Treg activity. These observations shed light on the complexity of disease mechanism, which involves an anti-inflammatory role played by MCs, IL-33 and IgE, traditionally known for their pro-inflammatory contributions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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