



Over-expression of IL-33 leads to spontaneous pulmonary inflammation in mIL-33 transgenic mice

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ABSTRACT

IL-33 plays an important role in inflammatory diseases including hypersensitive diseases like asthma, autoimmune diseases like rheumatoid arthritis, cardiovascular diseases like heart failure and neurodegenerative diseases like Alzheimer's disease. Here we reported the generation of an IL-33 transgenic mouse, in which mouse IL-33 full-length cDNA was controlled under the CMV promoter. The transgenic IL-33 was released as a cleaved form with molecular weight of 18 kDa in pulmonary, nephritic, cardiac and pancreatic tissues in transgenic mice and the pI of 18 kDa peptide was about pH 3–5 on the 2D PAGE which was similar with the activated peptide of IL-33. Histological analysis showed massive airway inflammation with infiltration of eosinophils around bronchi and small blood vessels, hyperplasia of goblet cells and accumulation of mucus-like material in pulmonary tissue of transgenic mice. An increase of IL-5, IL-8, IL-13 and IgE was detected in bronchoalveolar lavage fluid (BALF) of transgenic mice, which are inflammatory factors. These findings suggest transgenic IL-33 could be cleaved and secreted in an activated form and play an important role in the pathogenesis of pulmonary inflammation.

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1. Introduction

IL-33 was initially described as 'nuclear factor from high endothelial venules' (NF-HEV) because of its nuclear localization [1]. Over-expression study suggested its role in transcriptional repression *in vitro* [2], but the exact function of endogenous IL-33 in the nucleus has not been established. More importantly, IL-33 was identified as an extra cellular ligand for the orphan receptor ST2 [3]. IL-33 binding to the ST2 receptor on targeted cells can trigger the secretion of inflammatory cytokines [4]. More and more evidences show that IL-33 plays an important role in inflammatory diseases: hypersensitive diseases like asthma [5,6], autoimmune diseases like rheumatoid arthritis [7], cardiovascular diseases like heart failure [8] and neurodegenerative diseases like Alzheimer's disease [9]. Genetic analysis has located some SNP sites around human IL-33 and ST2 genes and correlated these sites with inflammatory diseases [10–12]. These findings paved the way to unravel the role of IL-33 in inflammatory diseases.

IL-33 contains an IL-1-like cytokine fold in its carboxyl-terminus and chromatin binding domain in amino-terminus. Without a sig-

nal peptide for secretion, Schmitz assumed the cleavage of IL-33 by caspase-1 between the two domains, and the truncated peptide (amino acid 112–270 in human IL-33 and amino acid 109–266 in mouse IL-33) was the mature cytokine [3]. But this hypothesis now meets challenges from a series of recent researches. Firstly, caspase-1 was not necessary for the release and cleavage of IL-33 [13,14]. Secondly, IL-33 may be cleaved at Asp 178 by caspase-3, and this cleavage suppressed IL-33 cytokine activity in apoptotic cells [15,16]. Finally, the full-length IL-33 can directly bind to its ST2 receptor, and the cleavage at Ser 111 was not necessary for its activity [15,16]. For better understanding of this contravention, endogenous study is needed.

The exogenous administration of recombinant IL33 (rIL-33) induced severe airway inflammation in mice [3,17,18]. In these reports, the mice were daily treated with 0.4–4 µg rIL-33 intraperitoneally. While the physiological and pathological concentration of IL-33 is between 30 and 100 pg/mg in mouse pulmonary tissue [19]. The differences at concentration and distribution of IL-33 between physiological or pathological condition and exogenous models may affect the evaluation of the IL-33 in inflammation. In this study, an IL-33 transgenic mouse model was created. Up regulation of cleaved peptide of IL-33 at molecular weight (MW) 18 kDa can be detected in pulmonary, nephritic, cardiac and pancreatic tissues. The increase of inflammatory cytokines, the infiltration of

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inflammatory cells can be seen in pulmonary tissue of transgenic mice. Here, we show that release of cleaved IL-33 promotes airway inflammation in transgenic mice.

2. Materials and methods

2.1. Animals

The proposals using animals were approved by the Institutional Animal Care and Use Committee of the Chinese Institute of Laboratory Animal Science (GC-08-2018) and the mice were hosted in AAALAC accredited facility. The transgenic founders (F0) mice were mated with wild-type C57BL/6J mice to produce F1 generation and IL-33 expression was detected in F1 transgenic mice. The F1 transgenic lines were mated with wild-type C57BL/6J mice to produce F2 mice, which were used for phenotypic analysis.

2.2. Plasmid construction and preparation of transgenic mice

The full-length mouse IL-33 (mIL-33) cDNA was cloned from neonate C57BL/6J mouse tissue by reverse transcription PCR (RT-PCR) using a kit from Invitrogen and confirmed by sequencing analysis. The primers were: sense 5'-GCAAGCTTCATGAGACCTAGAATGAAGTAT-3', antisense 5'-CGGGATCCTTTA CTGCATTAGATTTTCGAG-3'. The PCR was conducted using 35 cycles under the following conditions: denaturation at 94 °C for 30 s, annealing at 63 °C for 30 s and extension at 72 °C for 70 s, to amplify the desired 829 bp fragment of the mouse IL-33 gene. The mIL-33 cDNA was inserted downstream of the CMV promoter in the vector pCDNA3.1(+) between BamHI and XhoI cloning sites. The completed plasmid was linearized by Endonuclease BglII digestion. The entire transgenic expression cassette, comprised of the CMV promoter, mouse IL-33 full-length cDNA and BGH polyA signal was not disrupted. The DNA fragments were gel-purified, dissolved in Tris-HCl-EDTA at a final concentration of 5 ng/μl, and were injected into the pronucleus of fertilized zygotes harvested from C57BL/6J mice, using conventional methods for generation of transgenic mice [20].

2.3. Genomic PCR for genotyping identification

Mouse genomic DNA was extracted from tail biopsies [21]. Genomic PCR was performed in a 20 μl reaction system containing 1 μg genomic DNA by the same primers and conditions previously employed for IL-33 gene cloning. The primers can amplify the 829 bp of the IL-33 ORF, which was spliced from 9 kb of IL-33 gene. The endogenous IL-33 gene did not affect the genotyping of the transgenic mice. PCR products were examined by electrophoresis on 1% agarose gel.

2.4. Determination of copy number of transgene

The copy number of the transgene was detected in the method reported by Randy and Tom [22]. Briefly, standard curves were generated with IL-33 transgenic construct in a concentration gradient of 0.0001–0.1 ng/μl. Mouse genomic DNA from F1, F2 and F3 generation was diluted to 50 ng/μl. Real-time PCR was performed under the same condition used for standard curves with the Roche Light Cycler Instrument following the instructions for the SYBR Green Real Time PCR Master Mix (Qiagen). The reactions were cycled 40 times with denaturation at 94 °C for 5 s, annealing at 57 °C for 5 s and extension at 72 °C for 20 s per cycle. The fatty acid binding protein of the intestine (Fabpi) was used as the internal controls with primers of 5'-TGGACAGGACTGGACCTCTGCTTCCTAGA-3' and 5'-TAGAGCTTTGCCACATCACAGGTCATTAG-3' [23].

2.5. Western blot

Protein samples of lung, kidney, heart, pancreas, brain, liver, intestine and spleen had been extracted from 2-month-old mice in RIPA buffer containing NP-40 by homogenitor at 20,000 rpm for 2 min, and then centrifuged at 12,000 rpm at 4 °C for 30 min. The supernatant was incubated with loading buffer in boiling water for 5 min, and subsequently separated on SDS-PAGE. The proteins were transferred to a nitrocellulose (NC) membrane (Millipore) and were probed with polyclonal goat anti-mouse IL-33 antibody (R&D Systems). The primary antibody was detected with HRP-conjugated polyclonal rabbit anti-goat IgG antibody (Pierce). HRP-conjugated anti-β-actin (Boster, China) was used as normalization. The NC membrane was visualized using ECL (Santa Cruz) and exposed to X-ray film (Kodak).

2.6. 2D PAGE

Protein samples for 2D PAGE analysis were harvested from mouse pulmonary tissues in a protein extraction solution (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 2% NP-40). The tissue samples were homogenized on ice with homogenitor at 20,000 rpm for 2 min, and then kept for 30 min on ice. The homogenates were centrifuged at 12,000 rpm at 4 °C for 30 min, 60 μg samples were mixed with the same volume of IEF loading buffer (9.5 M urea, 2% NP-40, 10% 2-mercaptoethanol, 2% ampholyte) for each assay. Each sample was loaded onto a strip (pH 3–10). Isoelectric focusing was carried out on a Bio-Rad protean IEF cell using the following program: 200 V, linear ramp for 30 min; 300 V, linear ramp for 30 min; 450 V, linear ramp for 30 min; 600 V, linear ramp for 30 min; 800 V, linear ramp for 16 h. The strips were equilibrated with equilibration buffer (60 mM Tris-HCl, pH 6.8, 5% SDS, 10% glycerol, 5% 2-mercaptoethanol) for 30 min at room temperature. The strips were run in second dimension on 12% SDS-PAGE. The proteins were transferred to the same NC membrane. Western blot analysis was also carried out on 2D PAGE.

2.7. Histological analysis

Lung, brain, heart, spleen, liver, intestine, kidney and pancreas from 2-month-old animals were fixed in neutral buffered formalin. The tissues were dehydrated in alcohol gradient then embedded in paraffin and sliced at 4 μm of thickness. Hematoxylin and eosin (HE) staining and Periodic acid-Schiff (PAS) staining (for pulmonary tissue only) were performed on these slices.

2.8. Bronchoalveolar lavage

The mice were sacrificed and the bronchoalveolar lavage fluid was collected through three rinses with 0.6 ml PBS/rinse (NaCl 137 mM, KCl 2.7 mM, Na₂HPO₄ 4.3 mM, KH₂PO₄ 1.4 mM, pH 7.4). All of the bronchoalveolar lavage fluid was collected together and centrifuged at 4 °C, 400 × g for 5 min. The supernatant (BALF) was used for ELISA analysis. The precipitated cells of the fluid were counted by Wright's staining.

2.9. ELISA

The concentration of IL-33, soluble ST2, total IgE in BALF were analyzed by direct ELISA. The BALF was diluted to 1:5 with coating buffer (Na₂CO₃ 28.6 mM, NaHCO₃ 71.4 mM, pH 9.6) and distributed (100 μl/well) in 96-well EIA/RIA plates. The plates were then kept at 4 °C overnight. PBS was used as systematic control for BALF. After washing three times with PBST (Tween-20 0.05% in PBS), the wells were blocked with blocking solution (BSA 3% in PBS, 200 μl/well) at room temperature for 45 min, and then washed three times

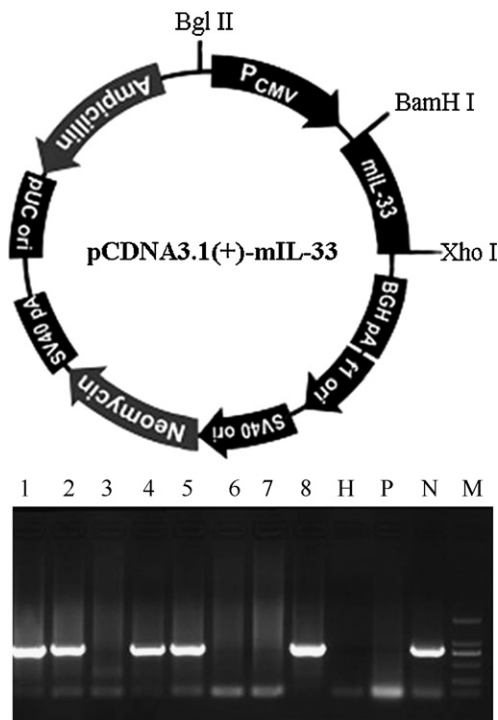


Fig. 1. IL-33 expression vector and genotyping of transgenic mice. The ORF of mIL-33 was linked into vector pCDNA3.1(+) by BamHI/XhoI to generate the transgenic construct (A); The transgenic mice were genotyped by PCR (B). Lines 1, 2, 4, 5 and 8 were 5 transgenic founders of #14, #50, #55, #63 and #79. Lines 3, 6 and 7 were non-transgenic littermates. Lines H and N were blank control and negative control. Line P was the transgenic construct as positive control. Line M is DNA marker DL2000.

with PBST. Primary antibodies against IL-33 (0.1 μ g/ml), against ST2 (AnaSpec, rabbit polyclonal antibody, 1 μ g/ml) and against mouse IgE (Biolegend, biotin conjugated Rat monoclonal antibody, 0.2 μ g/ml), each diluted with blocking solution, were then

added to the wells (100 μ l/well). After incubation at room temperature for 2 h, the primary antibodies were aspirated off and the wells were washed three times with PBST. Secondary antibodies (Pierce, HRP-conjugated rabbit anti-goat IgG polyclonal antibody, 1:10,000; HRP-conjugated goat anti-rabbit IgG polyclonal antibody 1:10,000; HRP-conjugated avidin, 2 μ g/ml) dissolved in blocking solution were then added (100 μ l/well) and incubated at room temperature for 1 h. After washing in the same manner, TMB (3,3',5,5'-tetramethylbenzidine) One Solution (Promega) was distributed (100 μ l/well) and the plates were incubated at 37 °C for 10 min. The enzyme reaction was terminated by the addition of 2 M H_2SO_4 (100 μ l/well), and absorbance at 450 nm was measured. The results were optimized by elimination of systematic background. IL-5, IL-8, IL-10, IL-13 were analyzed by ELISA kit from R&D Systems and Invitrogen company. These experiments were performed according to the kits instructions. Every sample was analyzed in triplicate.

2.10. Statistical analysis

Data are expressed as means $\pm$ SD. The differences were analyzed by Student's *t*-test using SPSS 13.0 for windows and *p* < 0.05 was considered significant.

3. Results

3.1. Transgenic construction and genotype of transgenic mice

The ORF of mIL-33 was amplified from neonate C57BL/6J mouse tissue and the sequence was consistent with GenBank record of mouse IL-33 mRNA (NM.133775). The mIL-33 ORF was linked into the plasmid pCDNA3.1(+) (Fig. 1A). The transgenic mice were created by microinjection of the linearized construct into the pronucleus of fertilized zygotes from C57BL/6J mice. Five transgenic founders (number #14, #50, #55, #63 and #79) were genotyped by PCR (Fig. 1B). The transgene copy number of founder #50 was detected in mice from F1, F2 and F3 generations separately as

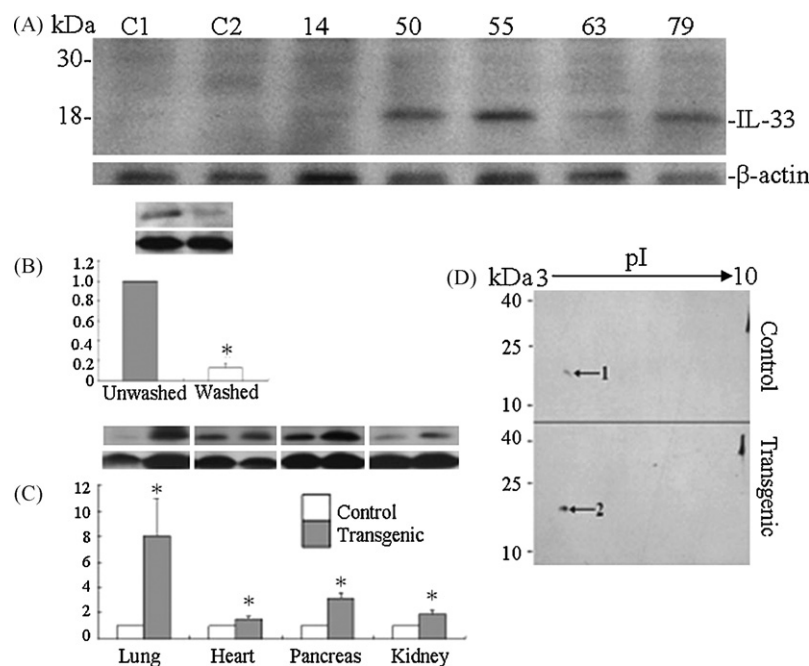


Fig. 2. IL-33 expression in different tissues. Five transgenic mice from different founders (#14, #50, #55, #63, #79) and 2 non-transgenic littermates (C1 and C2) were used in target protein expression screen by Western blot (A). The occurrence of IL-33 in unwashed and washed pulmonary tissue from transgenic mice of line #50 were compared by Western blot and evaluated by density analysis (B, *n* = 3, *p* < 0.05). The expression of transgenic IL-33 was also detected in heart, pancreas and kidney (C, *n* = 3, *p* < 0.05). The 18 kDa IL-33 peptides in non-transgenic control and transgenic mice were separated on 2D PAGE and their isoelectric point ranges were detected by western bolt (D).

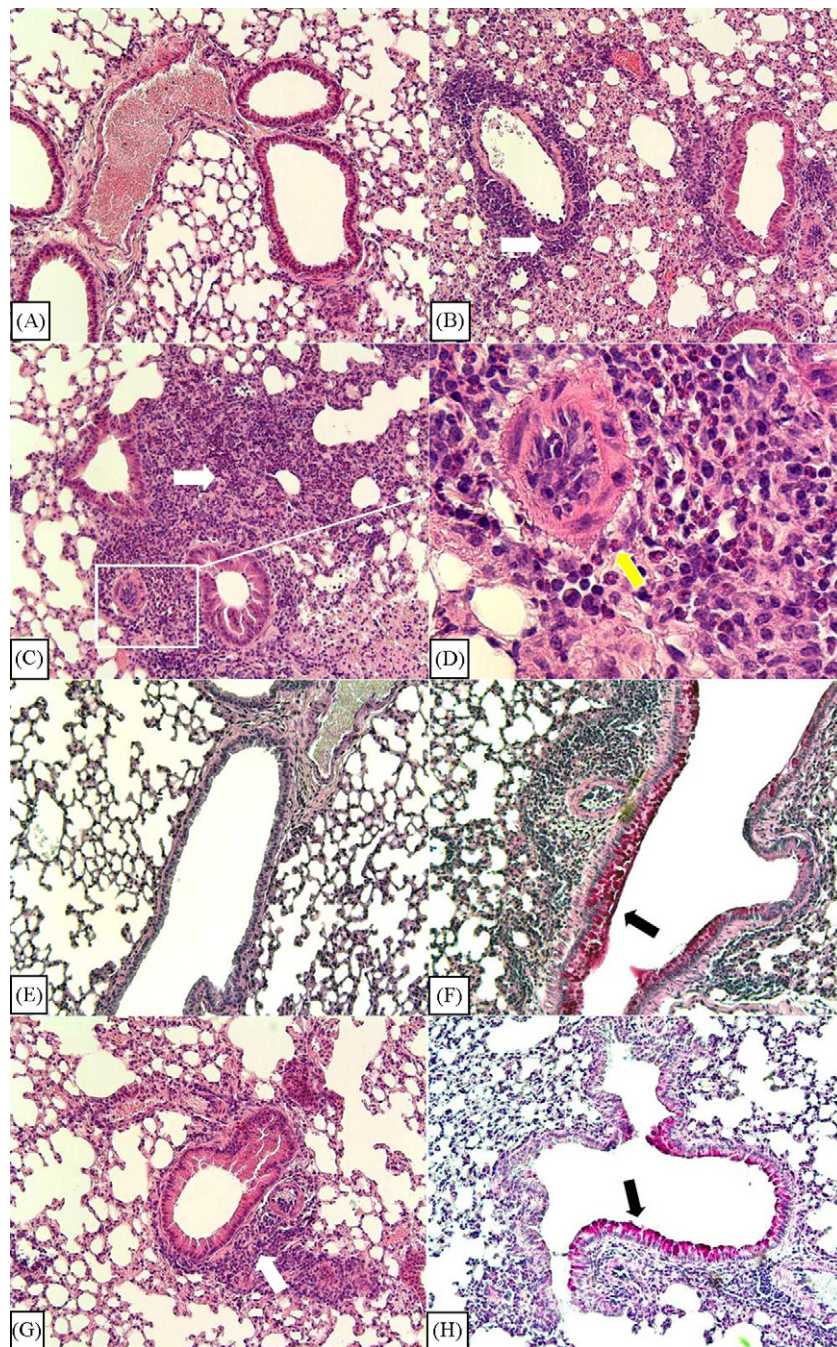


Fig. 3. Histological changes in pulmonary tissue. The pulmonary tissues from the two transgenic lines and non-transgenic littermates were stained with methods of HE (A–D and G) and PAS (E, F and H). A and E were the non-transgenic control. B–D and F were the line #50. G and H were the line #55. The pulmonary inflammations around the blood vessel and small bronchus were marked with white arrow. The eosinophili's infiltration was marked with yellow arrow. The hyperplasia of goblet cells and accumulation of mucus-like material were marked with black arrow. Original magnifications of A–C and E–H were 100 $\times$ and D was 400 $\times$.

16.5 $\pm$ 2.5, 17.2 $\pm$ 3.1 and 17.9 $\pm$ 3.7 (n = 4) suggesting that the transgene was stable during the passage of the transgenic mice.

3.2. The expression of transgenic mL-33 in mouse

The expression of mL-33 in pulmonary tissues from mice of F1 generation was detected by Western Blot analysis to screen the over-expression lines. The obvious expression of the mL-33 at MW 18 kDa was detected in mice from founders of #50, #55 and #79 compared with that of wild-type littermates (Fig. 2A) and the founders of #50 and #55 were selected for this study. The expression of predicated protein at MW 30 kDa can also be detected, but

there were no significant difference between transgenic and control mice. The most of the over-expressed IL-33 peptide was 18 kDa of molecular weight, which could be washed off by rinsing the pulmonary tissues (Fig. 2B, n = 3, p < 0.05). This result suggested that 18 kDa peptide was presented in extracellular space. Two-month-old mice of F2 generation from founder #50 were used for subsequent expression pattern analysis of the transgenic gene in different tissues and the over-expression of 18 kDa mL-33 were detected in the tissues of lung, heart, pancreas, and kidney (Fig. 2C, n = 3, p < 0.05), but not in the tissues of brain, liver, intestine, and spleen. The similar expression pattern was also detected in founder #55 (data not shown). To verify the isoelectric point character of IL-

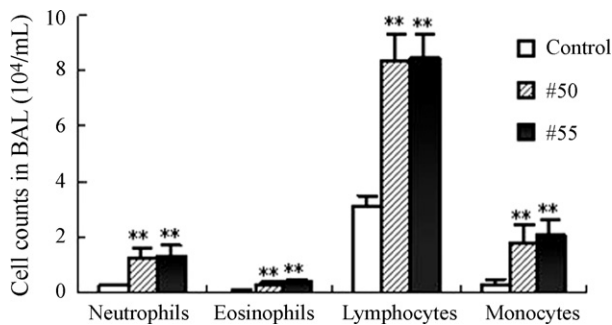


Fig. 4. Cell counts in BAL. Cells populations in BAL of non-transgenic control group and the two transgenic groups (#50 and #55) were counted by Wright's staining ($n=6$, $**p<0.01$).

33 of 18 kDa, two-dimensional gel electrophoresis was carried out (Fig. 2D). The positive IL-33 points located at the isoelectric point range pH 3–5.

3.3. The histological changes in the pulmonary tissue of IL-33 transgenic mice

The birth-rates, body weights, and sex ratios of line #50 and line #55 were recorded ($n=60$). Both of the two lines showed no difference compared with that of C57BL/6 wild-type mice. The gross anatomic and histological analysis were performed on the tissues of heart, liver, spleen, kidney, pancreas, intestine and brain from mice of line #50 and line #55 at 2-month-old. No obvious changes were observed compared with that of non-transgenic littermates. The histological changes of lung tissue were analyzed with HE staining and PAS staining from transgenic and non-transgenic littermates at 2-month-old. The inflammatory foci were observed around the blood vessels and the bronchi and the infiltration of mononucleated and multinucleated inflammatory cells were mainly assembled around airways and blood vessels in transgenic mice but no in control group (Fig. 3B, C and G). The multinucleated inflammatory cells were mainly eosinophils in inflammatory foci (Fig. 3D). The goblet cell hyperplasia and mucus accumulation were also observed in the transgenic mice with PAS staining, but no in control group (Fig. 3F and H).

3.4. The increase of inflammatory cells in BAL of IL-33 transgenic mouse

The cell population in BAL was isolated from the mice of F2 generation at 2-month-old and counted under a light microscope (Fig. 4). Two transgenic lines (#50 and #55) were used. Compared with non-transgenic littermates, the neutrophils were increased about 4.6-fold ($n=6$, $p<0.01$), the eosinophils were increased by 7.2-fold ($n=6$, $p<0.01$), the lymphocytes were increased about 2.7-fold ($n=6$, $p<0.01$) and the monocytes were increased about 6.2-fold ($n=6$, $p<0.01$) in both of the two transgenic lines. The results indicated that over-expression of IL-33 results in the accumulation of inflammatory cells in the airway of IL-33 transgenic mice.

3.5. The increased levels of inflammatory factors in BALF of IL-33 transgenic mouse

In BALF, the levels of IL-33, soluble ST2, total IgE and inflammatory factors including IL-5, IL-13, IL-8 and IL-10 were detected with ELISA (Fig. 5). Compared with non-transgenic littermates, the levels of IL-33 was increased about 5-fold ($n=6$, $p<0.01$), the level of soluble ST2 was increased about 2.6-fold ($n=6$, $p<0.01$) and total IgE was increased about 2-fold ($n=6$, $p<0.01$) in the two transgenic

lines. The levels of IL-5, IL-13 and IL-8 were increased about 38-fold, 21-fold and 20-fold ($n=6$, $p<0.01$) in the two transgenic lines compared with that of non-transgenic littermates. The IL-10 was also increased about 4.3-fold ($n=6$, $p<0.05$), which is an inflammation inhibitory factor.

4. Discussion

IL-33 plays an important role in inflammatory diseases including hypersensitive diseases like asthma [5,6], autoimmune diseases like rheumatoid arthritis [7], cardiovascular diseases like heart failure [8] and neurodegenerative diseases like Alzheimer's disease [9]. In this paper, we generated the mIL-33 transgenic mice to detect the effect of over-expressed IL-33 on inflammation and the endogenous cleavage of IL-33 in mice. In IL-33 mice, the over-expression of transgenic mIL-33 can be detected in lung, heart, pancreas and kidney at molecular weight of 18 kDa (Fig. 2A and C). The over-expressed 18 kDa peptide in pulmonary tissues could be washed off (Fig. 2B) and its pI was in pH 3–5 (Fig. 2D). The full-length IL-33 can be cleaved at Leu108 by caspase-1 to form the mature cytokine that is the peptide of 109–266 amino acid residues with MW 18 kDa in mice [3]. Some other proteases like calpain may participate the maturation of IL-33 [24], and the size of the peptide could be between 18 and 22 kDa [13,24]. IL-33 also can be cleaved at asp175 by caspase-3 to form an inactivated peptide of 1–175 amino acid residues also with MW 18 kDa [15,16]. The isoelectric point characters of the two kinds of peptide with MW 18 kDa were calculated by the composition of the amino acids with software Editseq 5.00 (DNASTAR Inc.). The pI of the activated peptide of 109–266 amino acid residues was pH 4.36, while pI of the inactivated peptide of 1–175 amino acid residues was pH 9.21. Therefore, the over-expressed 18 kDa IL-33 peptide in the IL-33 mice was the activated IL-33 and could be released into extracellular space.

The histological analysis of IL-33 mice revealed the pulmonary inflammation with infiltration of inflammatory cells around airways and blood vessels (Fig. 3A–D and G) and goblet cell hyperplasia and mucus accumulation in the airway of IL-33 mice (Fig. 3E, F and H). The pathologic changes in lung of the IL-33 mice were similar to the changes induced by exogenous administration of rIL-33 in mice [3,17,18]. The splenomegaly and pathological changes in digestive tract were not observed in IL-33 mice, which occurred in mice administrated with exogenous rIL-33 in dose of 0.4–4 μ g of rIL-33 daily for 3 days to a few weeks [3,17,18]. However, the IL-33 was about 30 pg/mg in pulmonary tissue in physiological condition [19], and the IL-33 was increased about 5 folds in the mIL-33 transgenic mice (Fig. 5). The differences at concentration and distribution of IL-33 between the transgenic mice and exogenous administration could be the reason that splenomegaly and pathological changes in digestive tract were not observed in transgenic mice.

In IL-33 mice, eosinophils, neutrophils and monocytes were present in greater quantity and percentage in BAL (Fig. 4). Increased expression levels of IL-5, IL-8 and IL-13 were detected in BALF (Fig. 5). These findings corroborated other researches on effect of IL-33 on allergic diseases [3,4,17]. The main cellular targets of the IL-33/ST2 signal pathway are Th2 cells, mast cells, basophils and eosinophils [25–29]. Some other cells like NKT cells may also be the target of IL-33 [30]. Besides the participation of Th2 cells, eosinophils play a central role in airway allergic diseases. Eosinophilic infiltration is a symptom in asthma, especially in severe asthma. Eosinophils play a key role in the initiation and propagation of inflammatory responses and therefore are major actors in the pathogenesis of inflammatory diseases. Recent research indicated that signaling through the IL-33-ST2 pathway

has an important role in eosinophil maturation, survival and activation [31], both by direct effects on eosinophils and indirectly through recruitment and regulation by Th2 cells. The SNP sites at IL-33 and ST2 had been associated with eosinophils count in inflammatory diseases [12]. Although neutrophils are not regarded as direct target cells of IL-33 because there is no ST2 receptor on its surface, through the recruitment of IL-8, IL-33 can promote neutrophil's accumulation.

As a pro-inflammatory cytokine, IL-33 promotes inflammatory response. There are, however, ways to neutralize IL-33 in order

to avoid or reduce severe inflammation. One way is through the soluble ST2 receptor, which may bind the soluble IL-33 and prevent it from interacting with the membrane-bound receptor on target cells. sST2 had been used to treat OVA challenged mice, to modulate IL-33's biological activity[32]. In IL-33 mice, with the increased levels of soluble IL-33, the sST2 in BALF was also up-regulated (Fig. 5). The increase of sST2 may functionally feed back to the increase of IL-33 to neutralize IL-33 in order to avoid or reduce severe inflammation. Moreover, there was an increase in IL-10 levels in IL-33 mice. As an anti-inflammatory cytokine [33], IL-10

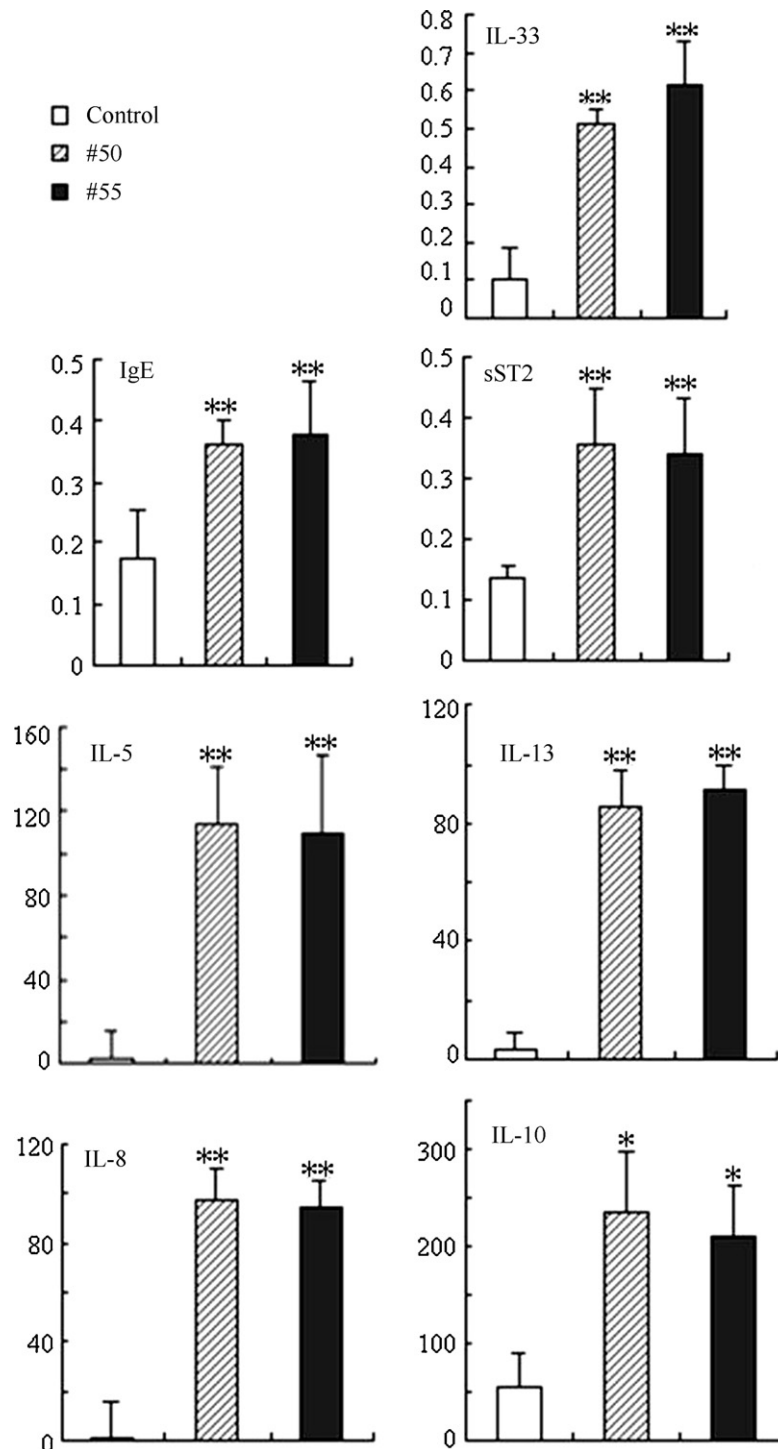


Fig. 5. ELISA of IL-33, sST2, IgE and Cytokines in BALF. The concentrations of IL-33, sST2, IgE, IL-5, 8, 10 and 13 in BALF were detected by ELISA. The IL-33, sST2, IgE were shown in OD ratios and the concentrations IL-5, 8, 10, 13 were shown in the pg/ml ($n=6$, * $p<0.05$; ** $p<0.01$).

may work as another way to counterbalance the over-expression of IL-33.

Collectively, our findings suggest transgenic IL-33 could be cleaved and secreted in an active form and play an important role in the pathogenesis of pulmonary inflammation.

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References

- [1] Baekkevold ES, Roussigne M, Yamanaka T, Johansen FE, Jahnsen FL, Amalric F, et al. Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules. *Am J Pathol* 2003;163:69–79.
- [2] Carriere V, Roussel L, Ortega N, Lacorre DA, Americh L, Aguilar L, et al. IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo. *Proc Natl Acad Sci USA* 2007;104:282–7.
- [3] Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, 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;23:479–90.
- [4] Chackerian AA, Oldham ER, Murphy EE, Schmitz J, Pflanz S, Kastelein RA. IL-1 receptor accessory protein and ST2 comprise the IL-33 receptor complex. *J Immunol* 2007;179:2551–5.
- [5] Smith DE. IL-33: a tissue derived cytokine pathway involved in allergic inflammation and asthma. *Clin Exp Allergy* 2009;40:200–8.
- [6] Prefontaine D, Lajoie-Kadach S, Foley S, Audusseau S, Olivenstein R, Halayko AJ, et al. Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. *J Immunol* 2009;183:5094–103.
- [7] Matsuyama Y, Okazaki H, Tamemoto H, Kimura H, Kamata Y, Nagatani K, et al. Increased levels of interleukin 33 in sera and synovial fluid from patients with active rheumatoid arthritis. *J Rheumatol* 2009.
- [8] Bartunek J, Delrue L, Van DF, Muller O, Casselman F, De WB, et al. Nonmyocardial production of ST2 protein in human hypertrophy and failure is related to diastolic load. *J Am Coll Cardiol* 2008;52:2166–74.
- [9] Chapuis J, Hot D, Hansmannel F, Kerdraon O, Ferreira S, Hubans C, et al. Transcriptomic and genetic studies identify IL-33 as a candidate gene for Alzheimer's disease. *Mol Psychiatry* 2009;14:1004–16.
- [10] Matsuda A, Okayama Y, Terai N, Yokoi N, Ebihara N, Tanioka H, et al. The role of interleukin-33 in chronic allergic conjunctivitis. *Invest Ophthalmol Vis Sci* 2009;50:4646–52.
- [11] Sakashita M, Yoshimoto T, Hirota T, Harada M, Okubo K, Osawa Y, et al. Association of serum interleukin-33 level and the interleukin-33 genetic variant with Japanese cedar pollinosis. *Clin Exp Allergy* 2008;38:1875–81.
- [12] Gudbjartsson DF, Bjornsdottir US, Halapi E, Helgadottir A, Sulem P, Jonsdottir GM, et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat Genet* 2009;41:342–7.
- [13] Talbot-Ayer D, Lamacchia C, Gabay C, Palmer G. Interleukin-33 is biologically active independently of caspase-1 cleavage. *J Biol Chem* 2009;284:19420–6.
- [14] Ohno T, Oboki K, Kajiura N, Morii E, Aozasa K, Flavell RA, et al. Caspase-1, caspase-8, and calpain are dispensable for IL-33 release by macrophages. *J Immunol* 2009;183:7890–7.
- [15] Luthi AU, Cullen SP, McNeela EA, Duriez PJ, Afonina IS, Sheridan C, et al. Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases. *Immunity* 2009;31:84–98.
- [16] Cayrol C, Girard JP. The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. *Proc Natl Acad Sci USA* 2009;106:9021–6.
- [17] Kondo Y, Yoshimoto T, Yasuda K, Futatsugi-Yumikura S, Morimoto M, Hayashi N, et al. Administration of IL-33 induces airway hyperresponsiveness and goblet cell hyperplasia in the lungs in the absence of adaptive immune system. *Int Immunol* 2008;20:791–800.
- [18] Kurowska-Stolarska M, Kewin P, Murphy G, Russo RC, Stolarski B, Garcia CC, et al. IL-33 induces antigen-specific IL-5+ T cells and promotes allergic-induced airway inflammation independent of IL-4. *J Immunol* 2008;181:4780–90.
- [19] Kearley J, Buckland KF, Mathie SA, Lloyd CM. Resolution of allergic inflammation and airway hyperreactivity is dependent upon disruption of the T1/ST2-IL-33 pathway. *Am J Respir Crit Care Med* 2009;179:772–81.
- [20] Hogan B, Costantini F, Lacy E. Manipulating the mouse embryo: a laboratory manual. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press; 1986.
- [21] Sambrook J, Fritsch E, Maniatis T. Molecular cloning: a laboratory manual. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press; 2001.
- [22] Rasmussen R, Morrison T, Herrmann M, Wittwer C. Quantitative PCR by continuous fluorescence monitoring of a double strand DNA specific binding dye. *Biochemistry* 1998;2:8–11.
- [23] Wang Y, Song Y, Liu Q, Liu C, Wang L, Liu Y, et al. Quantitative analysis of lentiviral transgene expression in mice over seven generations. *Transgenic Res*, doi:10.1007/s11248-009-9355-z.
- [24] Hayakawa M, Hayakawa H, Matsuyama Y, Tamemoto H, Okazaki H, Tominaga S. Mature interleukin-33 is produced by calpain-mediated cleavage in vivo. *Biochem Biophys Res Commun* 2009;387:218–22.
- [25] 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;113:1526–34.
- [26] 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;179:2051–4.
- [27] Iikura M, Suto H, Kajiura N, Oboki K, Ohno T, Okayama Y, et al. IL-33 can promote survival, adhesion and cytokine production in human mast cells. *Lab Invest* 2007;87:971–8.
- [28] Suzukawa M, Koketsu R, Iikura M, Nakae S, Matsumoto K, Nagase H, et al. Interleukin-33 enhances adhesion, CD11b expression and survival in human eosinophils. *Lab Invest* 2008;88:1245–53.
- [29] Suzukawa M, Iikura M, Koketsu R, Nagase H, Tamura C, Komiya A, et al. An IL-1 cytokine member, IL-33, induces human basophil activation via its ST2 receptor. *J Immunol* 2008;181:5981–9.
- [30] Bourgeois E, Van LP, Samson M, Bourgeois E, Van LP, Samson M, et al. The pro-Th2 cytokine IL-33 directly interacts with invariant NKT and NK cells to induce IFN-gamma production. *Eur J Immunol* 2009;39:1046–55.
- [31] Cherry WB, Yoon J, Bartemes KR, Iijima K, Kita H. A novel IL-1 family cytokine, IL-33, potently activates human eosinophils. *J Allergy Clin Immunol* 2008;121:1484–90.
- [32] Hayakawa H, Hayakawa M, Kume A, Tominaga S. Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation. *J Biol Chem* 2007;282:26369–80.
- [33] Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, O'Garra A. IL-10 inhibits cytokine production by activated macrophages. *J Immunol* 1991;147:3815–22.