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Endogenous IL-33 Is Highly Expressed in Mouse Epithelial Barrier Tissues, Lymphoid Organs, Brain, Embryos, and Inflamed Tissues: In Situ Analysis Using a Novel IL-33–LacZ Gene Trap Reporter Strain

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IL-33 (previously known as NF from high endothelial venules) is an IL-1 family cytokine that signals through the ST2 receptor and drives cytokine production in mast cells, basophils, eosinophils, invariant NKT and NK cells, Th2 lymphocytes, and type 2 innate immune cells (natural helper cells, nuocytes, and innate helper 2 cells). Little is known about endogenous IL-33; for instance, the cellular sources of IL-33 in mouse tissues have not yet been defined. In this study, we generated an IL-33–LacZ gene trap reporter strain (Il-33Gt/Gt) and used this novel tool to analyze expression of endogenous IL-33 in vivo. We found that the IL-33 promoter exhibits constitutive activity in mouse lymphoid organs, epithelial barrier tissues, brain, and embryos. Immunostaining with anti–IL-33 Abs, using Il-33Gt/Gt (IL-33-deficient) mice as control, revealed that endogenous IL-33 protein is highly expressed in mouse epithelial barrier tissues, including stratified squamous epithelia from vagina and skin, as well as cuboidal epithelium from lung, stomach, and salivary gland. Constitutive expression of IL-33 was not detected in blood vessels, revealing the existence of species-specific differences between humans and mice. Importantly, IL-33 protein was always localized in the nucleus of producing cells with no evidence for cytoplasmic localization. Finally, strong expression of the IL-33–LacZ reporter was also observed in inflamed tissues, in the liver during LPS-induced endotoxin shock, and in the lung alveoli during papain-induced allergic airway inflammation. Together, our findings support the possibility that IL-33 may function as a nuclear alarmin to alert the innate immune system after injury or infection in epithelial barrier tissues. The Journal of Immunology, 2012, 188: 3488–3495.

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Abbreviations used in this article: dpc, days postconitum; DSS, dextran sulfate sodium; E, embryonic day; ES, embryonic stem; FRC, fibroblastic reticular cell; Gt, gene trap; HEV, high endothelial venules; α-SMA, α-smooth muscle actin; vWF, von Willebrand factor.

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infection (20). In agreement with this model, we and other investigators recently reported that full-length IL-33–270 is biologically active (23–25) and that it can be released in the extracellular space after cellular damage (23, 24). We also showed that processing of full length IL-33–1–270 by caspases results in its extracellular space after cellular damage (23, 24). We also showed (39), that processing of full length IL-33–1–270 by caspases results in its extracellular space after cellular damage (23, 24). We also showed (39), that processing of full length IL-33–1–270 by caspases results in its extracellular space after cellular damage (23, 24).

Although the expression profile of endogenous IL-33 has been relatively well characterized in normal human tissues (20, 21), comparatively little is known about the expression of endogenous IL-33 protein in mouse tissues during homeostasis. Immunohistochemical analysis of lung tissue from wild-type and IL-33–deficient mice revealed that IL-33 exhibits constitutive nuclear expression in the alveolar epithelium (26). High levels of IL-33 mRNA have been reported in other mouse epithelial barrier tissues (i.e., stomach, skin) and lymphoid organs (i.e., lymph nodes, spleen) (3), but the cellular sources of IL-33 protein in these tissues have not been characterized. Similarly, nothing is known about the expression of IL-33 during embryogenesis. In the current study, we generated a novel IL-33–LacZ gene trap (Gt) reporter strain (IL-33–Gt/Gt) and used this mouse model to analyze IL-33 promoter activity in mouse adult tissues and embryos. In addition, we investigated expression of the IL-33 protein in mouse tissues by immunohistofluorescence, using IL-33–Gt/Gt mice (IL–33–deficient mice) as control. These analyses indicated that endogenous IL-33 is highly expressed in mouse epithelial barrier tissues, lymphoid organs, brain, embryos, and inflamed tissues. The results also revealed the existence of important species-specific differences between humans and mice, with a striking lack of IL-33 expression in mouse blood vessels during homeostasis.

Materials and Methods

Generation of C57BL/6:Il-33–LacZ Gt mice (IL-33Gt)

C57BL/6 mice were obtained from Charles River Laboratories. IL-33–Gt/Gt mice were generated by microinjecting an embryonic stem (ES) cell clone provided by Texas A&M Institute for Genomic Medicine (IST10946B6-TigmGt). The ES clone contains a unique Gt insertion in the first intron of the Il-33 locus. The locus has been disrupted by insertion of the Omnibank Gt Vector 76 where two viral long terminal repeats frame a splice acceptor upstream of the β-galactosidase and neomycin resistance fusion gene. Sequencing of the Il-33 gene, 3076 bp upstream of the Gt reporter strain (IL-33/14B ocular. Bright-field images were captured with a Nikon Coolpix 950 digital camera. All images were processed using Adobe Photoshop CS2 software.

Western blotting

Lung homogenates were lysed in RIPA lysis buffer solution (400 mM NaCl, 20 mM Tris [pH 7.4], 10 mM EDTA, 0.1% SDS, 1% Triton, 0.05 Tween 20) supplemented with a protease inhibitor mixture tablet (Roche). The supernatants were collected after centrifugation (16,000 × g) at 4°C for 10 min. Lung lysates were analyzed by SDS-PAGE and blotted. Membranes were blocked and incubated with goat anti-mouse IL-33 (1/1000; R&D Systems) and donkey anti-goat, HRP-conjugated (1/100,000; Promega) polyclonal Abs. The immunoreactive proteins were visualized with ECL plus reagents (ECL Western blotting Detection Reagents; Amersham).

β-Galactosidase detection

β-Galactosidase detection with the chromogenic substrate X-Gal (X-Gal staining) was done overnight on 10-μm cryosections of wild-type and mutant tissue staining kit (InvivoGen). Cryosections were counterstained by nuclear Fast Red (Sigma) and mounted with Coversave medium (Microm). Fifty-micrometer vibratome brain sections and embryo, liver, or lung whole-mount staining were realized on freshly dissected tissues, fixed for 2 or 1 h, respectively, with 4% paraformaldehyde; washed in PBS; permeabilized for 3 h in PBS, 2 mM MgCl2, 0.02% Nonidet P-40, and 0.01% sodium deoxycholate at 4°C; and stained from 30 min to overnight using a LacZ Tissue staining kit (InvivoGen). Clearing of embryos was done as described previously (27).

Immunohistofluorescence

Immunohistofluorescence was performed on formalin-fixed mouse tissues. Five-micrometer paraffin-embedded sections were deparaffinized in Histoclear (National Diagnostics) and rehydrated in graded alcohol series. After rehydration, the paraffin sections were boiled in a microwave oven for 5 min followed by epitope retrieval in Sodium Citrate Buffer (10 mM [pH 6]) for 20 min. Sections were equilibrated in PBS and incubated with blocking solution MAXblock (Active Motif) for 1 h at room temperature. Polyclonal goat Ab anti-mouse IL-33 (1/200, AF326; R&D Systems), polyclonal rabbit Ab anti-Sox2 (1/100, AB5603; Millipore), rat mAb anti-mouse CD3 (1/20, clone 1D3; 120, clone S231; DiaNovia), rat mAb MECA-79 (1/100, clone MECA-79; Pharmingen), polyclonal rabbit Ab anti-von Willebrand factor (vWF; InvivoGen, clone MECA-79; Dako), and mouse mAb anti–endothelial nitric oxide synthase (SNM; 1/100, clone 1A4; Dako) diluted in PBS, MAXblock 20% were incubated overnight at 4°C. Sections were washed in PBS for 30 min and incubated with biotin-conjugated anti-goat DyLight 488 (1/200; Jackson ImmunoResearch), donkey anti-rat IgG DyLight 488 (1/200; Jackson ImmunoResearch), donkey anti-rabbit IgG DyLight 488 (1/200; Jackson ImmunoResearch), or goat anti-mouse Cy2 (1/200; Amersham) secondary Abs, respectively, for 1 h at room temperature. Sections were counterstained with DAPI and mounted in Mowiol.

Immunohistofluorescence on normal human tissues was performed using two sources of formalin-fixed paraffin-embedded human tissue microarrays, as previously described (20).

Image acquisition and processing

Fluorescent images were visualized using an inverted microscope Eclipse TE300 Nikon with 40×/0.75 and 100×/0.5–1.3 objectives at room temperature and captured with a DXM 1200 digital camera using Nikon ACT1 software. Bright-field images were visualized using an Eclipse 80i Nikon microscope with 4×/0.10 and 40×/0.75 objectives at room temperature and captured through a Digital Sight DS 5M L1 Nikon camera using DS Software. All images were processed using Adobe Photoshop CS2 software.

Results

Generation of an IL-33–LacZ Gt reporter strain: a new tool to analyze IL-33 expression in vivo

To further characterize the in vivo expression and function of IL-33, we activated the IL-33 gene using a Gt-inactivation strategy (Fig. 1A; Gt insertion in intron 1 of the IL-33 gene). The βgeo insertion disrupts production of the IL-33 protein and is useful to visualize the activity of the endogenous IL-33 promoter, through X-Gal staining (β-galactosidase activity). Mice heterozygous for the mutation (IL-33Gt/Gt) have been intercrossed, and viable homozygous IL-33 null mice (IL-33Gt/Gt) have been obtained, indicating that IL-
33 is not essential for embryonic development, health, and fertility, as recently reported for other lines of Il-33–deficient mice generated independently (18, 26). Loss of IL-33 protein in the Il-33Gt/Gt mice was validated by Western blot analysis of lung tissue extracts (Fig. 1B). X-Gal staining in IL-33Gt/Gt adult mice revealed constitutive activity of the Il-33 promoter in mouse lymphoid organs, with strong signals in the T cell areas of peripheral lymph nodes and spleen (Fig. 1C). Similar β-galactosidase activity was observed in Il-33+/Gt heterozygous mice (Fig. 1C). In contrast, no signals were observed in lymphoid organs from wild-type mice, indicating that the X-Gal staining in Il-33Gt/Gt mice was specific. We concluded that the Il-33–LacZ Gt mouse is a useful tool to analyze IL-33 expression in vivo.

Endogenous IL-33 is highly expressed in the nuclei of fibroblastic reticular cells but not detected in HEVs from mouse lymph nodes and spleen

We next performed immunofluorescence staining of mouse peripheral lymph node sections, using human lymphoid tissues as controls and Il-33–deficient mice (Il-33Gt/Gt) to validate the specificity of the immunostaining (Fig. 2). As previously reported (20), IL-33 was expressed in the nuclei of both HEV endothelial cells and α-SMA+ fibroblastic reticular cells (FRCs) in human lymph node and tonsil (Fig. 2A, 2C). Surprisingly, IL-33 expression was not observed in HEVs from wild-type mouse peripheral lymph nodes (Fig. 2B). In contrast, IL-33 was strongly expressed in α-SMA+ FRCs from mouse lymph nodes (Fig. 2D), similarly to human tonsil (Fig. 2C). Staining of α-SMA+ FRCs with anti–IL-33 Abs was specific because it was not observed in Il-33–deficient mice (Fig. 2E). Double staining with the DNA-binding dye DAPI indicated that IL-33 accumulates in the nuclei of α-SMA+ FRCs, with no evidence for cytoplasmic localization (Fig. 2F–H). Like in lymph nodes, IL-33 was constitutively expressed in the nuclei of α-SMA+ FRCs from mouse spleen (Fig. 2I–K). We concluded that IL-33 is constitutively and highly expressed in FRCs from mouse lymph nodes and spleen, but, contrary to humans, it is not expressed in HEVs.

Species-specific expression of IL-33 in blood vessels: IL-33 is not constitutively expressed in the nuclei of endothelial cells from mouse adult tissues

We then extended our analyses to nonlymphoid tissues. Strikingly, although IL-33 is constitutively expressed at high levels in the endothelium from normal human tissues (20), constitutive expression of IL-33 in endothelial cells was not observed in mouse tissues (Fig. 3). For instance, strong nuclear staining of blood vessel endothelial cells with anti–IL-33Abs was observed in human colon, lung, cervix, and breast but not in the corresponding mouse tissues (Fig. 3A). Although IL-33 was not constitutively expressed in endothelial cells along the vascular tree, some sporadic expression in a few blood vessels from adipose tissue was occasionally seen in some mice. This observation suggested that expression of IL-33 in mouse blood vessels could be induced under certain conditions. Accordingly, we observed strong nuclear staining of IL-33 in vWF+ endothelial cells in the inflamed colon during DSS-induced colitis (Fig. 3B), a model for inflammatory
bowl diseases, which was shown to be dependent (in part) on endogenous IL-33 (18). Together, these results revealed the existence of important species-specific differences in the regulation of IL-33 in blood vessels between humans and mice.

Endogenous IL-33 is highly expressed in mouse epithelial barrier tissues

X-Gal staining of nonlymphoid tissues from Il-33Gt/Gt adult mice revealed abundant expression of β-galactosidase in several epithelial tissues, including lung alveoli, stratified squamous epithelium of the vagina, and salivary gland ducts (Fig. 4). In contrast, no β-galactosidase activity was observed in the corresponding tissues from wild-type mice, which do not contain the Il-33–LacZ Gt. We concluded that the Il-33 promoter is constitutively active in epithelial barrier tissues. We then analyzed expression of IL-33 at the protein level by immunohistofluorescence with anti–IL-33 Abs (Fig. 5). Constitutive expression of IL-33 was observed in the nuclei of epithelial cells in tissues exposed to the environment, in agreement with previous observations in human tissues (20). The highest levels of IL-33 were observed in the vagina, skin, lung, stomach, and salivary glands. Epithelial staining in all of these tissues was specific, because it was not observed in the corresponding tissues from Il-33–deficient mice (Il-33Gt/Gt). Double staining of tissue sections with the DNA-binding dye DAPI revealed that IL-33 was always localized in the nuclei of the epithelial cells (Fig. 5). No evidence was found for cytoplasmic localization of IL-33 in murine adult tissues. Cytoplasmic staining with the anti–IL-33 Abs was only observed in the lamina propria from the inflamed colon during DSS-induced colitis, but this staining turned out to be nonspecific because it was still present in Il-33–deficient mice (Supplemental Fig. 1). We concluded that IL-33 is a nuclear cytokine constitutively expressed at high levels in epithelial barrier tissues from adult mice.

Constitutive expression of IL-33 in nervous tissues

In addition to lymphoid organs and epithelial barrier tissues, IL-33 was abundantly expressed in the brain and the eye (Fig. 6). X-Gal staining revealed abundant expression of β-galactosidase in the brain from Il-33Gt/Gt adult mice (Fig. 6B, 6D) compared with the brain from wild-type mice (Fig. 6A, 6C). Strong activity of the Il-33 promoter was observed in the corpus callosum, hippocampus, and retina. Double staining of tissue sections with anti–IL-33 (red) and anti-β-galactosidase Abs (green) revealed that IL-33 was expressed in the nuclei of epithelial cells in the brain, but not in the cytoplasmic compartment. DNA was counterstained with DAPI (blue). Scale bars, 10 μm.
Endogenous IL-33 expression in adult tissues and embryos

Endogenous IL-33 is highly expressed in epithelial barrier tissues. IL-33 is constitutively expressed in the nuclei of epithelial cells from mouse tissues exposed to the environment, including stratified squamous epithelia from vagina and skin, stratified cuboidal epithelium from salivary gland, and simple cuboidal epithelium from lung and stomach. Tissue sections from wild-type (Il-33+/+) and Il-33-deficient mice (Il-33<sup>−/−</sup>) were stained with anti–IL-33 Abs (red). DNA was counterstained with DAPI (blue). The background staining observed in tissues from Il-33<sup>−/−</sup> mice was also observed with the secondary Ab alone (Supplemental Fig. 2). Scale bars are as indicated.

Endogenous IL-33 is highly expressed in embryonic tissues

X-Gal staining in Il-33–LacZ Gt mice revealed constitutive activity of the Il-33 promoter during embryonic development (Fig. 5). β-galactosidase activity was already detected in embryonic day (E)11.5 d postcoitum (dpc) embryo in the caudal part along the anteroposterior axis in Il-33<sup>−/−</sup>Gt/Gt mice but not in control wild-type mice (Fig. 7A), and it was further increased and restricted at E15.5 to specific tissues of the embryo (Fig. 7B). Strong expression of the Il-33–LacZ reporter was detected in the mesenchyme surrounding the eye, in the nasal cavity, in the grooves separating the body, from the ora serrata to the iris (Fig. 6L, 6M). Original magnification ×10 (A–D). Scale bars, 50 μm (E–H, L, M), 10 μm (I–K). cc, corpus callosum; dg, dentate gyrus; Hip, hippocampus; th, thalamus.

Endogenous IL-33 is highly expressed in inflamed tissues

We then used the Il-33–LacZ Gt reporter strain to analyze IL-33 expression during inflammation. LPS-induced endotoxin shock is characterized by a systemic inflammatory response associated with dysfunction of multiple organs, including the liver and the lung. This LPS-induced systemic inflammatory response is substantially decreased in Il-33–deficient mice (18). Interestingly, we found that expression of endogenous IL-33 in the liver, which is not observed in the absence of inflammation, is highly induced around blood vessels during LPS-induced endotoxin shock (Fig. 8A). We then analyzed a model of lung inflammation (papain-induced airway inflammation), which was shown to be dependent on endogenous IL-33 (18). Papain is a protease allergen considered a cause of occupational asthma, which induces innate-type allergic airway inflammation in mouse (28). Papain-induced airway inflammation (eosinophilia) is profoundly impaired in Il-
33–deficient mice (18). In contrast to the upregulation of IL-33 expression in the liver during LPS-induced endotoxin shock, we observed that expression of endogenous IL-33 in the lung alveoli, which is already very high under basal conditions (Fig. 8B, see also Fig. 4), is not increased further during papain-induced airway inflammation. Together, these results indicated that the II-33–LacZ Gt mouse is a useful tool to analyze IL-33 expression in inflamed tissues.

Discussion

In this study, we show that endogenous IL-33 is constitutively expressed at high levels in mouse adult tissues and embryos with a very refined expression pattern. Using a novel II-33–LacZ Gt reporter strain (II-33Gt/Gt), we demonstrate that the II-33 promoter exhibits constitutive activity in mouse lymphoid organs (lymph node, spleen), epithelial barrier tissues (lung, skin, vagina), brain, and embryos. Strong expression of the II-33–LacZ reporter was also observed in inflamed tissues, in the liver during LPS-induced endotoxin shock, and in the lung alveoli during papain-induced allergic airway inflammation. IL-33–producing cells in tissues were further characterized by indirect immunofluorescence staining with anti–IL-33 Abs, using II-33Gt/Gt mice (II-33–deficient mice) as control to validate the specificity of the staining. We found that the IL-33 protein is highly expressed in the nuclei of epithelial cells from mouse epithelial barrier tissues, such as the vagina, skin, lung, stomach, and salivary glands. The IL-33 protein was also strongly expressed in the nuclei of FRCs from mouse lymphoid organs but was not found in HEVs. Strikingly, IL-33 expression was also not detected in blood vessels from other mouse tissues during homeostasis, revealing the existence of important species-specific differences between humans and mice. In addition to epithelial barrier tissues and lymphoid organs, we identified the eye as another major site of IL-33 expression in mouse tissues. Nuclear expression of the IL-33 protein was found in Sox2+ Müller glial cells from the retina and epithelial cells from the ciliary body. Finally, strong expression of the IL-33 protein was also observed in olfactory epithelium and some mesenchymal cells from E15.5 embryonic tissues. Interestingly, in some organs (i.e., lymph nodes, salivary glands), IL-33 was constitutively expressed from late-gestation embryo to adult life. Importantly, IL-33 protein was always localized in the nucleus of producing cells, with no evidence for cytoplasmic localization. Together, our data indicate that endogenous IL-33 is a nuclear cytokine constitutively expressed at high levels in mouse epithelial barrier tissues, lymphoid organs, brain, eye, and embryo.

We believe that the findings reported in this article support the alarmin function of IL-33, which we initially proposed based on the high levels of constitutively expressed IL-33 in human blood vessels and epithelial barrier tissues (20). Like in humans, IL-33 is highly expressed in the nuclei of epithelial cells of mouse tissues in contact with the environment (lungs, stomach, skin), where pathogens, allergens, and other environmental agents are frequently encountered. Thus, IL-33, as an epithelial alarmin, could play important roles in the response to tissue injury or infection. IL-33 is likely to be a very good alarm signal: it is constitutively expressed at high levels in tissues in vivo (this study, 20); it can be released after cellular injury or necrosis (23, 24); it is active as a full-length molecule that does not require maturation for biological activity (23–25); and it has the capacity to activate many actors of the innate immune system (4, 5, 29). Mast cells (30) and type 2 innate immune cell populations, such as natural helper cells (13, 14), nuocytes (15), and innate helper 2 cells (16), are likely to play major roles in the response to the IL-33 alarm signal in vivo. Indeed, IL-33 signaling through the ST2 receptor was recently shown to play an important role in the activation of these type 2 innate immune cell populations, after helminth infection in the intestine (13, 15, 16) or influenza virus infection in the lungs (14, 17).

![Figure 7](image_url)
**FIGURE 7.** Endogenous IL-33 is constitutively expressed in mouse embryonic tissues. (A and B) Constitutive activity of the II-33 promoter in mouse embryonic tissues. Whole-mount from II-33+/+ (A) and II-33Gt/Gt (A, B) E11.5 or E15.5 dpc embryos were stained with X-Gal to analyze II-33 promoter driving β-galactosidase expression in embryonic tissues, black arrows indicate areas of strong expression. Scale bars, 1 mm. (C) II-33 protein is constitutively expressed in the nuclei of embryonic cells, in the olfactory epithelium, and in mesenchyme from thoracic dorsal part. Tissue sections from E15.5 dpc wild-type embryos were stained with anti–IL-33 Abs (red). DNA was counterstained with DAPI (blue). The last row of images represents higher magnification of the areas boxed upstream. Scale bars, 1 mm (A, B); others as indicated.

![Figure 8](image_url)
**FIGURE 8.** Expression of the II-33-LacZ reporter in inflamed tissues. (A) II-33 promoter-driven β-galactosidase expression in the liver after LPS-induced endotoxin shock. Whole-mount and liver tissue sections from II-33Gt/Gt mice (control or LPS treated) were stained with X-Gal to analyze II-33 promoter activity in the inflamed liver. (B) II-33 promoter-driven β-galactosidase expression in the lung after papain-induced allergic airway inflammation. Whole-mount and lung tissue sections from II-33+/+ and II-33Gt/Gt mice (control or papain treated) were stained with X-Gal to analyze II-33 promoter activity in the inflamed lung. Results are representative of three independent experiments. Scale bars are as indicated.
We previously proposed that IL-33, which is abundantly expressed in human blood vessels along the vascular tree (1, 2, 20, 21), may play important roles in immune surveillance in humans as an endothelial alarmin responsible for alerting the immune system of blood vessel damage (23). Surprisingly, we discovered that IL-33 is not constitutively expressed in endothelial cells from blood vessels in mouse tissues. Particularly striking was the absence of IL-33 in HEVs from mouse lymphoid organs, because we discovered IL-33/NF from HEVs based on its abundant expression in human HEVs (1, 2). These species-specific differences in IL-33 expression along the vascular tree indicate that the endothelial alarmin function of IL-33 may be absent in mice. Although constitutive expression of IL-33 in blood vessels was not detected in mouse tissues, we observed inducible expression of IL-33 in the nuclei of endothelial cells from the inflamed colon during DSS-induced colitis. Expression of IL-33 in blood vessels from inflamed mouse tissues was also reported in experimental atherosclerosis (31) and mouse liver fibrosis (32). Differences in the regulation of endothelial cell gene expression of IL-33 between humans (constitutive) and mice (inducible) will need to be carefully considered when extrapolating results obtained in mouse models to humans.

We found that the IL-33 promoter is highly active in the T cell areas from mouse lymphoid organs and that the IL-33 protein is constitutively expressed in the nuclei of α-SMA+ FRCs from lymph nodes and spleen. T-zone FRCs produce high levels of cytokine IL-7 (33) and chemokines CCL21 and CCL19 (34), which are essential for lymphocyte survival and migration, respectively. Our results indicate that T-zone FRCs also constitute the major cellular sources of IL-33 in mouse lymphoid organs. FRC-derived IL-33 may play important roles in the activation of nuocytes and innate helper 2 cells, which are found in lymph nodes and spleen (15, 16). Because IL-33 expression was not detected in fibroblasts from nonlymphoid tissues, the constitutive expression of IL-33 in T-zone FRCs is likely to be linked to their myofibroblastic features, including the expression of α-SMA, which is associated with increased generation of contractile forces (35). Interestingly, IL-33 expression was also reported in α-SMA+ myofibroblasts associated with tissue fibrosis and/or inflammatory diseases, including cardiac myofibroblasts in cardiac hypertrophy and fibrosis (36), activated hepatic stellate cells in fibrotic liver (32), pancreatic myofibroblasts in chronic pancreatitis (37, 38), and ulceration-associated myofibroblasts in ulcerative colitis (39, 40). Thus, α-SMA+ myofibroblasts may represent a major cellular source of IL-33 in vivo.

Our in situ observations suggest that IL-33 may play important roles in the eye and the brain. We found that endogenous IL-33 protein is abundantly expressed in the eye, in the nuclei of Sox2+ Müller glial cells in the retinal intergranular layer, and in epithelial cells from the ciliary body. We also observed high levels of IL-33 promoter activity in corpus callosum, hippocampus, thalamus, and cerebellum. Interestingly, the IL-33 gene has been identified as a candidate gene for Alzheimer’s disease (41), and expression of IL-33 was shown to be induced in glial cells from the brain (glial fibrillary acidic protein+ astrocytes) after treatment with pathogen-associated molecular patterns (LPS or dsRNA) (42). IL-33 may play critical roles in innate immune responses in the brain and the eye, and it will be important to further characterize these roles in future studies.

In conclusion, we believe that the IL-33–LacZ Gt reporter strain is a useful tool to characterize the cellular sources of endogenous IL-33 in vivo during homeostasis and inflammation. Our data indicate that epithelial cells from barrier tissues constitute the major cellular sources of IL-33 in normal mouse tissues. Myoﬁbroblasts and endothelial cells may also be important sources of IL-33 in inflamed mouse tissues. Other cell types have been proposed to produce high levels of IL-33 in inflamed tissues, including alveolar macrophages (14), and the IL-33–LacZ Gt reporter strain will represent an important tool to validate and determine the relative importance of these additional cellular sources of IL-33 during inflammation. In our analyses, we observed induction of the IL-33–LacZ reporter in the liver during LPS-induced endotoxin shock but no upregulation in the lung during papain-induced airway inflammation. This observation, which is likely explained by the fact that expression of endogenous IL-33 in the lung alveoli is already very high under basal conditions, suggests that papain may act by modulating IL-33 release or bioactivity rather than IL-33 expression in the inflamed airways. The IL-33–LacZ Gt reporter strain will also be very useful to investigate the localization of endogenous IL-33 protein in vivo. For instance, although cytoplasmic expression of IL-33 protein was reported in some studies (43), we observed that cytoplasmic staining with anti–IL-33 Abs in the inflamed colon during DSS-induced colitis was still present in the IL-33–LacZ Gt reporter strain, which is IL-33 deficient.

The fundamental mechanisms of IL-33 synthesis, localization, and release during inflammation or infection remain to be fully characterized, and we are confident that the IL-33–LacZ Gt reporter strain will be a critical tool to answer these important questions.

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Disclosures

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