TOXOPLASMA GONDII

Th1-type cytokine-associated immune responses are important for protection against another protozoan, *Toxoplasma gondii*.[82] ST2 mRNA expression was upregulated, but IL-33 mRNA expression was not altered, in brain lesions of mice infected with *T. gondii* compared with naïve mice.[83] ST2-deficient mice showed high susceptibility to *T. gondii* in comparison with wild-type BALB/c mice.[83] On the other hand, susceptibility to this protozoan was similar in wild-type, IL-4-deficient and IL-4Ra-deficient mice.[83] These observations suggest that the IL-33-IL-33R pathway is important for protection against *T. gondii*, independently of IL-4 and IL-13 production.

NIPPOSTRONGYLUS BRASILIENSIS

Th2-type cytokines are important for protection against *Nippostrongylus brasiliensis* and *Trichinella spiralis*, nematode parasites.[84] However, IL-4 production by splenic CD4+ T cells and the serum levels of total IgG1 and IgE were normal in ST2-deficient mice on the 129 x B6 mixed background after *N. brasiliensis* infection.[21,85] On the other hand, the proportion of IL-5-producing CD4+ T cells and the number of eosinophils in the lung were decreased in ST2-deficient mice on the 129 x B6 mixed background during *N. brasiliensis* infection, although the eggs of this parasite were normally cleared in these mutant mice.[85] These observations suggest that the IL-33-IL-33R pathway contributes to eosinophilia induced by *N. brasiliensis* infection, but is dispensable for protection against parasitic nematodes.

SCHISTOSOMA MANSONI

Th2 cells are considered to be key effector cells in the immune responses to a helminth parasite, *Schistosoma mansoni*.[86,87] ST2-deficient mice on the 129 x B6 mixed background showed impaired granuloma formation, characterized by eosinophil infiltration, in the lungs after the first *S. mansoni* egg injection.[22] On the other hand, these mutant mice, which had been sensitized with *S. mansoni* eggs, showed normal pulmonary granuloma formation and serum IgG1 and IgE levels after the second egg challenge, although they showed reduced IL-4 and IL-5 production by mediastinal lymph node cells in response to *S. mansoni* egg antigens.[22] These observations suggest that the IL-33-IL-33R pathway is involved in Th2 cytokine production but not antibody production during infection with *S. mansoni*.

TRICHURIS MURIS

It is also known that Th2-type cytokines are important for protection against a parasitic nematode, *Trichuris muris*.[88] During *T. muris* infection in mice, it was shown that IL-33 mRNA expression was increased in the cecum,[89] suggesting a contribution of IL-33 to host defense against *T. muris*. Indeed, administration of IL-33 to AKR mice, but not SCID mice, resulted in accelerated parasite clearance during *T. muris* infection.[89] Although T cells and/or B cells are required for IL-33-mediated parasite expulsion, as seen in SCID mice during *T. muris* infection, IL-33 induced pathological changes such as increased crypt length and intestinal epithelial cell proliferation independently of T cells and/or B cells.[89] In those settings, NK cells were increased in mesenteric lymph nodes of IL-33-injected, *T. muris*-infected SCID mice, suggesting a contribution of NK cells to the T/B cell-independent IL-33-mediated pathological changes during *T. muris* infection.

PSEUDOMONAS AERUGINOSA

ST2 mRNA/protein and IL-33 mRNA expression were increased in local inflammatory lesions infected with a gram-negative bacterium, *Pseudomonas aeruginosa*.[90,91] Administration of soluble ST2 to mice resulted in aggravation of the keratitis induced by *P. aeruginosa*,[90] suggesting that the IL-33-IL-33R pathway is important for protection against *P. aeruginosa*.

MYCOBACTERIUM TUBERCULOSIS

It is known that both Th1 and Th17 cytokines are crucial for defense against a bacterium, *Mycobacterium tuberculosis*.[92] Even though PPD-specific IFN-γ production was increased in the culture supernatants of spleen cells from *M. tuberculosis*-infected ST2-deficient mice, the survival, bacterial counts, granuloma formation and pulmonary inflammation score were comparable between ST2-deficient and -sufficient mice during *M. tuberculosis* infection.[93] On the other hand, SIGIRR/TIR8-deficient mice showed high susceptibility to *M. tuberculosis* infection.[94] These observations suggest that the IL-33-IL-33R1 pathway is not crucial for *M. tuberculosis* pathogenesis, while the IL-33-IL-33R2 pathway is important for host defense against this pathogen.

LEPTOSPIRA

It was shown that the level of soluble ST2 was increased in plasma from patients with leptospirosis due to a gram-negative *Leptospira* infection and was associated with the severity of the disease, bleeding and mortality, suggesting a contribution of the IL-33-IL-33R pathway to the pathogenesis of leptospirosis.[95] However, the precise role of the IL-33-IL-33R pathway in this disease remains unclear.

VIRUSES

The serum levels of soluble ST2 protein were increased in patients infected with dengue virus.[96] In mice, IL-33 was increased in the brain after infection with Theiler's murine encephalomyelitis virus.[97] These observations suggest a contribution of the IL-33-IL-33R pathway to the pathogenesis of certain viral infections.

Inhalation of respiratory syncytial virus (RSV) by mice that had been sensitized with recombinant vaccinia virus expressing the attachment protein G of RSV resulted in induction of Th2-type cytokine-associated eosinophilic airway inflammation. On the other hand, inhalation of RSV by mice sensitized with recombinant vaccinia virus expressing the fusion protein F of RSV led to induction of non-eosinophilic airway inflammation. In this model, administration of anti-ST2 mAb (clone 3E10) attenuated the development of Th2-type cytokine-associated eosinophilic airway inflammation, but not non-eosinophilic airway inflammation.[98] These observations suggest that the IL-33-IL-33R pathway is crucial for the development of virus-induced, Th2-type cytokine-associated eosinophilic inflammation.

IL-33-IL-33R IN ANGIOGENESIS AND TU-MORGENESIS

IL-33 is constitutively expressed in nuclei of vascular endothelial cells in various human tissues such as the skin, small intestine, umbilical veins and lungs.[99] On the other hand, constitutive expression of IL-33 was not observed in human angiogenic tumor vessels.[99] Induction of IL-33 was observed during confluent growth of endothelial cells. On the other hand, expression of IL-33 was down-regulated during migration of those cells. Expression of nuclear IL-33 was rapidly down-regulated at the onset of angiogenesis during wound healing.[99] Proinflammatory cytokines (e.g., IL-1β and TNF) and proangiogenic growth factors (e.g., VEGF), which are induced during wound healing, suppressed IL-33 expression in endothelial cells.[99] In addition, IL-33 promoted angiogenesis by promoting proliferation, migration and differentiation of endothelial cells, and vascular permeability by reducing cell-cell interactions via cadherin.[100] These observations suggest that IL-33 may be involved in tumorigenesis and the development of vascular diseases.

IL-33-IL-33R IN ALLERGIC DISEASES

ASTHMA
The levels of soluble ST2 protein and IL-33 mRNA/protein are increased in sera and tissues from patients with asthma.[27,72,101-103] Genome-wide association studies identified polymorphism in the ST2 and/or IL-33 genes in patients with asthma, suggesting an association with susceptibility to asthma.[104-106] In support of that notion, intraperitoneal or intranasal administration of IL-33 to mice led to induction of eosinophilic inflammation in the pulmonary and intestinal mucosa through the IL-13 and STAT6-dependent pathway.[3,23]

The levels of soluble ST2 protein and IL-33 mRNA were increased in sera and/or lungs in a murine asthma model of airway inflammation induced by ovalbumin (OVA).[107,108] However, the roles of ST2

and IL-33 in the induction of OVA-induced airway inflammation in mice remain controversial. Respiratory function, airway pathology, eosinophil number in bronchoalveolar lavage fluids (BALFs) and/or the levels of serum total IgG1 and IgE were normal in 129 × B6 mixed and BALB/c background-ST2-deficient mice sensitized twice with OVA emulsified with alum (Table 2).[21,24,109] On the other hand, airway inflammation was attenuated in BALB/c background-ST2-deficient mice sensitized once with OVA/alum (Table 2).[24] Moreover, it is noteworthy that OVA-induced airway inflammation was exacerbated in SI-GIRR/TIR8-deficient mice.[15]

Several investigators reported the effect of an anti-ST2 mAb (clone 3E10) on OVA-induced airway inflammation in BALB/c mice (sensitized twice with OVA/Alum). That mAb enhanced ST2-expressing Th2 cell proliferation and cytokine production in vitro, indicating that it acts as an agonistic Ab, at least on Th2 cells.[110] Despite its agonistic activity on Th2 cells in vitro, BALB/c mice treated with 3E10 mAb showed attenuated airway inflammation in response to OVA (Table 3).[111,112] Likewise, Th2 responses during OVA-induced airway inflammation (sensitized twice with OVA/Alum) were reduced in mice carrying a soluble ST2 gene expression vector or treated with an anti-IL-33 polyclonal Ab (Table 3).[107,113]

Adoptive transfer with in vitro-skewed DO11.10 Th2 cells, which express OVA-specific T-cell receptors, in mice resulted in Th2 cytokine-dependent eosinophilic airway inflammation after intranasal OVA challenge.[114] BALB/c wild-type mice or BALB/c-Rag-1-deficient mice injected with ST2-deficient DO11.10 Th2 cells showed exacerbated airway function and inflammation after OVA challenge in comparison with mice injected with ST2-sufficient DO11.10 Th2 cells (Table 4).[109] These observations suggest that IL-33 signals on Th2 cells have a regulatory function in OVA-induced airway inflammation in that animal model. In contrast with that study using ST2-deficient DO11.10 Th2 cells, administration of 3E10 (anti-ST2) mAb or soluble ST2-Fc fusion protein to mice injected with DO11.10 Th2 cells showed attenuated airway function and inflammation after OVA challenge (Table 4).[19,111] If 3E10 mAb acted as an agonistic Ab for Th2 cells in vivo as well as in vitro, the phenotypes seen in mice treated with this mAb may be consistent with the study using ST2-deficient DO11.10 Th2 cells (Table 4),[109] but inconsistent with those treated with soluble ST2-Fc fusion protein (Table 4) or other studies (Table 2, 3). The apparent discrepancy between the study using ST2-deficient mice and mice treated with anti-ST2 and ST2-Fc fusion proteins remains to be explained. However, perhaps ST2-expressing Th2 cells, macrophages and other immune cells have different roles in the induction of allergic airway inflammation, because airway eosinophilia was observed even in mice deficient in and/or

81

Table 2 Airway inflammation in ST2-deficient mice

Reference	Experimental protocol	Observations
Hoshino K *et al*. J Exp Med 190, 1541, 1999	129 x B6 ST2-/-mice. Day 0, 12, 24 26 28 30 31 → Analyze. 50 μg OVA in 1 mg alum i.p. 10 mg/ml OVA aerosol 30 min, 3 times at 1-h intervals	- Normal eosinophil count in BALFs - Normal pulmonary inflammation - Normal total IgG1 and IgE in sera
Mangan NE *et al*. Eur J Immunol 37, 1302-1312, 2007	BALB/c ST2-/- mice. Day 0, 14, 28 29 30 32 → Analyze. 20 μg OVA in 2 mg alum i.p. 1% OVA aerosol, 20 min.	- Normal AHR (penh and GL) - Normal pulmonary inflammation (airway mucous cell hyperplasia, pulmonary fibrosis or peribronchial cell inflammation) - Normal OVA-specific cytokine secretion by mediastinal LN cells in vitro - Normal eosinophils, but reduced macrophages in BALFs
Kurowska-Stolarska M *et al*. J Immunol 181, 4780, 2008	BALB/c ST2-/-mice. Day 0, 8 9 10 11 → Analyze. 100 μg OVA in 2% alum i.p. 10 μg OVA I.n.	- Reduced eosinophils and macrophages in BALFs - Reduced IL-5, but not IL-4 or IL-13, levels in BALFs - Reduced pulmonary inflammation - Normal levels of IgG1 and IgE in sera
	BALB/c ST2-/-mice. Day 0, 12, 24 26 28 30 31 → Analyze. 100 μg OVA in 2% alum i.p. 10 μg OVA I.n.	- Normal responses

Table 3 Effects of anti-ST2 mAb (3E10), soluble ST2 and anti-IL-33 Ab on mouse airway inflammation

Reference	Experimental protocol	Observations
Coyle AJ *et al*. J Exp Med 190, 895, 1999	Male BALB/c-WT mice. 100 μg anti-ST2 mAb (clone 3E10), or control rat IgG1 1 h before OVA sensitization and challenge, route ??? → Analyze. Day 0, 7, 14, 21, 22. 20 μg OVA in 1.5 mg alum i.p. 10 mg/ml OVA aerosol, 1 hr.	- Reduced eosinophil count and IL-5 level in BALFs - Reduced OVA-specific serum IgE level
Kearley J *et al*. Am J Respir Crit Care Med 179, 772, 2009	BALB/c WT mice. 25 μg anti-ST2 mAb (clone 3E10) or control rat IgG i.v. → Analyze. Day 0 12, 18 19 20 21 22 23, 25 27 29 30. 10 μg OVA In alum i.p. 5% OVA aerosol 20 min.	- Reduced AHR, mucus secretion and ST2+ T cell infiltration in lungs - Reduced IL-4 and IL-13 levels in BALFs - Normal IL-33 levels in lung tissues
Oikawa K *et al*. Clin Exp Allergy 32, 1520, 2002	Female BALB/c-WT mice. Soluble ST2 gene transfer → Analyze. Day -1, 0, 6 7, 13 14, 15. 100 μg OVA in 2 mg alum i.p. 1% OVA aerosol, 30 min, twice	- Reduced eosinophils and IL-4 and IL-5 levels in BALFs - Reduced OVA-specific IL-4 and IL-5 production by spleen cells from OVA-sensitized mice in vitro
Liu X *et al*. BBRC 386, 181, 2009	Female BALB/c-WT mice. 150 μg anti-IL-33 polyclonal Ab, or control rabbit IgG I.p 30 min before OVA sensitization and challenge → Analyze. Day 0, 14, 25 26 27 28. 20 μg OVA in 2 mg alum i.p. 1% OVA aerosol, 30 min.	- Reduced eosinophils and lymphocytes, and IL-4, IL-5 and IL-13 levels in BALFs - Reduced pulmonary inflammation - Reduced total and OVA-specific IgE in sera

Table 4 Effects of anti-ST2 mAb (3E10) and soluble ST2 on airway inflammation in adoptive Th2 cell transfer model

Reference	Experimental protocol	Observations
Lohning M et al. Proc Natl Acad Sci USA 95, 6930, 1998		- Reduced eosinophils and IL-4 and IL-5 levels in BALFs
Coyle AJ et al. J Exp Med 190, 895, 1999		- Reduced IL-4, IL-5, IL-6 and IL-13, but not IL-10 or IFN-γ, in BALFs - Reduced eosinophil count in BALFs - Reduced Penh responses and pulmonary inflammation
Mangan NE et al. Eur J Immunol 37, 1302-1312, 2007		- Exacerbated AHR and lung inflammation - Increased eosinophils in BALFs - Increased IL-5 expression - No differences in lung IL-33 mRNA expression - Normal OVA-specific cytokine secretion by mediastinal LN cells in vitro

depleted of T cells, B cells, mast cells, and/or NK cells after IL-33 inhalation.[23] Also, excessive IL-1 production, if it occurred in ST2-deficient mice, might contribute to the discrepant results between the effects of genetic ST2 deficiency and protein molecules on IL-33 signals, as mentioned before.

In addition, Rag-2-deficient mice, which lack B cells and T cells, including ST2-expressing Th2 cells and Tr1 cells,[32] showed aggravated airway inflammation induced by IL-33.[23] Together with the study of the adoptive Th2 cell transfer model using ST2-deficient DO11.10 Th2 cells,[109] these observations suggest that ST2-expressing T cells have a regulatory role in IL-33-mediated airway inflammation. On the other hand, IL-33 can enhance the development and activation of AAMφ/M2a, resulting in enhanced eosinophilic airway inflammation.[72]

DERMATITIS, RHINITIS, RHINOSINUSITIS AND CONJUNCTIVITIS

In addition to in asthma, polymorphism in the ST2 and/or IL-33 genes was also found in patients with atopic dermatitis,[115] rhinitis[116] and rhinosinusitis.[117] ST2 mRNA expression is increased in skin from rats with contact dermatitis induced with 2,4-dinitro-fluorobenzene.[118] IL-33 mRNA/protein levels are increased in specimens from patients with allergic conjunctivitis,[119] rhinitis[116] and atopic dermatitis.[39] These observations suggest involvement of the IL-33-IL-33R pathway in the development of such allergic disorders.

ANAPHYLAXIS

The serum levels of IL-33 were significantly increased

in atopic patients during anaphylaxis.[39] In mice, IL-33 deteriorated IgE-mediated anaphylaxis.[39]

IL-33-IL-33 R IN AUTOIMMUNE AND CHRONIC INFLAMMATORY DISEASES

ARTHRITIS

The levels of soluble ST2 were increased in the synovial fluid from patients with rheumatoid arthritis (RA),[120] which is considered to be a Th17 cell-mediated autoimmune disorder. IL-33 mRNA/proteins were also elevated in the sera, synovial fluid and/or inflamed lesions of the patients.[5,121-123]

Administration of polyclonal anti-ST2 antiserum, which has potential activity to deplete ST2-expressing cells, to mice resulted in exacerbation of collagen-induced arthritis (CIA), which is considered to be a mouse model for RA.[64] On the other hand, mice treated with soluble ST2-Fc fusion proteins or anti-ST2 mAb and mice deficient in ST2 showed attenuation of CIA,[121,122,124] while mice treated with IL-33 showed aggravated disorders. These observations suggest that certain ST2-expressing cells may act as effector cells and/or regulatory cells in the development of CIA. In fact, ST2-expressing mast cells were shown to act as an effector of aggravated CIA development.[121] Inhibition of IL-1 is beneficial for treatment of RA,[11] and inhibition of the IL-33-IL-33R pathway may be similarly useful for therapy of this disease.

DIABETES MELLITUS (DM) AND ATHEROSCLEROSIS

Diabetes mellitus (DM) is divided into insulin-dependent DM (IDDM)/type 1 DM (T1D) and non-

83

insulin-dependent DM/type 2 DM (T2D). T1D, which is due to insulin deficiency caused by destruction of beta cells in the pancreatic islets of Langerhans by autoreactive T cells, is considered to be a chronic autoimmune disease.

Antibiotic streptozocin, which is an analogue of N-acetylglucosamine (GluNAc), is transported into beta cells via the GLUT2 glucose transporter and inhibits the activity of O-GlcNAcase.[125,126] Since metabolism of the sugar in O-linked proteins is critical for beta cells, inhibition of O-GlcNAcase leads to apoptosis of beta cells.[125,126] Thus, it is known that administration of streptozocin to rodents results in symptom of T1D.[125,126] The destruction of islet beta cells during streptozocin-induced DM is known to be mediated by TRAIL, but not Fas.[127,128] Recently, it was shown that the development of streptozocin-induced DM was exacerbated in ST2-deficient mice,[129] suggesting involvement of the IL-33-IL-33R pathway in the development of human T1D.

Some patients with T2D characterized by hyperglycemia, and hyperinsulinemia due to insulin resistance, develop atherosclerosis associated with hyperlipidemia. Apolipoprotein E-deficient mice spontaneously develop atherosclerosis that resembles human atherosclerosis. Administration of IL-33 ameliorated atherosclerosis development in apolipoprotein E-deficient mice by enhancing IL-5 production and reciprocally suppressing Th1 cell activity.[130] On the other hand, blockade of the IL-33-IL-33R pathway by treatment of apolipoprotein E-deficient mice with soluble ST2 resulted in exacerbated disease.[130] These observations suggest that IL-33 plays a protective role in the development of atherosclerosis in apolipoprotein E-deficient mice.

ALZHEIMER'S DISEASE
In addition to the role of apolipoprotein E in atherosclerosis, polymorphism of the ε4 allele of the apolipoprotein E gene is considered to be a genetic determinant of the common forms of Alzheimer's disease.[131] As another genetic candidate, it is suggested that SNPs at loci in regions associated with the IL-33 gene may be involved in susceptibility to non-ε4-type Alzheimer's disease.[132]

INFLAMMATORY BOWEL DISEASE
Crohn's disease (CD) and ulcerative colitis (UC) are representative inflammatory bowel diseases (IBD). Soluble ST2 protein and/or IL-33 mRNA were detected in endothelial cells from patients with CD and/or UC,[5,133,134] suggesting involvement of the IL-33-IL-33R pathway in induction of IBD, although the precise role of IL-33 remains unclear.

Administration of dextran sodium sulfate (DSS) to mice resulted in development of colitis associated with destruction of colonic epithelial cells even in the absence of T cells, B cells, NK cells and mast cells,[135-137] dependent on TLR signals.[138] SIGIRR/TIR8-deficient mice showed high susceptibility to development of DSS-induced colitis,[17,139] suggesting that signaling through IL-33-IL-33R2 is involved in protection against colitis induced by DSS.

SYSTEMIC SCLEROSIS
IL-33 and ST2 mRNA/protein expression was increased in the inflamed skin of patients with systemic sclerosis.[140] Repeated subcutaneous IL-33 injection resulted in development of skin fibrosis similar to that seen in patients with systemic sclerosis (scleroderma).[141] The development of IL-33-mediated cutaneous fibrosis was dependent on IL-13 (probably derived from eosinophils), but not IL-4, and it required eosinophils and T and/or B cells, but not mast cells.[141] These observations suggest that IL-33-IL-33R pathways may be important for induction of the skin fibrosis seen in patients with systemic sclerosis (scleroderma).

SYSTEMIC LUPUS ERYTHEMATOSUS
Serum levels of soluble ST2 protein were increased in patients with systemic lupus erythematosus (SLE).[142,143] MRL$^{lpr/lpr}$ mice spontaneously develop autoimmune diseases resembling human SLE. SIGIRR/TIR8-deficient C57BL/6$^{lpr/lpr}$ mice showed exacerbated lung disease and lupus nephritis.[144] These observations suggest that the IL-33-IL-33R pathway is involved in the development of SLE, but the precise roles of IL-33 and IL-33Rs in that pathogenesis remain unclear.

CARDIAC DISEASES
The serum level of soluble ST2 protein was elevated in patients with heart failure, acute myocardial infarction, aortic stenosis and congestive cardiomyopathy.[145-147] In addition, IL-33 mRNA/protein was induced in cardiac fibroblasts after biomechanical stimulation.[148] It was shown that mortality, cardiac fibrosis and cardiomyocyte hypertrophy were increased in ST2-deficient mice, but decreased in mice administered IL-33, after transverse aortic constriction.[148] Treatment with IL-33 reduced ventricular dilation, improved contractile function and improved survival in mice after myocardial infarction by preventing cardiomyocyte apoptosis.[149] These observations suggest that the IL-33-IL-33R pathway plays a regulatory role in the induction of certain cardiac diseases.

LIVER INJURY AND FIBROSIS
Both IL-33 and ST2 mRNA were increased in fibrotic livers of humans and mice.[150] Sinusoidal endothelial cells in the normal liver and activated hepatic stellate cells in fibrotic livers are the main source of IL-33.[150] Administration of carbon tetrachloride (CCl4) induces liver injury and fibrosis in mice. In general, treatment with soluble ST2-Fc fusion proteins re-

sulted in inhibition of Th2-type responses such as Th2 cytokine production. During CCl4-induced liver injury in mice, however, soluble ST2 protein treatment enhanced production of Th2 cytokines such as IL-4 and IL-13.[151] CCl4-induced liver injury developed as usual in mice treated with soluble ST2-Fc fusion proteins, while liver fibrosis was accelerated and enhanced by increased production of IL-4 and IL-13 (derived from Th2 cells, not iNKT cells) in those mice. Although the molecular mechanism of the enhancement of Th2 cytokine production by soluble ST2 protein treatment in mice during CCl4-induced liver injury and fibrosis remains unclear, the IL-33-IL-33R pathway is important for the pathogenesis of liver fibrosis.

HYPERNOCICEPTION

Such IL-1 cytokines as IL-1 and IL-18 can provoke hypernociception.[152,153] Like IL-1 and IL-18, IL-33 injection can induce nociceptor sensitization (hypernociception), and IL-33 is also involved in antigen-induced hypernociception that is dependent on TNF, IL-1 and IFN-γ, but not IL-18.[154]

CONCLUSION

Although it was initially thought that IL-33 was a crucial cytokine for Th2 cytokine-mediated host defense as well as induction of Th2-type allergic disorders, it is now known that IL-33 has a pleiotropic, not a restricted, Th2 cytokine-mediated, role in various immune responses as a proinflammatory cytokine, similar to IL-1 and IL-18. Therefore, IL-33 may have potential as a therapeutic target in various diseases. Whereas the effects of IL-33 on various cell types have been extensively investigated, further studies are required to understand the biological significance of IL-33, the cellular source(s) of IL-33, the mechanisms involved in active IL-33 production and the role of IL-33 as a nuclear factor.

ACKNOWLEDGEMENTS

REFERENCES

1. Onda H, Kasuya H, Takakura K et al. Identification of genes differentially expressed in canine vasospastic cerebral arteries after subarachnoid hemorrhage. J Cereb Blood Flow Metab 1999;19:1279-88.
2. Baekkevold ES, Roussigne M, Yamanaka T et al. Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules. Am J Pathol 2003;163:69-79.
3. 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;23:479-90.
4. Ohno T, Oboki K, Kajiwara N et al. Caspase-1, caspase-8, and calpain are dispensable for IL-33 release by macro-

phages. J Immunol 2009;183:7890-7.
5. Carriere V, Roussel L, Ortega N 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 U S A 2007;104:282-7.
6. Roussel L, Erard M, Cayrol C, Girard JP. Molecular mimicry between IL-33 and KSHV for attachment to chromatin through the H2A-H2B acidic pocket. EMBO Rep 2008;9:1006-12.
7. 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.
8. Ali S, Huber M, Kollewe C, Bischoff SC, Falk W, Martin MU. IL-1 receptor accessory protein is essential for IL-33-induced activation of T lymphocytes and mast cells. Proc Natl Acad Sci U S A 2007;104:18660-5.
9. O'Neill LA. The interleukin-1 receptor/Toll-like receptor superfamily: 10 years of progress. Immunol Rev 2008;226:10-8.
10. Arend WP, Palmer G, Gabay C. IL-1, IL-18, and IL-33 families of cytokines. Immunol Rev 2008;223:20-38.
11. Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. Annu Rev Immunol 2009;27:519-50.
12. Iwahana H, Yanagisawa K, Ito-Kosaka A et al. Different promoter usage and multiple transcription initiation sites of the interleukin-1 receptor-related human ST2 gene in UT-7 and TM12 cells. Eur J Biochem 1999;264:397-406.
13. Tago K, Noda T, Hayakawa M et al. Tissue distribution and subcellular localization of a variant form of the human ST2 gene product, ST2V. Biochem Biophys Res Commun 2001;285:1377-83.
14. Iwahana H, Hayakawa M, Kuroiwa K et al. Molecular cloning of the chicken ST2 gene and a novel variant form of the ST2 gene product, ST2LV. Biochim Biophys Acta 2004;1681:1-14.
15. Bulek K, Swaidani S, Qin J et al. The essential role of single Ig IL-1 receptor-related molecule/Toll IL-1R8 in regulation of Th2 immune response. J Immunol 2009;182:2601-9.
16. Garlanda C, Anders HJ, Mantovani A. TIR8/SIGIRR: an IL-1R/TLR family member with regulatory functions in inflammation and T cell polarization. Trends Immunol 2009;30:439-46.
17. Garlanda C, Riva F, Polentarutti N et al. Intestinal inflammation in mice deficient in Tir8, an inhibitory member of the IL-1 receptor family. Proc Natl Acad Sci U S A 2004;101:3522-6.
18. Lecart S, Lecointe N, Subramaniam A et al. Activated, but not resting human Th2 cells, in contrast to Th1 and T regulatory cells, produce soluble ST2 and express low levels of ST2L at the cell surface. Eur J Immunol 2002;32:2979-87.
19. Lohning M, Stroehmann A, Coyle AJ et al. T1/ST2 is preferentially expressed on murine Th2 cells, independent of interleukin 4, interleukin 5, and interleukin 10, and important for Th2 effector function. Proc Natl Acad Sci U S A 1998;95:6930-5.
20. Nakae S, Iwakura Y, Suto H, Galli SJ. Phenotypic differences between Th1 and Th17 cells and negative regulation of Th1 cell differentiation by IL-17. J Leukoc Biol 2007;81:1258-68.
21. Hoshino K, Kashiwamura S, Kuribayashi K et al. The absence of interleukin 1 receptor-related T1/ST2 does not affect T helper cell type 2 development and its effector

function. *J Exp Med* 1999;**190**:1541-8.

22. Townsend MJ, Fallon PG, Matthews DJ, Jolin HE, McKenzie AN. T1/ST2-deficient mice demonstrate the importance of T1/ST2 in developing primary T helper cell type 2 responses. *J Exp Med* 2000;**191**:1069-76.

23. Kondo Y, Yoshimoto T, Yasuda K *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.

24. Kurowska-Stolarska M, Kewin P, Murphy G *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.

25. Guo L, Wei G, Zhu J *et al*. IL-1 family members and STAT activators induce cytokine production by Th2, Th17, and Th1 cells. *Proc Natl Acad Sci U S A* 2009;**106**:13463-8.

26. 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.

27. Smithgall MD, Comeau MR, Yoon BR, Kaufman D, Armitage R, Smith DE. IL-33 amplifies both Th1- and Th2-type responses through its activity on human basophils, allergen-reactive Th2 cells, iNKT and NK cells. *Int Immunol* 2008;**20**:1019-30.

28. Komai-Koma M, Xu D, Li Y, McKenzie AN, McInnes IB, Liew FY. IL-33 is a chemoattractant for human Th2 cells. *Eur J Immunol* 2007;**37**:2779-86.

29. Kropf P, Schopf LR, Chung CL *et al*. Expression of Th2 cytokines and the stable Th2 marker ST2L in the absence of IL-4 during Leishmania major infection. *Eur J Immunol* 1999;**29**:3621-8.

30. Kropf P, Herath S, Klemenz R, Muller I. Signaling through the T1/ST2 molecule is not necessary for Th2 differentiation but is important for the regulation of type 1 responses in nonhealing Leishmania major infection. *Infect Immun* 2003;**71**:1961-71.

31. Chan WL, Pejnovic N, Lee CA, Al-Ali NA. Human IL-18 receptor and ST2L are stable and selective markers for the respective type 1 and type 2 circulating lymphocytes. *J Immunol* 2001;**167**:1238-44.

32. McGuirk P, McCann C, Mills KH. Pathogen-specific T regulatory 1 cells induced in the respiratory tract by a bacterial molecule that stimulates interleukin 10 production by dendritic cells: a novel strategy for evasion of protective T helper type 1 responses by Bordetella pertussis. *J Exp Med* 2002;**195**:221-31.

33. Moritz DR, Rodewald HR, Gheyselinck J, Klemenz R. The IL-1 receptor-related T1 antigen is expressed on immature and mature mast cells and on fetal blood mast cell progenitors. *J Immunol* 1998;**161**:4866-74.

34. Chen CC, Grimbaldeston MA, Tsai M, Weissman IL, Galli SJ. Identification of mast cell progenitors in adult mice. *Proc Natl Acad Sci U S A* 2005;**102**:11408-13.

35. Iikura M, Suto H, Kajiwara N *et al*. IL-33 can promote survival, adhesion and cytokine production in human mast cells. *Lab Invest* 2007;**87**:971-8.

36. 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.

37. Ho LH, Ohno T, Oboki K *et al*. IL-33 induces IL-13 production by mouse mast cells independently of IgE-FcepsilonRI signals. *J Leukoc Biol* 2007;**82**:1481-90.

38. Moulin D, Donze O, Talabot-Ayer D, Mezin F, Palmer G, Gabay C. Interleukin (IL)-33 induces the release of pro-inflammatory mediators by mast cells. *Cytokine* 2007;**40**:216-25.

39. Pushparaj PN, Tay HK, H'Ng SC *et al*. The cytokine interleukin-33 mediates anaphylactic shock. *Proc Natl Acad Sci U S A* 2009;**106**:9773-8.

40. Min B, Le Gros G, Paul WE. Basophils: a potential liaison between innate and adaptive immunity. *Allergol Int* 2006;**55**:99-104.

41. Sullivan BM, Locksley RM. Basophils: a nonredundant contributor to host immunity. *Immunity* 2009;**30**:12-20.

42. Perrigoue JG, Saenz SA, Siracusa MC *et al*. MHC class II-dependent basophil-CD4+ T cell interactions promote T (H)2 cytokine-dependent immunity. *Nat Immunol* 2009; **10**:697-705.

43. Sokol CL, Chu NQ, Yu S, Nish SA, Laufer TM, Medzhitov R. Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. *Nat Immunol* 2009;**10**:713-20.

44. Yoshimoto T, Yasuda K, Tanaka H *et al*. Basophils contribute to T(H)2-IgE responses in vivo via IL-4 production and presentation of peptide-MHC class II complexes to CD4+ T cells. *Nat Immunol* 2009;**10**:706-12.

45. Suzukawa M, Iikura M, Koketsu R *et al*. An IL-1 cytokine member, IL-33, induces human basophil activation via its ST2 receptor. *J Immunol* 2008;**181**:5981-9.

46. Schneider E, Petit-Bertron AF, Bricard R *et al*. IL-33 Activates unprimed murine basophils directly in vitro and induces their in vivo expansion indirectly by promoting hematopoietic growth factor production. *J Immunol* 2009; **183**:3591-7.

47. Kroeger KM, Sullivan BM, Locksley RM. IL-18 and IL-33 elicit Th2 cytokines from basophils via a MyD88- and p38 alpha-dependent pathway. *J Leukoc Biol* 2009;**86**:769-78.

48. 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.

49. Suzukawa M, Koketsu R, Iikura M *et al*. Interleukin-33 enhances adhesion, CD11b expression and survival in human eosinophils. *Lab Invest* 2008;**88**:1245-53.

50. 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.

51. Mayuzumi N, Matsushima H, Takashima A. IL-33 promotes DC development in BM culture by triggering GM-CSF production. *Eur J Immunol* 2009;**39**:3331-42.

52. Rank MA, Kobayashi T, Kozaki H, Bartemes KR, Squillace DL, Kita H. IL-33-activated dendritic cells induce an atypical TH2-type response. *J Allergy Clin Immunol* 2009; **123**:1047-54.

53. Saenz SA, Taylor BC, Artis D. Welcome to the neighborhood: epithelial cell-derived cytokines license innate and adaptive immune responses at mucosal sites. *Immunol Rev* 2008;**226**:172-90.

54. Soumelis V, Reche PA, Kanzler H *et al*. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002;**3**:673-80.

55. Ito T, Wang YH, Duramad O *et al*. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J Exp Med* 2005;**202**:1213-23.

56. Wang YH, Angkasekwinai P, Lu N *et al*. IL-25 augments type 2 immune responses by enhancing the expansion and functions of TSLP-DC-activated Th2 memory cells. *J Exp Med* 2007;**204**:1837-47.

57. Omori M, Ziegler S. Induction of IL-4 expression in CD4

(+) T cells by thymic stromal lymphopoietin. *J Immunol* 2007;**178**:1396-404.

58. Angkasekwinai P, Park H, Wang YH *et al.* Interleukin 25 promotes the initiation of proallergic type 2 responses. *J Exp Med* 2007;**204**:1509-17.

59. Sweet MJ, Leung BP, Kang D *et al.* A novel pathway regulating lipopolysaccharide-induced shock by ST2/T1 via inhibition of Toll-like receptor 4 expression. *J Immunol* 2001;**166**:6633-9.

60. Oshikawa K, Yanagisawa K, Tominaga S, Sugiyama Y. ST 2 protein induced by inflammatory stimuli can modulate acute lung inflammation. *Biochem Biophys Res Commun* 2002;**299**:18-24.

61. Kumar S, Tzimas MN, Griswold DE, Young PR. Expression of ST2, an interleukin-1 receptor homologue, is induced by proinflammatory stimuli. *Biochem Biophys Res Commun* 1997;**235**:474-8.

62. Saccani S, Polentarutti N, Penton-Rol G, Sims JE, Mantovani A. Divergent effects of LPS on expression of IL-1 receptor family members in mononuclear phagocytes in vitro and in vivo. *Cytokine* 1998;**10**:773-80.

63. Espinassous Q, Garcia-de-Paco E, Garcia-Verdugo I *et al.* IL-33 enhances lipopolysaccharide-induced inflammatory cytokine production from mouse macrophages by regulating lipopolysaccharide receptor complex. *J Immunol* 2009;**183**:1446-55.

64. Xu D, Chan WL, Leung BP *et al.* Selective expression of a stable cell surface molecule on type 2 but not type 1 helper T cells. *J Exp Med* 1998;**187**:787-94.

65. Brint EK, Xu D, Liu H *et al.* ST2 is an inhibitor of interleukin 1 receptor and Toll-like receptor 4 signaling and maintains endotoxin tolerance. *Nat Immunol* 2004;**5**:373-9.

66. Glaccum MB, Stocking KL, Charrier K *et al.* Phenotypic and functional characterization of mice that lack the type I receptor for IL-1. *J Immunol* 1997;**159**:3364-71.

67. Hirsch E, Irikura VM, Paul SM, Hirsh D. Functions of interleukin 1 receptor antagonist in gene knockout and overproducing mice. *Proc Natl Acad Sci U S A* 1996;**93**:11008-13.

68. Gordon S. Alternative activation of macrophages. *Nat Rev Immunol* 2003;**3**:23-35.

69. Ma J, Chen T, Mandelin J *et al.* Regulation of macrophage activation. *Cell Mol Life Sci* 2003;**60**:2334-46.

70. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* 2004;**25**:677-86.

71. Sica A, Larghi P, Mancino A *et al.* Macrophage polarization in tumour progression. *Semin Cancer Biol* 2008;**18**:349-55.

72. Kurowska-Stolarska M, Stolarski B, Kewin P *et al.* IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. *J Immunol* 2009;**183**:6469-77.

73. Allakhverdi Z, Comeau MR, Smith DE *et al.* CD34+ hemopoietic progenitor cells are potent effectors of allergic inflammation. *J Allergy Clin Immunol* 2009;**123**:472-8.

74. Moro K, Yamada T, Tanabe M *et al.* Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. *Nature* 2010;**463**:540-4.

75. Bianchi ME. DAMPs, PAMPs alarmins: all we need to know about danger. *J Leukoc Biol* 2007;**81**:1-5.

76. Lotze MT, Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. *Nat Rev Immunol* 2005;**5**:331-42.

77. Luthi AU, Cullen SP, McNeela EA *et al.* Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases. *Immunity* 2009;**31**:84-98.

78. Cayrol C, Girard JP. The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. *Proc Natl Acad Sci U S A* 2009;**106**:9021-6.

79. Talabot-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.

80. Sacks D, Anderson C. Re-examination of the immunosuppressive mechanisms mediating non-cure of Leishmania infection in mice. *Immunol Rev* 2004;**201**:225-38.

81. Kropf P, Bickle Q, Herath S, Klemenz R, Muller I. Organ-specific distribution of CD4+ T1/ST2+ Th2 cells in Leishmania major infection. *Eur J Immunol* 2002;**32**:2450-9.

82. Aliberti J, Jankovic D, Sher A. Turning it on and off: regulation of dendritic cell function in Toxoplasma gondii infection. *Immunol Rev* 2004;**201**:26-34.

83. Jones LA, Roberts F, Nickdel MB *et al.* IL-33 receptor (T 1/ST2) signalling is necessary to prevent the development of encephalitis in mice infected with Toxoplasma gondii. *Eur J Immunol* 2010;**40**:426-36.

84. Finkelman FD, Shea-Donohue T, Morris SC *et al.* Interleukin-4- and interleukin-13-mediated host protection against intestinal nematode parasites. *Immunol Rev* 2004;**201**:139-55.

85. Senn KA, McCoy KD, Maloy KJ *et al.* T1-deficient and T1-Fc-transgenic mice develop a normal protective Th2-type immune response following infection with Nippostrongylus brasiliensis. *Eur J Immunol* 2000;**30**:1929-38.

86. Pearce EJ, M Kane C, Sun J, J Taylor J, McKee AS, Cervi L. Th2 response polarization during infection with the helminth parasite Schistosoma mansoni. *Immunol Rev* 2004;**201**:117-26.

87. Wynn TA, Thompson RW, Cheever AW, Mentink-Kane MM. Immunopathogenesis of schistosomiasis. *Immunol Rev* 2004;**201**:156-67.

88. Cliffe LJ, Grencis RK. The Trichuris muris system: a paradigm of resistance and susceptibility to intestinal nematode infection. *Adv Parasitol* 2004;**57**:255-307.

89. Humphreys NE, Xu D, Hepworth MR, Liew FY, Grencis RK. IL-33, a potent inducer of adaptive immunity to intestinal nematodes. *J Immunol* 2008;**180**:2443-9.

90. Huang X, Du W, Barrett RP, Hazlett LD. ST2 is essential for Th2 responsiveness and resistance to pseudomonas aeruginosa keratitis. *Invest Ophthalmol Vis Sci* 2007;**48**:4626-33.

91. Hazlett LD, McClellan SA, Barrett RP *et al.* IL-33 shifts macrophage polarization, promoting resistance against Pseudomonas aeruginosa keratitis. *Invest Ophthalmol Vis Sci* 2010;**51**:1524-32.

92. Cooper AM. Cell-mediated immune responses in tuberculosis. *Annu Rev Immunol* 2009;**27**:393-422.

93. Wieland CW, van der Windt GJ, Florquin S, McKenzie AN, van der Poll T. ST2 deficient mice display a normal host defense against pulmonary infection with Mycobacterium tuberculosis. *Microbes Infect* 2009;**11**:524-30.

94. Garlanda C, Di Liberto D, Vecchi A *et al.* Damping excessive inflammation and tissue damage in Mycobacterium tuberculosis infection by Toll IL-1 receptor 8/single Ig IL-1-related receptor, a negative regulator of IL-1/TLR signaling. *J Immunol* 2007;**179**:3119-25.

95. Wagenaar JF, Gasem MH, Goris MG *et al.* Soluble ST2 levels are associated with bleeding in patients with severe Leptospirosis. *PLoS Negl Trop Dis* 2009;**3**:e453.

96. Becerra A, Warke RV, de Bosch N, Rothman AL, Bosch I. Elevated levels of soluble ST2 protein in dengue virus infected patients. Cytokine 2008;41:114-20.

97. Hudson CA, Christophi GP, Gruber RC, Wilmore JR, Lawrence DA, Massa PT. Induction of IL-33 expression and activity in central nervous system glia. J Leukoc Biol 2008; 84:631-43.

98. Walzl G, Matthews S, Kendall S et al. Inhibition of T1 ST 2 during respiratory syncytial virus infection prevents T helper cell type 2 (Th2)- but not Th1-driven immunopathology. J Exp Med 2001;193:785-92.

99. Kuchler AM, Pollheimer J, Balogh J et al. Nuclear interleukin-33 is generally expressed in resting endothelium but rapidly lost upon angiogenic or proinflammatory activation. Am J Pathol 2008;173:1229-42.

100. Choi YS, Choi HJ, Min JK et al. IL-33 induces angiogenesis and vascular permeability through ST2/TRAF6-mediated endothelial NO production. Blood 2009;114: 3117-26.

101. Kuroiwa K, Li H, Tago K et al. Construction of ELISA system to quantify human ST2 protein in sera of patients. Hybridoma 2000;19:151-9.

102. Oshikawa K, Kuroiwa K, Tago K et al. Elevated soluble ST2 protein levels in sera of patients with asthma with an acute exacerbation. Am J Respir Crit Care Med 2001;164: 277-81.

103. Prefontaine D, Lajoie-Kadoch S, Foley S et al. Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. J Immunol 2009;183: 5094-103.

104. Gudbjartsson DF, Bjornsdottir US, Halapi E et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. Nat Genet 2009; 41:342-7.

105. Reijmerink NE, Postma DS, Bruinenberg M et al. Association of IL1RL1, IL18R1, and IL18RAP gene cluster polymorphisms with asthma and atopy. J Allergy Clin Immunol 2008;122:651-4. e8.

106. Ali M, Zhang G, Thomas WR et al. Investigations into the role of ST2 in acute asthma in children. Tissue Antigens 2009;73:206-12.

107. Oshikawa K, Yanagisawa K, Tominaga S, Sugiyama Y. Expression and function of the ST2 gene in a murine model of allergic airway inflammation. Clin Exp Allergy 2002;32: 1520-6.

108. 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.

109. Mangan NE, Dasvarma A, McKenzie AN, Fallon PG. T1/ ST2 expression on Th2 cells negatively regulates allergic pulmonary inflammation. Eur J Immunol 2007;37:1302-12.

110. Meisel C, Bonhagen K, Lohning M et al. Regulation and function of T1/ST2 expression on CD4+ T cells: induction of type 2 cytokine production by T1/ST2 cross-linking. J Immunol 2001;166:3143-50.

111. Coyle AJ, Lloyd C, Tian J et al. Crucial role of the interleukin 1 receptor family member T1/ST2 in T helper cell type 2-mediated lung mucosal immune responses. J Exp Med 1999;190:895-902.

112. 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.

113. Liu X, Li M, Wu Y, Zhou Y, Zeng L, Huang T. Anti-IL-33 antibody treatment inhibits airway inflammation in a murine model of allergic asthma. Biochem Biophys Res Commun 2009;386:181-5.

114. Cohn L, Homer RJ, Marinov A, Rankin J, Bottomly K. Induction of airway mucus production By T helper 2 (Th2) cells: a critical role for interleukin 4 in cell recruitment but not mucus production. J Exp Med 1997;186:1737-47.

115. Shimizu M, Matsuda A, Yanagisawa K et al. Functional SNPs in the distal promoter of the ST2 gene are associated with atopic dermatitis. Hum Mol Genet 2005;14:2919-27.

116. Sakashita M, Yoshimoto T, Hirota T 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.

117. Castano R, Bosse Y, Endam LM, Desrosiers M. Evidence of association of interleukin-1 receptor-like 1 gene polymorphisms with chronic rhinosinusitis. Am J Rhinol Allergy 2009;23:377-84.

118. Hartmann B, Staedtler F, Hartmann N, Meingassner J, Firat H. Gene expression profiling of skin and draining lymph nodes of rats affected with cutaneous contact hypersensitivity. Inflamm Res 2006;55:322-34.

119. Matsuda A, Okayama Y, Terai N et al. The Role of interleukin-33 in chronic allergic conjunctivitis. Invest Ophthalmol Vis Sci 2009;50:4646-52.

120. Fraser A, Moore M, Jongbloed S, Gracie A, McInnes IB. Elevated soluble ST2 and cytokine levels in synovial fluids of patients with inflammatory synovitis. Ann Rheum Dis 2006;65(Suppl 1):A10.

121. Xu D, Jiang HR, Kewin P et al. IL-33 exacerbates antigen-induced arthritis by activating mast cells. Proc Natl Acad Sci U S A 2008;105:10913-8.

122. Palmer G, Talabot-Ayer D, Lamacchia C et al. Inhibition of interleukin-33 signaling attenuates the severity of experimental arthritis. Arthritis Rheum 2009;60:738-49.

123. Matsuyama Y, Okazaki H, Tamemoto H et al. Increased levels of interleukin 33 in sera and synovial fluid from patients with active rheumatoid arthritis. J Rheumatol 2010; 37:18-25.

124. Leung BP, Xu D, Culshaw S, McInnes IB, Liew FY. A novel therapy of murine collagen-induced arthritis with soluble T1/ST2. J Immunol 2004;173:145-50.

125. Tesch GH, Allen TJ. Rodent models of streptozotocin-induced diabetic nephropathy. Nephrology (Carlton) 2007; 12:261-6.

126. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia 2008;51:216-26.

127. Lamhamedi-Cherradi SE, Zheng SJ, Maguschak KA, Peschon J, Chen YH. Defective thymocyte apoptosis and accelerated autoimmune diseases in TRAIL-/- mice. Nat Immunol 2003;4:255-60.

128. Pakala SV, Chivetta M, Kelly CB, Katz JD. In autoimmune diabetes the transition from benign to pernicious insulitis requires an islet cell response to tumor necrosis factor alpha. J Exp Med 1999;189:1053-62.

129. Zdravkovic N, Shahin A, Arsenijevic N, Lukic ML, Mensah-Brown EP. Regulatory T cells and ST2 signaling control diabetes induction with multiple low doses of streptozotocin. Mol Immunol 2009;47:28-36.

130. Miller AM, Xu D, Asquith DL et al. IL-33 reduces the development of atherosclerosis. J Exp Med 2008;205:339-46.

131. Farrer LA, Cupples LA, Haines JL et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA 1997;278:1349-56.

132. Chapuis J, Hot D, Hansmannel F *et al*. Transcriptomic and genetic studies identify IL-33 as a candidate gene for Alzheimer's disease. *Mol Psychiatry* 2009;**14**:1004-16.

133. Seidelin JB, Bjerrum JT, Coskun M, Widjaya B, Vainer B, Nielsen OH. IL-33 is upregulated in colonocytes of ulcerative colitis. *Immunol Lett* 2010;**128**:80-5.

134. Beltran CJ, Nunez LE, Diaz-Jimenez D *et al*. Characterization of the novel ST2/IL-33 system in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2010. In press.

135. Axelsson LG, Landstrom E, Goldschmidt TJ, Gronberg A, Bylund-Fellenius AC. Dextran sulfate sodium (DSS) induced experimental colitis in immunodeficient mice: effects in CD4(+)-cell depleted, athymic and NK-cell depleted SCID mice. *Inflamm Res* 1996;**45**:181-91.

136. Ueno Y, Tanaka S, Sumii M *et al*. Single dose of OCH improves mucosal T helper type 1/T helper type 2 cytokine balance and prevents experimental colitis in the presence of valpha14 natural killer T cells in mice. *Inflamm Bowel Dis* 2005;**11**:35-41.

137. Minocha A, Thomas C, Omar R. Lack of crucial role of mast cells in pathogenesis of experimental colitis in mice. *Dig Dis Sci* 1995;**40**:1757-62.

138. Heimesaat MM, Fischer A, Siegmund B *et al*. Shift towards pro-inflammatory intestinal bacteria aggravates acute murine colitis via Toll-like receptors 2 and 4. *PLoS One* 2007;**2**:e662.

139. Xiao H, Gulen MF, Qin J *et al*. The Toll-interleukin-1 receptor member SIGIRR regulates colonic epithelial homeostasis, inflammation, and tumorigenesis. *Immunity* 2007;**26**:461-75.

140. Manetti M, Ibba-Manneschi L, Liakouli V *et al*. The IL-1-like cytokine IL-33 and its receptor ST2 are abnormally expressed in the affected skin and visceral organs of patients with systemic sclerosis. *Ann Rheum Dis* 2010;**69**:598-605.

141. Rankin AL, Mumm JB, Murphy E *et al*. IL-33 Induces IL-13-Dependent Cutaneous Fibrosis. *J Immunol* 2010;**184**:1526-35.

142. Kuroiwa K, Arai T, Okazaki H, Minota S, Tominaga S. Identification of human ST2 protein in the sera of patients with autoimmune diseases. *Biochem Biophys Res Commun* 2001;**284**:1104-8.

143. Mok MY, Huang FP, Ip WK *et al*. Serum levels of IL-33 and soluble ST2 and their association with disease activity in systemic lupus erythematosus. *Rheumatology (Oxford)* 2010;**49**:520-7.

144. Lech M, Kulkarni OP, Pfeiffer S *et al*. Tir8/Sigirr prevents murine lupus by suppressing the immunostimulatory effects of lupus autoantigens. *J Exp Med* 2008;**205**:1879-88.

145. Weinberg EO, Shimpo M, Hurwitz S, Tominaga S, Rouleau JL, Lee RT. Identification of serum soluble ST2 receptor as a novel heart failure biomarker. *Circulation* 2003;**107**:721-6.

146. Shimpo M, Morrow DA, Weinberg EO *et al*. Serum levels of the interleukin-1 receptor family member ST2 predict mortality and clinical outcome in acute myocardial infarction. *Circulation* 2004;**109**:2186-90.

147. Bartunek J, Delrue L, Van Durme F *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.

148. Sanada S, Hakuno D, Higgins LJ, Schreiter ER, McKenzie AN, Lee RT. IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. *J Clin Invest* 2007;**117**:1538-49.

149. Seki K, Sanada S, Kudinova AY *et al*. Interleukin-33 prevents apoptosis and improves survival after experimental myocardial infarction through ST2 signaling. *Circ Heart Fail* 2009;**2**:684-91.

150. Marvie P, Lisbonne M, L'Helgoualc'h A *et al*. Interleukin-33 overexpression is associated with liver fibrosis in mice and humans. *J Cell Mol Med*. Epub 2009 Jun 5.

151. Amatucci A, Novobrantseva T, Gilbride K, Brickelmaier M, Hochman P, Ibraghimov A. Recombinant ST2 boosts hepatic Th2 response in vivo. *J Leukoc Biol* 2007;**82**:124-32.

152. Verri WA Jr, Schivo IR, Cunha TM, Liew FY, Ferreira SH, Cunha FQ. Interleukin-18 induces mechanical hypernociception in rats via endothelin acting on ETB receptors in a morphine-sensitive manner. *J Pharmacol Exp Ther* 2004;**310**:710-7.

153. White FA, Jones KJ. IL-1beta signaling initiates inflammatory hypernociception. *Brain Behav Immun* 2008;**22**:1014-5.

154. Verri WA Jr, Guerrero AT, Fukada SY *et al*. IL-33 mediates antigen-induced cutaneous and articular hypernociception in mice. *Proc Natl Acad Sci U S A* 2008;**105**:2723-8.

IL-33 is a crucial amplifier of innate rather than acquired immunity

Keisuke Oboki[a], Tatsukuni Ohno[a], Naoki Kajiwara[a,b], Ken Arae[a,c], Hideaki Morita[a,d], Akina Ishii[a], Aya Nambu[b], Takaya Abe[e], Hiroshi Kiyonari[e], Kenji Matsumoto[a], Katsuko Sudo[f], Ko Okumura[b], Hirohisa Saito[a,b,1], and Susumu Nakae[a,b,g,1]

[a]Department of Allergy and Immunology, National Research Institute for Child Health and Development, Tokyo 157-8535, Japan; [b]Atopy Research Center, Juntendo University, Tokyo 113-8412, Japan; [c]Department of Immunology, Faculty of Health Science, Kyorin University, Tokyo 192-8508, Japan; [d]Department of Pediatrics, School of Medicine, Keio University, Tokyo 160-8582, Japan; [e]Laboratory for Animal Resources and Genetic Engineering, Center for Developmental Biology, Institute of Physical and Chemical Research, Kobe 650-0047, Japan; [f]Animal Research Center, Tokyo Medical University, Tokyo 160-8402, Japan; and [g]Frontier Research Initiative, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan

IL-33, a member of the IL-1-related cytokines, is considered to be a proallergic cytokine that is especially involved in Th2-type immune responses. Moreover, like IL-1α, IL-33 has been suggested to act as an "alarmin" that amplifies immune responses during tissue injury. In contrast to IL-1, however, the precise roles of IL-33 in those settings are poorly understood. Using IL-1- and IL-33-deficient mice, we found that IL-1, but not IL-33, played a substantial role in induction of T cell-mediated type IV hypersensitivity such as contact and delayed-type hypersensitivity and autoimmune diseases such as experimental autoimmune encephalomyelitis. Most notably, however, IL-33 was important for innate-type mucosal immunity in the lungs and gut. That is, IL-33 was essential for manifestation of T cell-independent protease allergen-induced airway inflammation as well as OVA-induced allergic topical airway inflammation, without affecting acquisition of antigen-specific memory T cells. IL-33 was significantly involved in the development of dextran-induced colitis accompanied by T cell-independent epithelial cell damage, but not in streptozocin-induced diabetes or Con A-induced hepatitis characterized by T cell-mediated apoptotic tissue destruction. In addition, IL-33-deficient mice showed a substantially diminished LPS-induced systemic inflammatory response. These observations indicate that IL-33 is a crucial amplifier of mucosal and systemic innate, rather than acquired, immune responses.

asthma | colitis | cytokine | interleukin-33 | sepsis

IL-33, which is a member of the IL-1 family of cytokines that includes IL-1 and IL-18, induces Th2-type immune responses such as eosinophil-rich inflammation in the intestines and lungs associated with elevated numbers of blood eosinophils and increases in serum concentrations of IgE, IgA, IL-5, and IL-13 in mice, dependent on IL-13 (1). IL-33 is important for Th2 cytokine-associated host defense against nematode infection through signals from IL-33 receptor (IL-33R), which consists of ST2 (also called T1, DER-4, Fit-1, or IL-1RL1) and IL-1R accessory protein (IL-1RAcP) (2, 3). As a Th2-inducing cytokine, IL-33 is considered to be involved in the development of allergic diseases such as atopic asthma (2, 3). IL-33 is also known to ameliorate the development of Th1 cytokine-associated atherosclerosis in apolipoprotein E-deficient mice (4) and accelerate Th17 cell-mediated murine arthritis (5). Thus, it is now thought that IL-33 acts not only as a Th2-inducing cytokine, but also as a proinflammatory cytokine, like IL-1 and IL-18, in various immune responses. Using ST2-deficient (ST2$^{-/-}$) mice and mice treated with anti-ST2 Ab or soluble ST2-Fc-fusion proteins, however, it was reported that the roles of ST2 were not identical in certain immune responses such as allergic airway inflammation (6–8) and LPS-induced endotoxin shock (9, 10). Therefore, we generated IL-33$^{-/-}$ mice to comprehensively examine the roles of IL-33 in the following disease models: allergic airway inflammation; LPS-induced endotoxin shock, contact, and delayed-type hypersensitivity; experimental autoimmune encephalomyelitis; Con A-induced hepatitis; streptozocin-induced diabetes; and T cell-independent dextran sodium sulfate-induced colitis.

Results and Discussion

IL-33 in Allergic Airway Inflammation. Allergic airway inflammation induced by OVA is a well-established mouse model of human asthma. IgE, mast cells, and IL-1 are responsible for the development of airway inflammation in mice sensitized with OVA in the absence of alum (IgE-dependent protocol), but they are not essential for that event in the presence of alum (IgE-independent protocol) (11). At present, the role of IL-33/ST2 in allergic airway inflammation using ST2$^{-/-}$ mice remains controversial (6–8). That is, OVA-induced airway inflammation developed normally in ST2$^{-/-}$ mice that had been sensitized twice with OVA emulsified in alum (6–8), whereas it was attenuated in ST2$^{-/-}$ mice that had been sensitized once with OVA emulsified in alum (8) and exacerbated in wild-type or Rag-1$^{-/-}$ mice that had undergone adoptive transfer of ST2$^{-/-}$ DO11.10 Th2 cells (7).

During airway inflammation induced by two sensitizations with OVA emulsified in alum, IL-33$^{-/-}$ mice, which were newly generated (Fig. S1), showed attenuated eosinophil influx into the bronchoalveolar lavage (BAL) fluid, airway hyperresponsiveness, and pulmonary inflammation (Fig. 1 A-C). The number of lymphocytes in BAL fluids was also reduced in IL-33$^{-/-}$ mice compared with IL-33$^{+/+}$ mice, although their proportions of CD3$^+$CD4$^+$ST2$^+$ cells were comparable (IL-33$^{+/+}$: 2.2 ± 0.5%, $n = 9$ and IL-33$^{-/-}$: 2.0 ± 0.4%, $n = 9$) after the last OVA challenge. In contrast, the IL-4 and IL-5 levels in the BAL fluid and serum OVA-specific IgE production were only slightly (i.e., not significantly) reduced in IL-33$^{-/-}$ mice after the last OVA challenge (Fig. S2 A and B). Interestingly, IL-33 deficiency showed no effect on OVA-specific spleen cell proliferation or cytokine secretion (Fig. S2C).

We also found that IL-33 deficiency significantly diminished inflammatory cell influx into the BAL fluid during airway inflammation induced by an extract derived from house dust mites (HDM) (Fig. 2A). HDM is a major source of allergens in allergic patients and can provoke allergic airway inflammation resembling human asthma in mice by facilitating barrier disruption, inflammation, and allergen sensitization of the airways through TLR4-dependent innate and acquired immunity (12–14).

90

Fig. 1. IL-33 is required for development of an IgE/mast cell-independent OVA-induced allergic airway hypersensitivity response. Mice were sensitized twice with OVA emulsified in alum on days 0 and 14. The mice were challenged intranasally with OVA or saline alone on days 28, 29, and 30. The number of cells in BAL fluids (A), airway hypersensitivity to methacholine (Mch) (B), and lung sections stained with hematoxylin-eosin (C) (100×; representative data from three to five mice are shown) at 24 h after the last OVA or PBS inhalation. Data show the mean ± SE: *$P < 0.05$ vs. corresponding values for saline-treated mice; †$P < 0.05$ vs. OVA-treated IL-33$^{-/-}$ mice.

These findings suggest that IL-33 is important for inducing antigen-dependent Th2-associated local airway inflammation but is mostly dispensable for antigen-specific Th2 cell differentiation.

Indeed, IL-33 can directly enhance eosinophil activation in vitro (15) and induces airway inflammation without Th2 cell activation dependent on IL-13 (16). In support of this notion, IL-33 induced
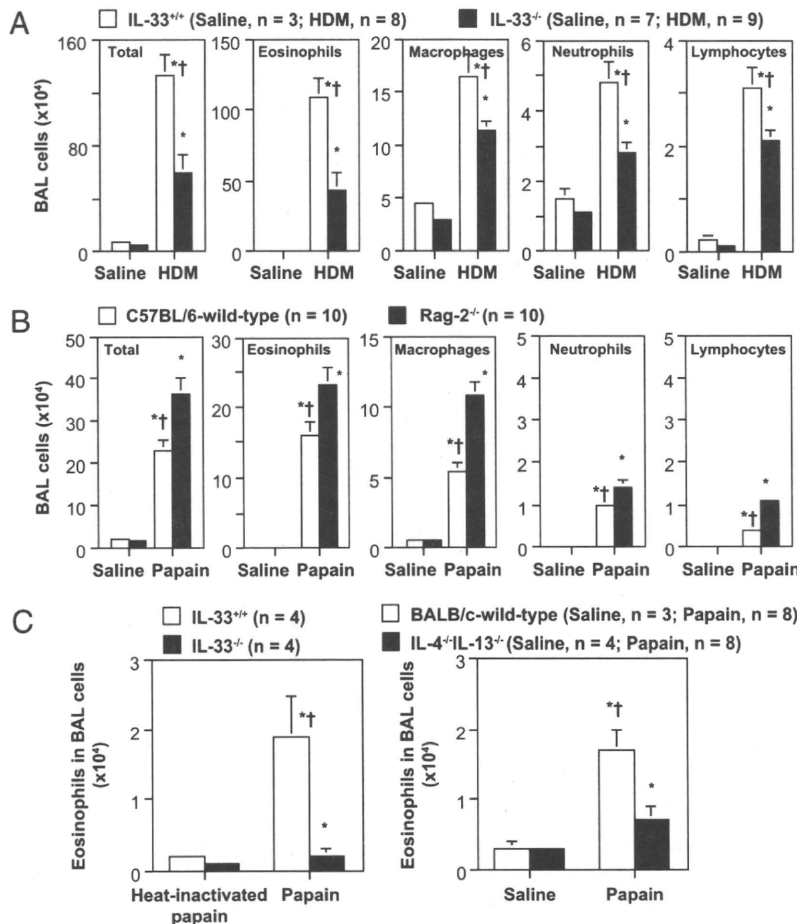
Fig. 2. IL-33 is essential for the development of innate-type airway inflammation. The number of cells in BAL fluids during airway inflammation induced by HDM in IL-33$^{+/+}$ and IL-33$^{-/-}$ mice (A) and induced by papain in C57BL/6-wild-type and Rag-2$^{-/-}$ mice (B), IL-33$^{+/+}$ and IL-33$^{-/-}$ mice (C, Left), and BALB/c-wild-type and IL-4$^{-/-}$ IL-13$^{-/-}$ mice (C, Right) at 24 h after the last OVA, HDM, papain, heat-inactivated papain, or saline inhalation. Data show the mean ± SE: *$P < 0.05$ vs. corresponding values for saline- or heat-inactivated papain-treated mice; †$P < 0.05$ vs. HDM- or papain-treated gene-deficient mice.

airway eosinophilia even in T/B cell-deficient Rag-2$^{-/-}$ mice (Fig. S3), indicating that acquired immune cells are not essential for the setting. Likewise, we found that inhalation of a protease allergen, papain, which is regarded as a cause of occupational asthma (17), induced strong airway eosinophilia in naïve Rag-2$^{-/-}$ mice and naïve wild-type mice (i.e., without prior immunization with papain) (Fig. 2B), although papain-induced airway inflammation has been considered to be mediated by Th2 cells when mice were first sensitized with papain (18).

Although IL-33 proteins were constitutively expressed in the lungs of naïve mice (Fig. S1C), papain inhalation resulted in increased IL-33 mRNA expression in the lungs of wild-type mice but not Rag-2$^{-/-}$ mice (Fig. S4A). The increased papain-induced inflammation seen in Rag-2$^{-/-}$ mice may be caused by the lack of Treg cells, as described (19). T/B cell-independent papain-induced innate-type airway inflammation developed in both C57BL/6 and BALB/c mouse strains, although the BALB/c strain was less sensitive to both papain and IL-33 than the C57BL/6 strain (Fig. S4 C and D). Interestingly, papain-induced airway eosinophilia was profoundly impaired in IL-33$^{-/-}$ mice and IL-4$^{-/-}$IL-13$^{-/-}$ mice (Fig. 2C), suggesting that IL-4 and/or IL-13 derived from IL-33-stimulated innate cells (i.e., Siglec-F$^+$ cells, but not Gr1$^+$, c-Kit$^+$, FcεRIα$^+$, DX5$^+$, or CD11c$^+$ cells; Fig. S5), but not T cell-derived IL-4 and/or IL-13, is important for the event. These findings indicate that IL-33 is crucial for induction of innate-type allergic airway inflammation, whereas it is mostly indispensable for papain-induced airway eosinophilia.

IL-33 in Type IV Hypersensitivity. Type IV hypersensitivity responses such as contact hypersensitivity (CHS) developed normally in TLR4$^{-/-}$ mice (20) but were abolished in Rag-2$^{-/-}$ mice (Fig. S6A), indicating that acquired, rather than innate, immune cells are an important effector for the induction of CHS. Moreover, it is thought that Th2 cytokines are involved in the pathogenesis of CHS (11), suggesting a contribution of IL-33 to the induction of CHS. After FITC and 2,4-dinitrofluorobenzene challenge, ear swelling was also normally induced in IL-33$^{-/-}$ mice but attenuated in IL-1α/β$^{-/-}$ mice (Fig. S6 B and C). IL-33$^{-/-}$ mice also showed normal responses in terms of the levels of skin DC migration and FITC-specific proliferation, IL-4 production by LN cells after FITC sensitization, the degree of skin inflammation, myeloperoxidase (MPO) activity, eosinophil peroxidase (EPO) activity, and serum FITC-specific IgG1 and IgG2a levels after FITC challenge (Fig. S7 A–E).

Moreover, methylated BSA (mBSA)-induced delayed-type hypersensitivity (DTH), which is another type IV hypersensitivity that is mediated by Th17 cells (21) and suppressed by Th1 cells (22), was also normally induced in IL-33$^{-/-}$ mice but impaired in IL-1α/β$^{-/-}$ mice (Fig. S8A). IL-33$^{-/-}$ mice also showed normal levels of Ag-specific T cell responses, a normal Th17/Treg proportion after Ag sensitization and normal serum levels of mBSA-specific IgG1 and IgG2a after Ag challenge (Fig. S8 B–D). Therefore, IL-33 seems to be unnecessary for the development of these T cell-dependent acquired immune responses.

IL-33 in Autoimmunity. ST2 was shown to contribute to the development of a Th17 cell-mediated autoimmune disease, collagen-induced arthritis (5). However, myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE), which is another Th17 cell-mediated autoimmune disease, developed normally in IL-33$^{-/-}$ mice but was attenuated in IL-1α/β$^{-/-}$ mice (Fig. S9A). The levels of MOG-specific LN cell proliferation and IL-17 and IFN-γ production, the proportions of Th17 and Treg cells among LN cells after MOG sensitization, and the levels of MOG-specific IgG1 and IgG2a in sera after MOG challenge were comparable between IL-33$^{-/-}$ and IL-33$^{+/+}$ mice (Fig. S9 B–D). These observations indicate that IL-33 is not essential for the pathogenesis of this Th17 cell-mediated EAE.
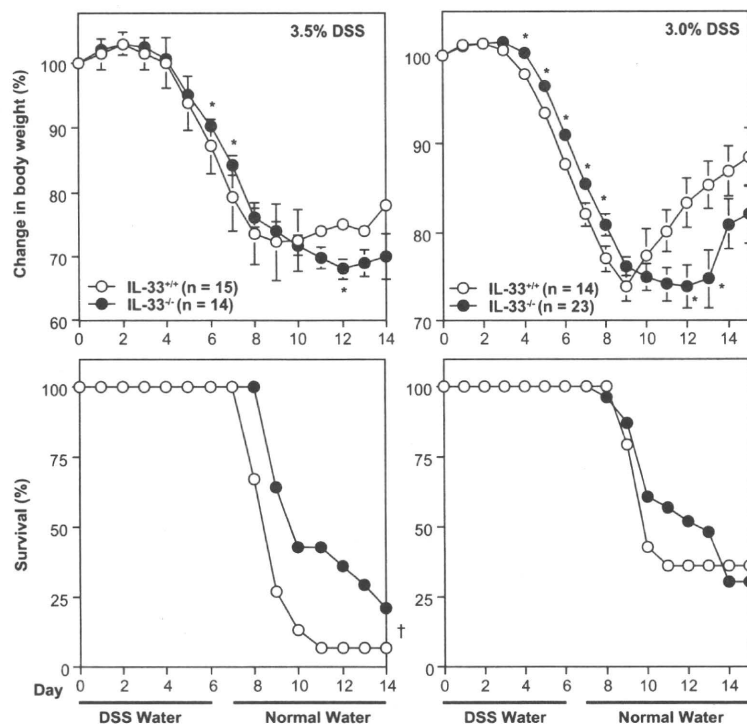

Fig. 3. IL-33 is crucial for T/NKT cell-independent DSS-induced colitis. Mice were provided sterile water containing 3.5% or 3.0% DSS ad libitum for 7 d, followed by 14–15 d of regular drinking water. The change in body weight and survival during 3.5% or 3.0% DSS-induced colitis are compared. Data show the mean ± SE: * and $^†P < 0.05$ vs. corresponding values for IL-33$^{+/+}$ mice.

IL-33 in Tissue Injury. Like pro-IL-1α, pro-IL-33, but not caspase-cleaved IL-33 (23–25), has biological activity and is suggested to act as an alarmin, an endogenous danger signal that alerts immune cells, in necrosis-, rather than apoptosis-, associated tissue injury during trauma and/or infection (23, 24). Streptozocin-induced diabetes and Con A-induced hepatitis are well-established models of apoptotic tissue injury via TRAIL and Fas on T cells and/or NKT cells (26, 27). Hyperglycemia during streptozocin-induced diabetes and the serum levels of GOT and GPT activities during Con A-induced hepatitis were comparable between IL-33$^{+/+}$ and IL-33$^{-/-}$ mice (Fig. S10), again indicating that IL-33 is not essential for the development of T/NKT cell-mediated tissue injury during these acquired immune responses.

Development of dextran sodium sulfate (DSS)-induced colitis is known to be triggered by enterobacteria via TLR (28), independently of T cells, NK cells, NKT cells, and mast cells (29–31). We found that IL-33$^{-/-}$ mice showed higher viability than IL-33$^{+/+}$ mice until approximately day 12 during 3.5% and 3.0% DSS-induced colitis, whereas the final viability on days 14 and 15 was nearly the same (Fig. 3). After treatment with 3.0% DSS, body weight loss, inflammation, and decreased MPO activity in the colon were significantly more pronounced in IL-33$^{-/-}$ mice compared with IL-33$^{+/+}$ mice until day 8 (Fig. 4 A–C). These observations suggest that IL-33 is important for induction of local inflammation at the onset of DSS-induced colitis. However, body weight recovery was markedly delayed in IL-33$^{-/-}$ mice compared with IL-33$^{+/+}$ mice after changing from drinking water containing 3.0% DSS to plain drinking water (Fig. 3). On day 15, the lesion was almost recovered but was still observed in a small proportion of the colons of both IL-33$^{+/+}$ and IL-33$^{-/-}$ mice (Fig. 4A), and the severity of tissue damage in the colon lesions was similar in IL-33$^{+/+}$ and IL-33$^{-/-}$ mice (Fig. 4B). On day 15, the MPO activity in the homogenates of whole colons from both strains returned to the level seen in naïve mice (Fig. 4C). The levels of IL-33 and proinflammatory mediators (i.e., IL-1β, TNF, KC, and MIP-2) that are involved in neutrophilia were similarly increased in the colon of both IL-33$^{+/+}$ and IL-33$^{-/-}$ mice compared with naïve mice on day 8 after DSS treatment (Fig. S11). The expression levels of IL-1β, KC, and MIP-2 in the colon of IL-33$^{+/+}$ mice on day 15 were higher than on day 8 (Fig. S11A), whereas the expression levels of IL-33 and TNF were reduced on day 15 (Fig. S11). In the setting, KC and MIP-2 expression was significantly decreased in the colon of IL-33$^{-/-}$ mice on day 15 after DSS treatment (Fig. S11). Normal expression of potent proinflammatory cytokines such as TNF and IL-1β may induce significant inflammation in IL-33$^{-/-}$ mice during DSS-induced colitis. However, these observations suggest that IL-33 deficiency leads to delayed local inflammation by reducing neutrophil-chemoattractant factors, resulting in delayed resolution of tissue damage during DSS-induced "innate" colitis.

IL-33 in Endotoxin Shock. The LPS-induced systemic inflammatory response is characterized by dysfunction of multiple organs, including the liver and lung. LPS-stimulated macrophages release IL-33 (32), and IL-33 enhances IL-6 (Fig. S12A) and TNF production (33) by LPS-stimulated macrophages, suggesting involvement of IL-33 in the setting. Indeed, IL-33$^{-/-}$ mice were resistant to endotoxin shock in comparison with IL-33$^{+/+}$ mice after LPS injection (Fig. 5A). We also found that production of IL-6, IL-1α, and IL-1β, but not TNF, by thioglycolate-induced peritoneal macrophages of IL-33$^{-/-}$ mice was reduced compared with IL-33$^{+/+}$ mice at 9 and/or 48 h after LPS stimulation (Fig. 5B). LPS-mediated IL-6 and TNF production by macrophages is known to be differentially regulated by nuclear IκB proteins such as IκBNS (34) and IκBζ (35), which was originally identified as an IL-1-, but not TNF-, inducible nuclear protein (36, 37). Therefore, like IL-1, IL-33 may be involved in host defense against bacteria and in innate inflammatory responses triggered
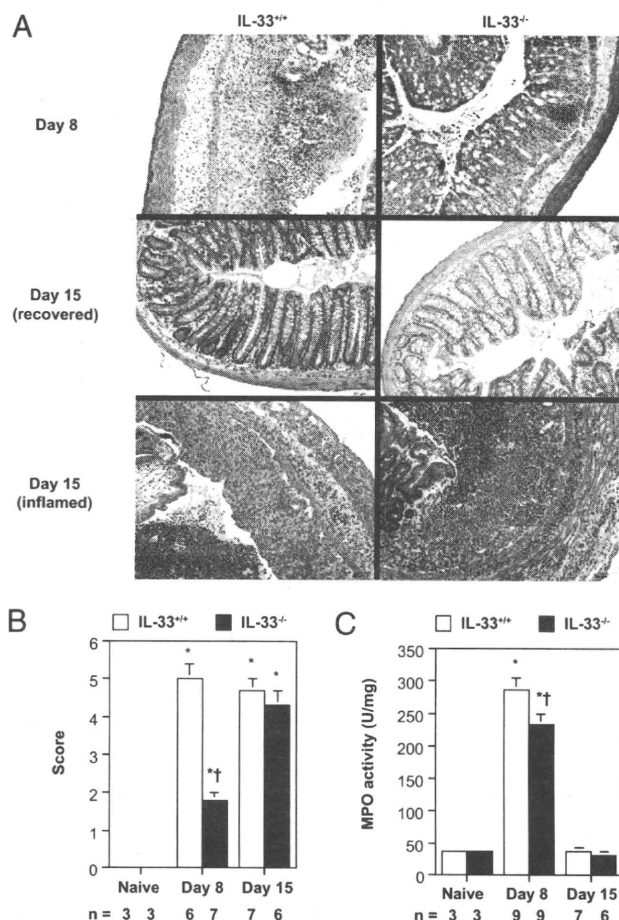
Fig. 4. IL-33 is involved in the induction of inflammation during DSS-induced colitis. Colon sections (A) (hematoxylin-eosin, 100×. Representative data from three to five mice are shown), score of the severity of colitis (B) (only inflamed sites were evaluated on day 15), and the levels of MPO activity (C) in colon homogenates from IL-33$^{+/+}$ and IL-33$^{-/-}$ mice on day 8 or 15 during 3.0% DSS-induced colitis or in naïve mice. Data show the mean + SE: *$P < 0.05$ vs. corresponding values for naive mice; †$P < 0.05$ vs. corresponding values for IL-33$^{+/+}$ mice.

by bacteria by inducing production of IL-6 and IL-1, rather than TNF, by LPS-stimulated macrophages via distinct nuclear protein activation.

Unlike endotoxin shock induced by a single LPS injection (Fig. 5A), the susceptibility to a lethal dose of LPS was comparable in IL-33$^{+/+}$ and IL-33$^{-/-}$ mice that had been injected once with a low dose of LPS (Fig. S12C) or had been made tolerant to LPS by repeated injection of a low dose of LPS (Fig. S12D). These observations suggest that IL-33 is important for acute responses, rather than secondary responses and tolerance, to LPS.

However, as in the case of OVA-induced airway inflammation (6–8), the contribution of ST2 to that response is controversial. Production of each of IL-6, IL-12, and TNF by LPS-stimulated macrophages was suppressed by blockade of ST2 signals using soluble ST2-Fc fusion proteins, and BALB/c mice treated with soluble ST2-Fc fusion proteins were resistant to LPS-induced endotoxemia (9). Conversely, production of these cytokines by LPS-stimulated BALB/c-ST2$^{-/-}$ macrophages was increased, and BALB/c-ST2$^{-/-}$ mice were highly susceptible to LPS-induced endotoxemia (10). On the other hand, it was reported that LPS-induced TNF production was normal in BALB/c-ST2$^{-/-}$ macrophages (33). Thus, in contrast to treatment with soluble ST2-
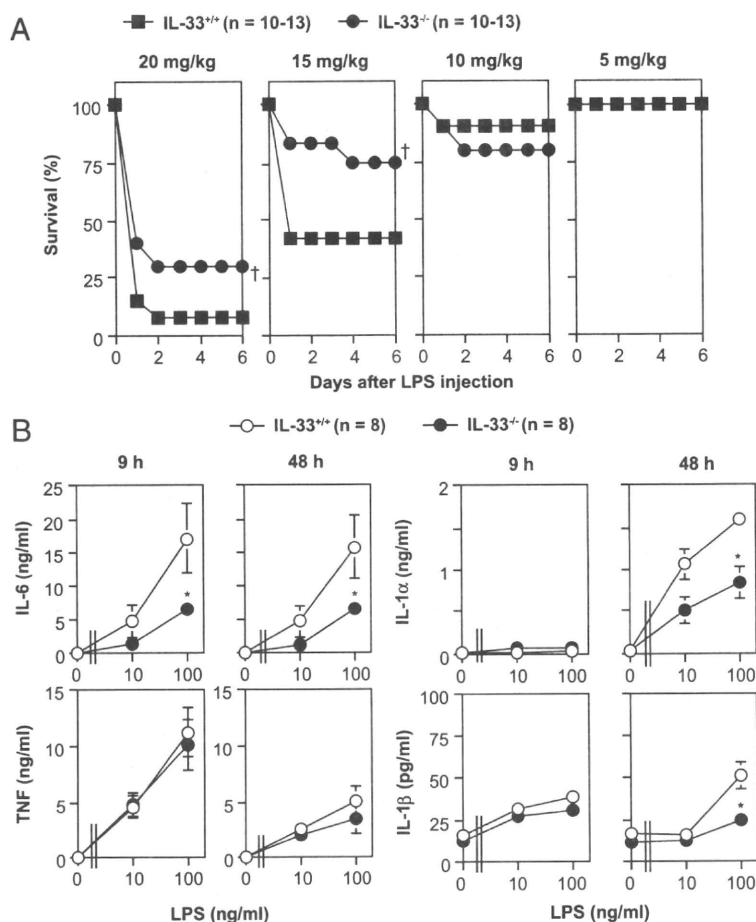
Fig. 5. IL-33 is important for LPS-induced endotoxin shock. (*A*) Mice were injected intraperitoneally with LPS (5–20 mg/kg; *n* = 10–13), and survival was monitored. (*B*) Thioglycolate-induced peritoneal macrophages were stimulated with LPS for 9 or 48 h. IL-1α, IL-1β, IL-6, and TNF levels in the culture supernatants were determined by ELISA. Data show the mean ± SE: *, $^{\dagger}P < 0.05$ vs. corresponding values for IL-33$^{+/+}$ mice.

Fc fusion proteins, ST2 deficiency may result in increased formation of IL-1R (IL-1R1 and IL-1RAcP) due to failed formation of IL-33R (ST2 and IL-1RAcP), causing cytokine hyperproduction by ST2$^{-/-}$ macrophages in response to IL-1. Notably, ST2$^{-/-}$ macrophages produced larger amounts of cytokines than wild-type macrophages after IL-1α or IL-1β treatment (10). Although IL-1R1$^{-/-}$ mice showed normal susceptibility to LPS-induced endotoxin shock (38), IL-1R antagonist-deficient mice, which have excessive IL-1-signaling, showed heightened susceptibility (39). Thus, IL-1 is not required for induction of LPS-induced endotoxin shock, but excessive IL-1 production leads to amplified susceptibility to LPS, as seen in ST2$^{-/-}$ mice. Distinct phenotypes were also seen in IL-18$^{-/-}$ and IL-18Rα$^{-/-}$ mice: EAE developed normally in IL-18$^{-/-}$ mice but was dramatically reduced in IL-18Rα$^{-/-}$ mice (40), suggesting a contribution by a ligand(s) other than IL-18, i.e., IL-1F7 (41), in the setting. As is well known, the function of the IL-1 family of cytokines is elaborately controlled by functional/decoy receptors and antagonists (41). Therefore, like IL-18R, ST2 may be a component of receptors for other ligand(s) besides IL-33. Alternatively, IL-33 may bind to other functional receptors besides ST2. Accordingly, for elucidation of the precise roles of IL-33 in immune responses, studies using IL-33$^{-/-}$, rather than ST2$^{-/-}$, mice have been highly anticipated.

Taken all together, IL-33 seems to play a critical role in various types of innate-type inflammation in the lung and gut, whereas it is dispensable for acquired immune responses. Of note, IL-33 is indispensable for innate airway inflammation induced by papain. We were also able to show that IL-33 is crucial as an alarmin for innate-type, but not acquired-type, tissue injury-related inflammatory responses such as LPS-induced endotoxin shock.

Methods

LPS-Induced Sepsis. LPS (*Escherichia coli* serotype 0111:B4; Sigma-Aldrich)-induced sepsis was evaluated as described (38).

DSS-Induced Colitis. Mice were provided sterile water containing 3.0% or 3.5% DSS (reagent grade, MW = 36,000–50,000; MP Biomedicals) ad libitum for 7 d, followed by 14 d of plain drinking water.

Other Methods. The details are provided in *SI Methods*.

1. Schmitz J, et al. (2005) 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* 23:479–490.
2. Smith DE (2010) IL-33: A tissue derived cytokine pathway involved in allergic inflammation and asthma. *Clin Exp Allergy* 40:200–208.
3. Liew FY, Pitman NI, McInnes IB (2010) Disease-associated functions of IL-33: The new kid in the IL-1 family. *Nat Rev Immunol* 10:103–110.
4. Miller AM, et al. (2008) IL-33 reduces the development of atherosclerosis. *J Exp Med* 205:339–346.
5. Xu D, et al. (2008) IL-33 exacerbates antigen-induced arthritis by activating mast cells. *Proc Natl Acad Sci USA* 105:10913–10918.
6. Hoshino K, et al. (1999) The absence of interleukin 1 receptor-related T1/ST2 does not affect T helper cell type 2 development and its effector function. *J Exp Med* 190:1541–1548.
7. Mangan NE, Dasvarma A, McKenzie AN, Fallon PG (2007) T1/ST2 expression on Th2 cells negatively regulates allergic pulmonary inflammation. *Eur J Immunol* 37:1302–1312.
8. Kurowska-Stolarska M, et al. (2008) IL-33 induces antigen-specific IL-5^{+} T cells and promotes allergic-induced airway inflammation independent of IL-4. *J Immunol* 181:4780–4790, and erratum (2008) 181:8170.
9. Sweet MJ, et al. (2001) A novel pathway regulating lipopolysaccharide-induced shock by ST2/T1 via inhibition of Toll-like receptor 4 expression. *J Immunol* 166:6633–6639.
10. Brint EK, et al. (2004) ST2 is an inhibitor of interleukin 1 receptor and Toll-like receptor 4 signaling and maintains endotoxin tolerance. *Nat Immunol* 5:373–379.
11. Oboki K, Ohno T, Saito H, Nakae S (2008) Th17 and allergy. *Allergol Int* 57:121–134.
12. Johnson JR, et al. (2004) Continuous exposure to house dust mite elicits chronic airway inflammation and structural remodeling. *Am J Respir Crit Care Med* 169:378–385.
13. Hammad H, et al. (2009) House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat Med* 15:410–416.
14. Trompette A, et al. (2009) Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein. *Nature* 457:585–588.
15. Suzukawa M, et al. (2008) Interleukin-33 enhances adhesion, CD11b expression and survival in human eosinophils. *Lab Invest* 88:1245–1253.
16. Kondo Y, et al. (2008) Administration of IL-33 induces airway hyperresponsiveness and goblet cell hyperplasia in the lungs in the absence of adaptive immune system. *Int Immunol* 20:791–800.
17. Milne J, Brand S (1975) Occupational asthma after inhalation of dust of the proteolytic enzyme, papain. *Br J Ind Med* 32:302–307.
18. Sokol CL, Barton GM, Farr AG, Medzhitov R (2008) A mechanism for the initiation of allergen-induced T helper type 2 responses. *Nat Immunol* 9:310–318.
19. D'Alessio FR, et al. (2009) CD4^{+}CD25^{+}Foxp3^{+} Tregs resolve experimental lung injury in mice and are present in humans with acute lung injury. *J Clin Invest* 119:2898–2913.
20. Martin SF, et al. (2008) Toll-like receptor and IL-12 signaling control susceptibility to contact hypersensitivity. *J Exp Med* 205:2151–2162.
21. Nakae S, et al. (2002) Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity* 17:375–387.
22. Irmler IM, Gajda M, Bräuer R (2007) Exacerbation of antigen-induced arthritis in IFN-γ-deficient mice as a result of unrestricted IL-17 response. *J Immunol* 179:6228–6236.
23. Cayrol C, Girard JP (2009) The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. *Proc Natl Acad Sci USA* 106:9021–9026.
24. Lüthi AU, et al. (2009) Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases. *Immunity* 31:84–98.
25. Talabot-Ayer D, Lamacchia C, Gabay C, Palmer G (2009) Interleukin-33 is biologically active independently of caspase-1 cleavage. *J Biol Chem* 284:19420–19426.
26. Lamhamedi-Cherradi SE, Zheng SJ, Maguschak KA, Peschon J, Chen YH (2003) Defective thymocyte apoptosis and accelerated autoimmune diseases in TRAIL^{-/-} mice. *Nat Immunol* 4:255–260.
27. Seino K, et al. (1997) Contribution of Fas ligand to T cell-mediated hepatic injury in mice. *Gastroenterology* 113:1315–1322.
28. Heimesaat MM, et al. (2007) Shift towards pro-inflammatory intestinal bacteria aggravates acute murine colitis via Toll-like receptors 2 and 4. *PLoS ONE* 2:e662.
29. Axelsson LG, Landström E, Goldschmidt TJ, Grönberg A, Bylund-Fellenius AC (1996) Dextran sulfate sodium (DSS) induced experimental colitis in immunodeficient mice: Effects in CD4(+) -cell depleted, athymic and NK-cell depleted SCID mice. *Inflamm Res* 45:181–191.
30. Ueno Y, et al. (2005) Single dose of OCH improves mucosal T helper type 1/T helper type 2 cytokine balance and prevents experimental colitis in the presence of valpha14 natural killer T cells in mice. *Inflamm Bowel Dis* 11:35–41.
31. Minocha A, Thomas C, Omar R (1995) Lack of crucial role of mast cells in pathogenesis of experimental colitis in mice. *Dig Dis Sci* 40:1757–1762.
32. Ohno T, et al. (2009) Caspase-1, caspase-8, and calpain are dispensable for IL-33 release by macrophages. *J Immunol* 183:7890–7897.
33. Espinassous Q, et al. (2009) IL-33 enhances lipopolysaccharide-induced inflammatory cytokine production from mouse macrophages by regulating lipopolysaccharide receptor complex. *J Immunol* 183:1446–1455.
34. Kuwata H, et al. (2006) IkappaBNS inhibits induction of a subset of Toll-like receptor-dependent genes and limits inflammation. *Immunity* 24:41–51.
35. Yamamoto M, et al. (2004) Regulation of Toll/IL-1-receptor-mediated gene expression by the inducible nuclear protein IkappaBzeta. *Nature* 430:218–222.
36. Haruta H, Kato A, Todokoro K (2001) Isolation of a novel interleukin-1-inducible nuclear protein bearing ankyrin-repeat motifs. *J Biol Chem* 276:12485–12488.
37. Yamazaki S, Muta T, Takeshige K (2001) A novel IkappaB protein, IkappaB-zeta, induced by proinflammatory stimuli, negatively regulates nuclear factor-kappaB in the nuclei. *J Biol Chem* 276:27657–27662.
38. Glaccum MB, et al. (1997) Phenotypic and functional characterization of mice that lack the type I receptor for IL-1. *J Immunol* 159:3364–3371.
39. Hirsch E, Irikura VM, Paul SM, Hirsh D (1996) Functions of interleukin 1 receptor antagonist in gene knockout and overproducing mice. *Proc Natl Acad Sci USA* 93:11008–11013.
40. Gutcher I, Urich E, Wolter K, Prinz M, Becher B (2006) Interleukin 18-independent engagement of interleukin 18 receptor-α is required for autoimmune inflammation. *Nat Immunol* 7:946–953.
41. Dinarello CA (2009) Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol* 27:519–550.

Supporting Information

Oboki et al. 10.1073/pnas.1003059107

SI Methods

Generation of IL-33–Deficient Mice. A BAC clone, RP23-355N22, which contains the translational start site of the IL-33 gene, was obtained from BACPAC Resources (http://bacpac.chori.org). The targeting vector was constructed as described (http://www.cdb.riken.jp/arg/protocol.html). The second exon was replaced with a cassette consisting of the *GFP* gene and the *neomycin resistance* gene (neo^r) flanked by loxP sequences (http://www.cdb.riken.jp/arg/cassette.html). The locations of 5′ and 3′ homologous recombinations of the targeting vector are shown in Fig. S1*A*. The targeting vector was electroporated into ES cells (TT2, derived from CBA × C57BL/6 mice). Chimera mice were obtained from two distinct clones out of three independently identified targeted ES cells and mated with C57BL/6 female mice (1). For confirmation of correct targeting, Southern blot hybridization was performed with 5′ probes located outside of the regions used in the targeting vector (Fig. S1*B*). As we reported (2), constitutive expression of IL-33 proteins could be detected in homogenates of lungs from IL-33-sufficient, but not IL-33-deficient, mice by Western blot analysis (Fig. S1*C*). IL-33-deficient mice were obtained at the expected Mendelian ratio from the intercross of heterozygous mice on the C57BL/6 background (N2). IL-33-deficient mice were fertile and did not show any gross phenotypic abnormalities under specific pathogen-free housing conditions. Genotyping of mice was performed by PCR of tail genomic DNAs using the following PCR primers: ex2-F (5′-cactaagactactcagcctcag), WT-R (5′-cggtgatgctgtgaagtctg), and KO-R (5′-gtgttctgctggtagtggtcg). The ex2-F and WT-R primers were used for detection of wild-type alleles, and the ex2-F and KO-R primers were used for detection of mutant alleles. Detailed information regarding the IL-33-deficient mice (accession number CDB0631K) is available at http://www.cdb.riken.jp/arg/mutant%20mice%20list.html.

Mice. C57BL/6-wild-type mice (SLC Japan), BALB/c-wild-type mice (SLC Japan), C57BL/6-Rag2-deficient mice (Taconic), and BALB/c-4-get mice (The Jackson Laboratory) were used. C57BL/6-IL-1α/β$^{-/-}$ mice and BALB/c-IL-4$^{-/-}$IL-13$^{-/-}$ mice were kindly provided by Yoichiro Iwakura and Andrew McKenzie, respectively. All mice were housed under specific pathogen-free conditions at the National Research Institute for Child Health and Development, and the animal protocols were approved by the Institutional Review Boards of the National Research Institute for Child Health and Development and the Institute of Medical Science, University of Tokyo.

Contact Hypersensitivity (CHS). FITC-induced CHS and 2,4-dinitrofluorobenzene (DNFB)-induced CHS were examined as described (3, 4). Briefly, 2 d after shaving the dorsal hair with clippers, mice were sensitized with 200 μL of a 2.0% FITC isomer I suspension (Sigma) in a mixture of acetone and dibutyl phthalate (1:1) or 25 μL of 0.5% DNFB solution (Wako) in a mixture of acetone and olive oil (1:4). Five days after sensitization, the mice were challenged with 40 μL of 0.5% FITC isomer I solution in a mixture of acetone and dibutyl phthalate (1:1) (the left ear, 20 μL on each surface) and 40 μL of the vehicle alone (the right ear, 20 μL on each surface) or with 20 μL of a 0.2% DNFB solution in a mixture of acetone and olive oil (1:4) (the left ear, 20 μL on the outside skin surface) and 20 μL of the vehicle alone (the right ear, 20 μL on the outside skin surface). Ear thickness was measured before and after FITC or DNFB challenge by using an engineer's calipers (Ozaki) by an investigator who was blinded to the mouse genotypes. At 24 h after

FITC challenge, ear tissues were harvested for histological analysis and measurement of the myeloperoxidase (MPO) and eosinophil peroxidase (EPO) activities in ear skin homogenates, as described below. One week after FITC challenge, sera were collected for measurement of the FITC-specific Ig levels, as described below.

Skin DC Migration. Skin DC migration was determined as described (5, 6). Mice were treated epicutaneously with 40 μL of a 0.5% (wt/vol) FITC isomer I solution in a mixture of acetone and dibutyl phthalate (1:1) (the left ear, 20 μL on each surface) and the vehicle alone (the right ear, 20 μL on each surface). Twenty-four hours later, submaxillary lymph nodes (LNs) were collected separately from both the FITC-treated left and vehicle-treated right ears. After incubation with anti-CD16/CD32 mAb (2.4G2; BD Biosciences), LN cells were incubated with PE-anti-mouse CD11c mAb (N418; eBioscience) and APC-anti-mouse I-A/I-E mAb (M5/114.15.2; eBioscience). The proportion of FITC$^+$ cells among 7-aminoactinomycin D-negative, MHC class II^{hi}, CD11c$^+$ cells was determined by using a FACSCalibur (BD Biosciences).

Delayed-Type Hypersensitivity (DTH). Methyl-BSA (mBSA)-induced CHS was examined as described (4, 7). Briefly, 200 μL of 1.25 mg/mL mBSA (Sigma) emulsified with complete Freund's adjuvant (CFA; Difco) was injected s.c. to the backs of mice. Seven days later, the mice were challenged intradermally by injection of 20 μL of 10 mg/mL mBSA in PBS to one footpad and 20 μL of PBS alone to another footpad as a control. Footpad thickness was measured before and after mBSA or PBS challenge by using an engineer's calipers (Ozaki) by an investigator who was blinded to the mouse genotypes. One week after the challenge, sera were collected for measurement of the mBSA-specific Ig levels, as described below.

Airway Inflammation. OVA-induced airway inflammation was examined as described (8, 9). For the IgE-independent protocol (OVA with alum), mice were immunized intraperitoneally with 200 μL of 0.5 mg/mL OVA (grade V; Sigma) emulsified with alum (Alum Immuject; Pierce) (1 mg/mL OVA:alum = 1:1) on days 0 and 14. The mice were then challenged intranasally with 20 μL of 10 mg/mL OVA in saline or saline alone on days 28, 29, and 30. For the IgE-dependent protocol (OVA without alum), mice were treated intraperitoneally with 200 μL of 50 μg/mL OVA in saline on days 0, 2, 4, 6, 8, 10, and 12. The mice were then challenged intranasally with 20 μL of 10 mg/mL OVA in saline or saline alone on days 40, 43, and 46. House dust mite (HDM)-induced airway inflammation was established as described elsewhere (10, 11). Mice were treated intranasally with 20 μL of 1.25 mg/mL HDM extract (Greer Laboratories) in saline or saline alone on 5 d per week for up to three consecutive weeks. For IL-33-induced airway inflammation, mice were treated intranasally with 20 μL of 25 μg/mL recombinant human IL-33 (PeproTech) in saline or saline alone once per day for 3 d. For papain-induced airway inflammation, mice were treated intranasally with 20 μL of 5 mg/mL papain (Wako) or heat-inactivated papain (at 100 °C for 15 min) in saline or saline alone once per day for 3 d. Twenty-four hours after the last Ag or saline inhalation, bronchoalveolar lavage (BAL) fluids and lungs were collected for examination of the BAL cell profiles and lung histology, respectively. After centrifugation, the BAL cells were resuspended in 200 μL of Hanks' buffer, and the total cell number and leukocyte profile were determined by using a hemocytometer (XT1800iV; Sysmex). Lung function during OVA-induced airway

inflammation was determined by an invasive approach (Elan Series Mouse RC Site; Buxco Electronics), as described (9, 12).

Experimental Autoimmune Encephalomyelitis (EAE). MOG-induced EAE was examined as described (13, 14). Briefly, mice were immunized s.c. with 200 μL of 1.5 mg/mL MOG35-55 peptide (MEVGWYRSPFSRVVHLYRNGK) emulsified with CFA, which consisted of incomplete Freund's adjuvant (Difco) and 5 mg/mL *Mycobacterium tuberculosis* H37RA (Difco), by injection to one flank on day 0 and the other flank on day 7. The mice were injected i.v. with 200 μL of 2.5 μg/mL pertussis toxin (Allexis) on days 0 and 2. After the first MOG treatment, the severity of EAE was monitored daily and graded on a scale of 0–5 by an investigator who was blinded to the mouse genotypes. The scale was as follows: 0, no disease; 1, limp tail; 2, hind limb weakness; 3, hind limb paralysis; 4, hind and fore limb paralysis; 5, moribundity and death.

Streptozocin-Induced Diabetes. Streptozocin-induced diabetes was examined as described (15), with minor modification. Briefly, mice were injected intraperitoneally with 4 mg/mL streptozocin in 0.05 M citrate buffer (pH 4.5) (50 mg/kg) once per day for 5 d. On days 0, 10, 17, and 24, blood was collected by cutting the tail vein, and the blood glucose levels were measured with an Accu-CHEK Aviva system (Roche Diagnostics).

Con A (ConA)-Induced Hepatitis. ConA-induced hepatitis was examined as described (16). Briefly, mice were i.v. administered 2 mg/mL ConA (Sigma) in saline (20 mg/kg). One day later, sera were collected, and the levels of GOT and GPT were measured by using Transaminase CII-test Wako (Wako).

Ag-Specific LN or Spleen Cell Responses. The FITC-, mBSA-, OVA- and MOG-specific LN cell proliferative responses were examined as described (4, 6, 14). Briefly, for FITC-specific LN cell responses, mice were treated epicutaneously with 2.0% FITC on both the left and right ears (20 μL on one surface of each ear). Five days later, submaxillary LNs were collected. For mBSA-specific LN cell responses, the backs of mice were injected s.c. with 200 μL of 1.25 mg/mL mBSA emulsified with CFA. Five days later, inguinal LNs were harvested. For OVA-specific LN and spleen cell responses, mice were immunized intraperitoneally with 200 μL of 0.5 mg/mL OVA emulsified with alum (1 mg/mL OVA:alum = 1:1) on days 0 and 14. Seven days later, mesenteric LNs and the spleen were collected. For MOG-specific LN cell responses, the backs of mice were s.c. injected with 200 μL of 1.5 mg/mL MOG35-55 peptide emulsified with CFA. Seven days later, inguinal LNs were harvested.

LN or spleen cells (4×10^5 cells per well in 96-well flat-bottom plates) were cultured in the presence and absence of 40 μg/mL FITC, mBSA, OVA, or MOG at 37 °C for 72 h. Cell proliferative responses were determined by pulsing with 0.25 μCi/mL [^{3}H]-labeled thymidine for 6 h.

The intracellular cytokine profiles during mBSA- and MOG-specific LN cell responses were determined as described (14) with minor modification. LN cells (5×10^6 cells per well in a 24 well-plate) were cultured in the presence of 40 μg/mL mBSA or MOG at 37 °C for 72 h, and then stimulated with 1 μg of ionomycin (Sigma) and 0.1 μg/mL PMA in the presence of 1 μM monensin (Sigma) for 5 h. After washing, the cells were incubated with anti-CD16/CD32 mAb (2.4G2; BD Biosciences) in FACS buffer (Hanks' buffer containing 2% FCS) for FcR blocking on ice for 15 min, and then incubated with APC-conjugated anti-mouse CD4 mAb (GK1.5; eBioscience) on ice for 30 min. After washing, the cells were treated with Fix Buffer I (BD Biosciences) at room temperature for 15 min. Then the cells were washed with 0.1% saponin (Sigma) in FACS buffer and incubated with FITC anti-mouse Foxp3 mAb (FJK-16s; eBiosciences) and PE anti-mouse IL-17 mAb (TC11-18H10, BD Biosciences) at 4 °C for 30 min. IL-17 and Foxp3 expressions in CD4$^+$ T cells were analyzed

on a FACSCalibur (Becton Dickinson) by using CellQuest software (Becton Dickinson).

Measurement of Cytokines. Cytokine levels in the culture supernatants of Ag-specific LN cells and BAL fluids during OVA-induced airway inflammation were determined with mouse IFN-γ, IL-1α, IL-1β, IL-4, IL-5, IL-13, and IL-17 ELISA kits obtained from BD Biosciences or eBioscience. The level of IL-33 in colon homogenates (prepared as described below) was measured with an ELISA kit (BioLegend).

Measurement of MPO and EPO Activities. The levels of MPO and EPO activities in tissues were examined as described (9, 12). Briefly, tissues (ear and colon) were homogenized in a 0.5% cetyltrimethylammonium chloride solution (Sigma). After centrifugation, the supernatants of the homogenates were collected. Total protein levels in the tissue homogenates were measured with a Bio-Rad DC protein assay kit. Recombinant human MPO and EPO (Calbiochem) were used as standard proteins. The MPO and EPO activities per milligram of total protein in tissue homogenates were calculated.

Measurement of Ag-Specific Ig Levels. Ninety-six-well ELISA plates (Nunc; 442404) were coated with 2 μg/mL FITC-OVA (6), 10 μg/mL mBSA, 10 μg/mL OVA, or 10 μg/mL MOG at 4 °C overnight. After the wells were blocked with PBS containing 10% FCS, optimally diluted serum samples (IgG1 = 1:10,000, IgG2a = 1:100, and IgE = undiluted for FITC-specific Igs; IgG1 = 1:50, IgG2a = 1:10, IgG2b = 1:50, and IgG3 = 1:2 for mBSA-specific Igs; IgE = undiluted for OVA-specific IgE; and IgG1 = 1:2 and IgG2a = 1:2 for MOG-specific Igs) were applied, and the plates were incubated at room temperature for 1 h. After washing, biotinylated anti-mouse IgG1 (A85-1; BD Biosciences), IgG2a (R19-15; BD Biosciences), IgG2b (R12-3; BD Biosciences), IgG3 (R40-82; BD Biosciences), or IgE (R35-118; BD Biosciences) mAb was added, followed by incubation at room temperature for 1 h. Then, after washing, HRP-conjugated streptavidin (BD Biosciences) was added, followed by incubation at room temperature for 1 h. For enzymatic reaction, TMB substrate (KPL) was used as the substrate. The reaction was stopped by addition of 1 M H$_2$SO$_4$, and then the absorbance at 450 nm was measured by using a plate reader. Data show the absorbance value at 450 nm. The levels of OVA-specific serum IgE were normalized by using an anti-OVA IgE mAb (TOS-2; kindly provided by Mamoru Kiniwa, Taiho Pharmaceutical, Saitama, Japan) as the standard antibody (12).

Quantitative PCR. Total RNA in the colon and lung specimens was isolated by using ISOGEN (Nippon Gene) and RNeasy Mini Kit (Qiagen). Using the isolated RNA, cDNA was obtained by RT-PCR with an iScript cDNA Synthesis Kit (Bio-Rad). Quantitative real-time PCR was performed with THUNDERBIRD SYBR qPCR Mix (Toyobo) and an Applied Biosystems 7300 real-time PCR system. The relative gene expression was normalized against GAPDH gene expression. PCR primers were designed as shown in Table S1.

Histology. Tissues were fixed in Carnoy's fluid and embedded in paraffin. Then sections were prepared and stained with hematoxylin-eosin.

Score During DSS-Induced Colitis. The severity of diseases was scored as described (17).

Statistical Analyses. Data show the mean ± SE. Differences were evaluated by the Kaplan–Meier test (survival), two-way ANOVA followed by the Holm–Sidak post hoc test (airway hypersensitivity), the Mann–Whitney u test (score in EAE), or the two-tailed Student's t test (other studies, unless otherwise specified).

1. Murata T, et al. (2004) ang is a novel gene expressed in early neuroectoderm, but its null mutant exhibits no obvious phenotype. *Gene Expr Patterns* 5:171–178.
2. Ohno T, et al. (2009) Caspase-1, caspase-8, and calpain are dispensable for IL-33 release by macrophages. *J Immunol* 183:7890–7897.
3. Zabel BA, et al. (2008) Mast cell-expressed orphan receptor CCRL2 binds chemerin and is required for optimal induction of IgE-mediated passive cutaneous anaphylaxis. *J Exp Med* 205:2207–2220.
4. Nakae S, et al. (2002) Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity* 17:375–387.
5. Nakae S, et al. (2001) IL-1 α, but not IL-1 β, is required for contact-allergen-specific T cell activation during the sensitization phase in contact hypersensitivity. *Int Immunol* 13:1471–1478.
6. Suto H, et al. (2006) Mast cell-associated TNF promotes dendritic cell migration. *J Immunol* 176:4102–4112.
7. Nambu A, Nakae S, Iwakura Y (2006) IL-1β, but not IL-1α, is required for antigen-specific T cell activation and the induction of local inflammation in the delayed-type hypersensitivity responses. *Int Immunol* 18:701–712.
8. Williams CM, Galli SJ (2000) Mast cells can amplify airway reactivity and features of chronic inflammation in an asthma model in mice. *J Exp Med* 192:455–462.
9. Nakae S, et al. (2007) TNF can contribute to multiple features of ovalbumin-induced allergic inflammation of the airways in mice. *J Allergy Clin Immunol* 119:680–686.
10. Johnson JR, et al. (2004) Continuous exposure to house dust mite elicits chronic airway inflammation and structural remodeling. *Am J Respir Crit Care Med* 169:378–385.
11. Rydell-Törmänen K, Johnson JR, Fattouh R, Jordana M, Erjefält JS (2008) Induction of vascular remodeling in the lung by chronic house dust mite exposure. *Am J Respir Cell Mol Biol* 39:61–67.
12. Nakae S, et al. (2007) Mast cell-derived TNF contributes to airway hyperreactivity, inflammation, and TH2 cytokine production in an asthma model in mice. *J Allergy Clin Immunol* 120:48–55.
13. Matsuki T, Nakae S, Sudo K, Horai R, Iwakura Y (2006) Abnormal T cell activation caused by the imbalance of the IL-1/IL-1R antagonist system is responsible for the development of experimental autoimmune encephalomyelitis. *Int Immunol* 18:399–407.
14. Komiyama Y, et al. (2006) IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. *J Immunol* 177:566–573.
15. Lukic ML, Mensah-Brown E, Wei X, Shahin A, Liew FY (2003) Lack of the mediators of innate immunity attenuate the development of autoimmune diabetes in mice. *J Autoimmun* 21:239–246.
16. Tagawa Y, Sekikawa K, Iwakura Y (1997) Suppression of concanavalin A-induced hepatitis in IFN-γ⁽⁻/⁻⁾ mice, but not in TNF-α⁽⁻/⁻⁾ mice: Role for IFN-γ in activating apoptosis of hepatocytes. *J Immunol* 159:1418–1428.
17. Siegmund B, Lehr HA, Fantuzzi G (2002) Leptin: A pivotal mediator of intestinal inflammation in mice. *Gastroenterology* 122:2011–2025.
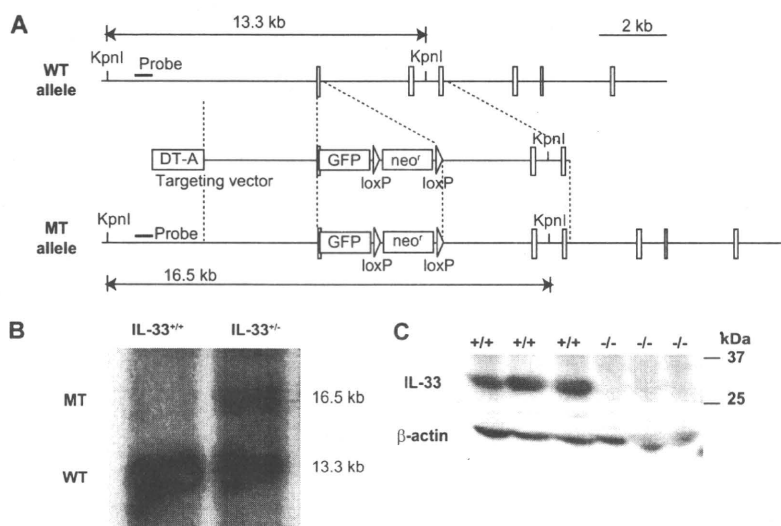
Fig. S1. Generation of IL-33-deficient mice. (*A*) IL-33 gene targeting strategy. The second exon containing a translational start codon was replaced with a tandemly arrayed promoter-less *GFP* gene and a floxed *neomycin resistance* gene (neoʳ) (targeted allele). (*B*) Southern blot analysis of genomic DNA obtained from wild-type or mutant ES cells. The DNA probes used for Southern blot analysis are shown in *A*. By digestion of genomic DNA with KpnI, the probes detected endogenous wild-type (WT; 13.3 kb) and/or targeted (MT; 16.5 kb) fragments. (*C*) Western blot analysis of whole-lung homogenates.

98
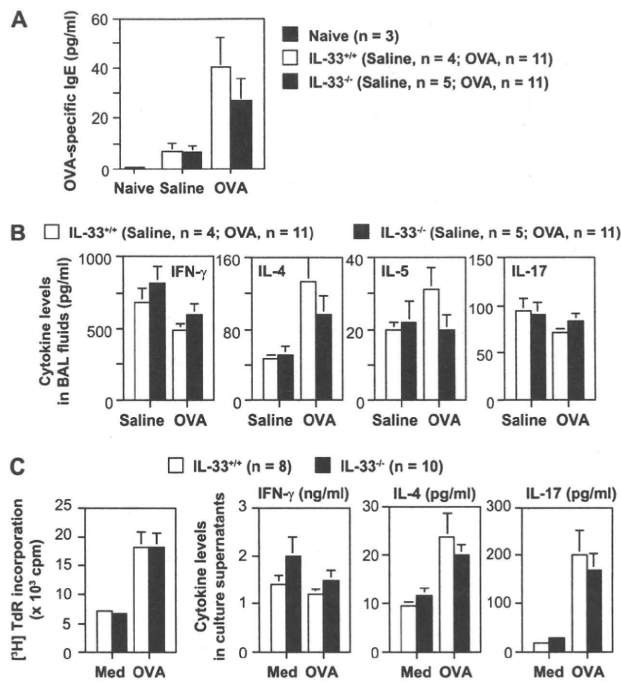
Fig. S2. IL-33 is not required for induction of antigen-specific T and B cell function. The levels of OVA-specific IgE in sera (*A*) and the levels of IFN-γ, IL-4, IL-5, and IL-17 in BAL fluids (*B*) from the mice shown in Fig. 1 or naïve mice. (*C*) OVA-specific proliferative responses and cytokine secretion of spleen cells from mice sensitized twice with OVA emulsified in alum. Data show the mean ± SE. No significant differences were found between IL-33$^{+/+}$ and IL-33$^{-/-}$ groups.
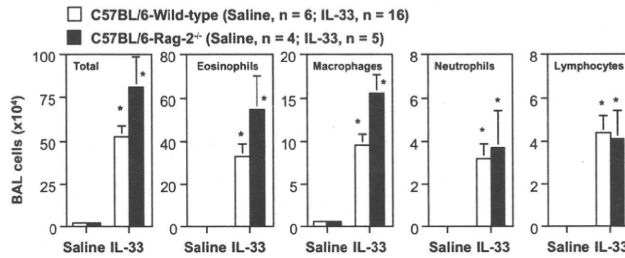
Fig. S3. IL-33 induces airway eosinophilia independently of T and B cells. At 24 h after the last IL-33 or saline inhalation, the number of inflammatory cells in BAL fluids from wild-type and Rag-2-deficient mice on the C57BL/6 background was determined. Data show the mean ± SE. *$P < 0.05$ vs. corresponding values for saline-treated mice.

99