
REVIEW

The Role of Interleukin-33 in Pathogenesis of Bronchial Asthma. New Experimental Data

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Abstract—Interleukin-33 (IL-33) belongs to the IL-1 cytokine family and plays an important role in modulating immune system by inducing Th2 immune response via the ST2 membrane receptor. Epithelial cells are the major producers of IL-33. However, IL-33 is also secreted by other cells, e.g., bone marrow cells, dendritic cells, macrophages, and mast cells. IL-33 targets a broad range of cell types bearing the ST2 surface receptor. Many ST2-positive cells, such as Th2 cells, mast cells, basophils, and eosinophils, are involved in the development of allergic bronchial asthma (BA). This suggests that IL-33 directly participates in BA pathogenesis. Currently, the role of IL-33 in pathogenesis of inflammatory disorders, including BA, has been extensively investigated using clinical samples collected from patients, as well as asthma animal models. In particular, numerous studies on blocking IL-33 and its receptor by monoclonal antibodies in asthma mouse model have been performed over the last several years; IL-33- and ST2-deficient transgenic mice have also been generated. In this review, we summarized and analyzed the data on the role of IL-33 in BA pathogenesis and the prospects for creating new treatments for BA.

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THE HISTORY OF IL-33 DISCOVERY

Interleukin-33 (IL-33) had been described by three independent research groups. In 1999, Onda et al. [1] were the first to identify IL-33 as a protein encoded by the *DVS27* gene that was activated during the cerebral artery spasm in a canine model of subarachnoid hemorrhage. It was found that *DVS27* codes for a nuclear protein, that turned out to be involved in inflammation. The proinflammatory role of *DVS27* was implied based on the fact that the level of the *DVS27* mRNA in human umbilical arterial smooth muscle cells (HUASMC) was upregulated in response to some proinflammatory factors. The authors also sequenced the *DVS27* gene and predicted the amino acid sequence of the encoded protein [1].

In 2003, Baekkevold et al. [2] independently described the NF-HEV protein (nuclear factor expressed

in human high endothelial venules), that was expressed in some endothelial cells. Sequencing of the corresponding gene revealed that it was similar to the *DVS27* gene, which suggested that human NF-HEV was homologous to canine *DVS27*. Moreover, murine orthologs of *NF-HEV* were found in the Ensembl genome database (www.ensembl.org). By aligning amino acid sequences of the human and mouse NF-HEV proteins (48% identity) and canine *DVS27* (56% identity), it was found that the NF-HEV/*DVS27* protein consists of two evolutionarily conserved regions separated by a variable fragment in the center of the open reading frame. Both Baekkevold et al. and Onda et al. demonstrated that the studied protein (NF-HEV and *DVS27*, respectively) was located in the nucleus, thereby suggesting that it might be a transcription regulator [2].

In 2005, IL-33 was identified as a member of the IL-1 cytokine family, that includes IL-1 α , IL-1 β , and IL-18 [3], based on the similarity of its 3D-structure to those of the IL-1 family proteins, and was classified as IL-1F11 or IL-33 according to the currently accepted nomenclature of interleukins [4, 5].

Abbreviations: a.a., amino acid residue; BA, bronchial asthma; BAL, bronchoalveolar lavage; bp, base pair; Ig, immunoglobulin; IL, interleukin.

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MOLECULAR AND GENETIC CHARACTERISTICS OF IL-33

In humans, the *IL-33* gene is located on chromosome 9p24.1, while its mouse counterpart can be found on the syntenic chromosome 19qC1 region [3]. The length of the coding sequence in the open reading frames of the human and mouse *IL-33* genes is 2641 and 2544 base pair (bp), respectively. The presence of exons in the *IL-33* genes suggests the possibility of the alternative splicing. Indeed, five alternatively spliced mRNA transcripts were identified for human and murine *IL-33* genes. The exons display high similarity in their lengths and positions in the open reading frames; however, the number of exons in the human and murine *IL-33* genes differs (seven and eight exons, respectively).

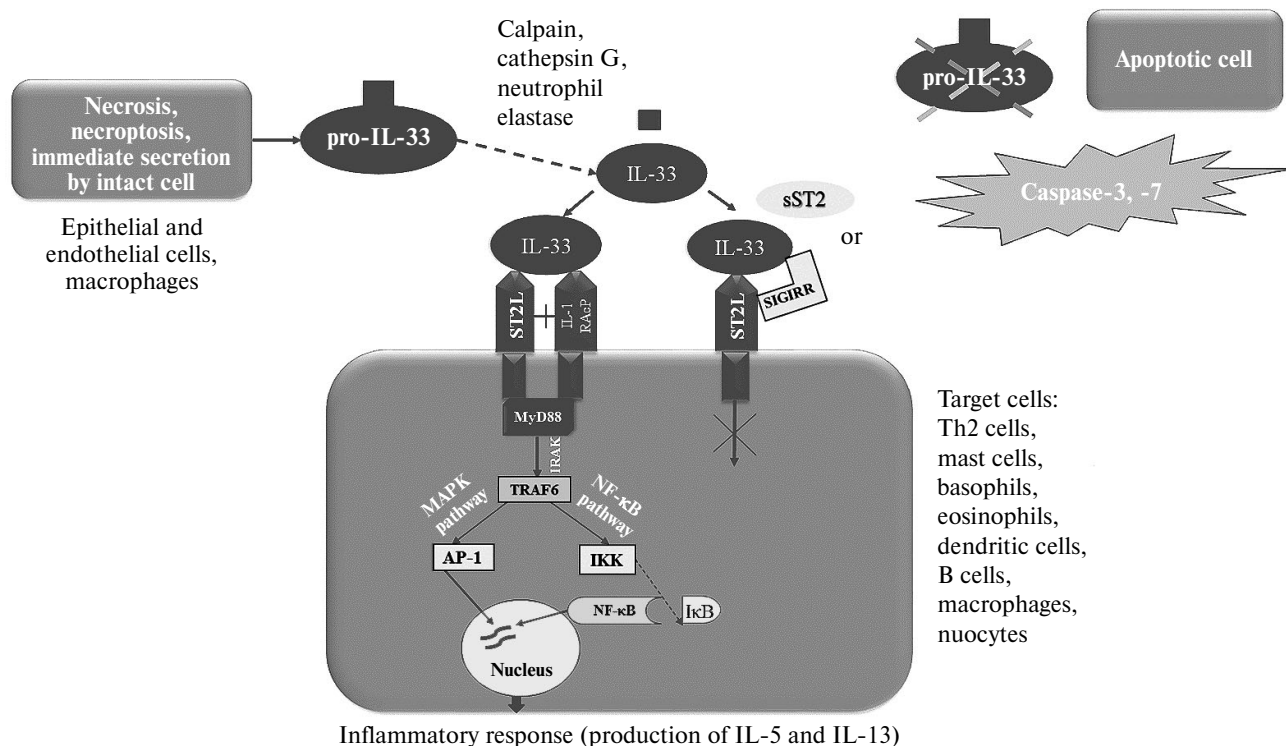
Human *IL-33* gene encodes the full-length IL-33 protein containing 270 amino acid residues (a.a.) with a calculated molecular weight of 30.759 kDa; mouse IL-33 consists of 266 a.a. and has a molecular weight of 29.992 kDa [3].

Schmitz et al. [3] deciphered the amino acid sequence of IL-33 and its tertiary structure that was later confirmed by Lingel et al. [6]. Nuclear magnetic resonance (NMR) studies of the recombinant human IL-33

provided more detailed insight into the IL-33 spatial structure and demonstrated that IL-33 adopts the β -trefoil fold, where 12 β -strands are arrayed in three pseudorepeats of four β -strand units, with two short α -helices ($\alpha 1$ and $\alpha 2$) preceding $\beta 8$ - and $\beta 12$ -strands. Similar β -trefoil structure was also found in all members of IL-1 superfamily [6, 7].

As mentioned above, IL-33 belongs to the IL-1 superfamily of regulatory cytokines [8, 9] that are secreted into the extracellular space during maturation. For the majority of cytokines belonging to this family, the mechanism of secretion and maturation includes synthesis of the inactive polypeptide precursor in the cytosol with subsequent proteolytic cleavage by caspases at specific sites with the formation of mature biologically active cytokine [10, 11].

It was found that IL-33 mRNA is translated into the full-length pro-IL-33 protein. It had been believed that recombinant pro-IL-33 protein may be cleaved *in vitro* by caspase 1 [3, 12]; however, no caspase-catalyzed cleavage of the IL-33 precursor was observed *in vivo* due to the lack of the caspase 1-specific cleavage site in pro-IL-33. Later, it was demonstrated that unlike other members of the IL-1 family, the full-length IL-33 exhibits its biological activity even in the absence of proteolytic processing [13, 14].



Signaling pathways, inactivation and negative regulation of IL-33 (see the texts for details): IL-1RAcP, IL-1R accessory protein; *MyD88*, myeloid differentiation primary response gene 88; IRAK, interleukin-1 receptor-associated kinase 1; TRAF6, TNF receptor-associated factor 6; MAPK, mitogen-activated protein kinase; NF- κ B, nuclear factor κ -light chain-enhancer of activated B cells; AP-1, activator protein 1; IKK, I κ B kinase; SIGIRR, single-immunoglobulin interleukin-1 receptor-related; ST2L, full-length transmembrane form of the ST2 receptor for IL-33; sST2, truncated soluble secreted form of ST2; I κ B, inhibitor of NF- κ B transcription factor

For example, extracellular IL-33 may act as an endogenous alarm signal in response to the mechanical strain on the plasma membrane [15], whereas inside the cells, the full-length IL-33 can translocate into the nucleus, bind to heterochromatin, and act as a transcriptional repressor [16]. Nevertheless, some proteases, such as calpain [17], neutrophil elastase, and cathepsin G [18], are able to process IL-33 to shorter biologically active fragments. Treatment of the full-length IL-33 with neutrophil elastase and cathepsin G produces mature IL-33 with ~10-times higher biological activity than the precursor molecule [18].

During apoptosis, the full-length IL-33 is cleaved by apoptotic proteases (caspases 3 and 7) with the formation of biologically inactive products [14]. Therefore, IL-33 can exhibit its biological activity as the full-length protein (endogenous alarm signal, intracellular transcription regulator) or the processed form that acts as a classic cytokine via complex formation with the corresponding receptor and regulation of the cell signaling pathways (figure).

IL-33 RECEPTOR COMPLEX AND SIGNALING PATHWAYS

IL-1 family cytokines (including IL-33) manifest their biological activity via interacting with the Toll-like IL-1 receptors (TLR-IL-1R) containing intracellular TIR (Toll/interleukin-1 receptor) domains [19]. It was found that IL-33 is a ligand of the membrane Toll-like ST2 receptor (also known as IL-33R, IL-1R4) [3]. However, in addition to ST2, the fully functional IL-33 receptor complex also contains the accessory IL-1RAcP polypeptide (IL-1R accessory protein) that acts as a co-receptor [20]. It was shown that IL-1RAcP binds IL-33 with a low affinity and participates in the formation of the fully functional complex only after IL-33 has interacted with ST2 [6]. Therefore, the IL-33 receptor (IL-33R) represents an ST2/IL-1RAcP heterodimer.

The detailed structure of IL-33R and its properties have been studied by several research groups. In particular, it was found that the extracellular ectodomain of ST2 consists of three IgG-like domains (D1-D3), where D1 and D2 domains form a single D1D2 module linked to the D3 domain. When ST2 forms complex with IL-33, all three IgG-like domains wrap around IL-33 like a grasping hand, after which the IL-33/ST2 complex interacts with the IL-1RAcP extracellular ectodomains [6].

After the fully functional IL-33/ST2/IL-1RAcP complex is assembled, it transduces signals via the IL-1RAcP TIR domain [20]. IL-33R signaling is mediated by the adaptor molecules common for the IL-1 receptor family, such as MyD88 (myeloid differentiation primary response gene 88) protein. MyD88 accumulation leads to the TRAF6 (TNF receptor-associated factor 6) acti-

vation through phosphorylation by IRAK (interleukin-1 receptor-associated kinase). In its turn, activated TRAF6 stimulates two independent signaling pathways: (i) the MAPK (mitogen-activated protein kinase) pathway and (ii) the NF- κ B-pathway [21, 22]. Signal transduction via the MAPK pathway results in the activation of the AP-1 (activator protein 1) transcription factor that enters the nucleus and regulates activity of certain genes. In the NF- κ B-pathway, IKK (I κ B kinase) phosphorylates the inhibitor of NF- κ B, thereby inducing its degradation. After being liberated from the complex with the inhibitor protein, activated NF- κ B modulates the activity of IL-33-regulated genes via its DNA-binding domain [21]. The involvement of IL-33 in the NF- κ B-pathway distinguishes it from the classic Th2 cytokines that exhibit their biological activity through the JAK-STAT pathway.

It is known that TRAF6 is essential for the NF- κ B signaling; however, IL-33 also mediates TRAF6-independent ERK activation, after which activated ERK can enter the nucleus and initiate transcription of certain genes. Moreover, IL-33 can activate AP-1 independently of the NF- κ B pathway [21].

As mentioned above, the fully functional IL-33 receptor complex contains ST2 and IL-1RAcP polypeptides. However, alternative splicing of the ST2 mRNA can generate two protein products: (i) the full-length ST2 transmembrane receptor (also designated as ST2L) described earlier and (ii) the short soluble secreted ST2 isoform (sST2) [23]. The full-length transmembrane ST2L is a functional component of IL-33R that binds IL-33 and mediates its biological activity. Since the soluble sST2 isoform (expressed by fibroblasts and mast cells) still contains three extracellular IgG-like domains (D1-D3), it is capable of IL-33 binding, but not of anchoring in the cell membrane and downstream signal transduction [23]. Therefore, the sST2 isoform can sequester IL-33 in the extracellular medium and negatively regulate the IL-33 effects [22]. Also, IL-33 can be negatively regulated by SIGIRR (single-immunoglobulin interleukin-1 receptor-related) [24] that suppresses signal transduction. In particular, SIGIRR forms a complex with ST2, thereby inhibiting the IL-33/ST2 interactions and, hence, the downstream signaling [22]. Inhibition of the IL-33/ST2 signaling pathway by SIGIRR was confirmed both *in vitro* and *in vivo* [24].

IL-33-EXPRESSING CELLS

Quantitative PCR analysis of human and murine cDNA libraries revealed that IL-33 mRNA is present in many tissue types. In mice, the highest level of IL-33 mRNA was observed in the spinal cord, brain, lungs, skin, and stomach. Lower amounts of IL-33 mRNA were also found in the lymph nodes, kidneys, spleen, and heart [3].

More detailed experiments identified IL-33 mRNA in various cell types, such as bone marrow cells, dendritic cells, LPS (lipopolysaccharide)-stimulated macrophages [3], and IgE-stimulated mast cells [25]. Glial cells and astrocytes express IL-33 mRNA after activation with TNF, IL-1, and a combination of double-stranded RNA (dsRNA) with LPS [26]. Peritoneal macrophages and splenic dendritic cells stimulated with LPS also express IL-33 mRNA [27].

Expression of the IL-33 mRNA was studied in mice with allergic bronchial asthma induced by ovalbumin (OVA) as a model allergen. IL-33 mRNA was found in the lungs, lymph nodes, thymus, and in female and male reproductive organs [28]. The expression profiles of the IL-33 gene were also examined in other experimental models. In particular, IL-33 mRNA expression was observed in the synovial fluid collected from the inflammation site in a murine arthritis model [29].

In humans, IL-33 expression was examined in clinical samples from patients with various disorders. Thus, upregulated IL-33 mRNA was found in the skin endothelial cells of patients with atopic dermatitis [30], heart and coronary arteries of patients with congestive cardiomyopathy [31], brain tissues of patients with Alzheimer's disease [32], and lung tissues of patients with bronchial asthma (BA) [33, 34]. Because respiratory viral infections are among the main triggers of BA [35, 36], IL-33 expression was also investigated in human subjects with rhinovirus-induced BA complications. It was shown that type 16 rhinovirus is able to induce IL-33 production in the nasal mucosa (as determined by a novel airway sampling technique) [37].

Therefore, epithelial and endothelial cells and macrophages are the major IL-33-producing cells *in vivo*. Mast cells continuously express low levels of IL-33 protein. This expression is significantly upregulated by the IgE-mediated stimulation of these cells via the IgE/FcεRI signaling and does not depend on the ST2-mediated signaling [38]. Because lung tissue is one of the major sites of IL-33 biosynthesis in humans, it is reasonable to assume that it is involved in various airway inflammatory pathologies, such as BA and respiratory viral infections.

CELL TARGETS OF IL-33

Because IL-33 exhibits biological activity through binding to the ST2 receptor, the targets of IL-33 should be cells with constitutive surface expression of ST2.

Th2 cells. Type 2 T helper cells (Th2 cells) express proinflammatory cytokines, such as IL-4, IL-5, and IL-13, and play a crucial role in pathogenesis of allergic disorders, including BA [39, 40]. IL-33 can affect these cells either directly or indirectly. Several Th cell subsets differentiating from naïve CD4⁺ T lymphocytes have been

described. Th2 cells, which express ST2, represent one of the most important targets for IL-33. ST2 is not detected on naïve T cells, Th1, Treg, and Th17 cells [41, 42]. It is known that naïve CD4⁺ T cells differentiate into Th2 cells after exposure to IL-4 and transcription factors STAT6 and GATA3 [33]; however, Th cells expressing ST2 were found in mice deficient by the *IL-4* gene [41]. These data suggest that naïve Th0 cells can differentiate into Th2 cells in response to IL-33 by the IL-4-independent mechanism. Furthermore, Th2 cells polarized by IL-33 were able to produce IL-5 and IL-13 (independently of GATA3, STAT6, and IL-4), but not IL-4 and were named atypical Th2 cells [33]. Differentiation of Th2 cell is not affected in the ST2- and IL-33-deficient mice [43, 44]. Interestingly, in humans, IL-33 activates expression not only of Th2 cytokines, but Th1-cytokines (e.g., IFNγ) as well [45]. Nevertheless, the role of IL-33 in Th0 cell differentiation into Th2 cells has not been fully elucidated.

Mast cells. Mast cells are found in many tissues, especially those that contact with the environment (e.g., epithelium). When activated, mast cells secrete a large number of soluble mediators and ensure a rapid organism's response to tissue or cell damage, often resulting in inflammation. Mast cells bind IgE antibodies with high affinity via the surface FcεRI receptors. Formation of the FcεRI–IgE complex induces mast cell degranulation and subsequent inflammatory response.

ST2 is expressed on the surface of human and murine mast cells. In humans, ST2 expression is upregulated by the GATA2 transcription factor. IL-33 interacts with ST2 at the surface of mast cells and promotes production of cytokines (IL-1β, IL-6, IL-8, IL-13, TNFα), chemokines (CCL1 and CXCL8), and growth factors (granulocyte macrophage colony stimulating factor) by activating the NF-κB transcription factor. *In vitro* experiments confirmed that IL-33 has an impact on mast cells; in particular, incubation of CD34⁺ cells with IL-33 accelerates maturation of mast cells [46, 47]. Despite the fact that mast cells respond to IL-33, this cytokine *per se* does not cause mast cell degranulation, neither histamine and leukotriene release (unlike the IgE-mediated mechanism observed in sensitized persons) [48].

Basophils. Similarly to mast cells, basophils contain granules filled with proinflammatory mediators and play a significant role in allergic inflammation. Basophils rapidly migrate from the peripheral circulatory system into various body tissues (e.g., lungs during allergic BA) and release inflammatory mediators, such as IL-4, IL-5, IL-8, and IL-13. Both basophils and mast cells express surface FcεRI for binding IgE and ST2 for binding IL-33. Unlike Th2 and mast cells, human and murine basophils express ST2 at much lower levels [49]. However, IL-33 exerts a pronounced biological effect on basophils. In particular, IL-33 stimulates expression of IL-4, IL-5, IL-8, and IL-13 by human basophils *in vitro*. At the same

time, IL-33 directly activates murine basophils for preferential IL-6 secretion [45, 49].

Eosinophils. Eosinophils are proinflammatory granulocytes that play a key role in the antiparasitic immunity. They are also involved in pathogenesis of BA. Eosinophils are primarily located in the mucosal tissues, but can also be found in the peripheral circulatory system [50].

Only insignificant amounts of ST2 molecules were found on the plasma membrane surface in eosinophils. Despite this fact, IL-33 can influence eosinophils by inducing production of inflammatory mediators, such as CCL2/MCP-1 and CXCL8/IL-8. Moreover, IL-33 prolongs eosinophil life cycle, promotes superoxide production, and enhances their adhesion to the blood vessel endothelium by upregulating the CD11b expression. Elevated adhesion ensures efficient exit of eosinophils from the circulation into the lung bronchoalveolar space, where they exhibit their damaging effects [51]. *In vivo* studies confirmed that IL-33 has an impact on eosinophils; in particular, systemic administration of IL-33 to mice resulted in the development of IL-5-dependent eosinophilia in the lungs of experimental animals [52]. However, IL-33 does not trigger eosinophil degranulation [49].

Dendritic cells. Dendritic cells are located in the submucosal layer in a close contact with the airway epithelial cells. They capture antigens that enter the submucosal layer during respiration and present them to naïve lymphocytes. Therefore, dendritic cells are important mediators involved in the adaptive immune response initiation.

It was found that IL-33 regulates maturation of the bone marrow-derived dendritic cells by upregulating costimulatory molecules, such as CD40, CD80, and OX40L, but not CD86 or MHC class II molecules [53]. However, other studies demonstrated that IL-33 upregulates the surface expression of MHC class II molecules and CD86 in dendritic cells [54]. Therefore, by promoting expression of the co-stimulatory molecules, IL-33 shifts the immune response toward the Th2 type.

B cells. B cells are subdivided into a smaller subpopulation of B1 cells and major subpopulation of B2 cells. B1 lymphocytes include CD5⁺ B1a and CD5⁻ B1b cells located in the peritoneal and pleural cavities. B1 cells are responsible for the immune response to thymus-independent antigens and facilitate induction of the innate immunity [55]. Out of the entire B cell population, only B1 cells express ST2. *In vitro* experiments showed that IL-33 upregulates production of IgM antibodies and Th2 cytokines (IL-5 and IL-13) by B1 cells [56]. However, IL-33-mediated B1 cell proliferation and IgM biosynthesis requires IL-5, which is one of the major cytokines in B1 cell development [57].

Macrophages. Murine peritoneal, alveolar, and bone marrow-derived macrophages express ST2 and, therefore, can be targeted by IL-33 [58]. It was found that IL-

33 changes the phenotype of alveolar macrophages by reprogramming them toward alternatively activated M2 macrophages that express mannose receptor and IL-4RA and produce chemokines CCL24 and CCL17, thereby playing an important role in the development of allergic inflammation [59]. IL-33 does not directly activate cytokine production by the macrophages, but promotes expression of TLR2, TLR4, MD2, CD14, and MyD88 on peritoneal macrophages that eventually results in the elevated secretion of IL-6 and/or TNF [60].

Nuocytes. Recently, a new type of cell was discovered – innate lymphoid cells (ILCs), also known as lung natural helper cells or nuocytes. Nuocytes were identified as a separate cell type in an attempt to find new producers of Th2 cytokines, particularly, IL-13. It was found that administration of IL-13 and IL-25 to mice of the IL13eGFP line specifically developed for the study promoted emergence of eGFP⁺ cells in the mesenteric lymph nodes. The majority (80%) of these cells could not be assigned to any of the known cell lineages (T cells, B cells, natural killers, dendritic cells, neutrophils, eosinophils, mast cells, basophils, or macrophages) based on their surface markers [61].

More detailed studies demonstrated that nuocytes are located in the lung tissue, where they directly contact with bronchial epithelial cells. The number of nuocytes increases with the development of pulmonary allergic inflammation. Also, bronchial epithelial cells, macrophages, and dendritic cells release markedly elevated amounts of IL-33 during pulmonary allergic inflammation or respiratory viral infections [62, 63]. Because nuocytes have ST2 on their surface, they become activated in response to IL-33 and produce significant amounts of IL-5 and IL-13, which enhances the Th2 immune response to allergens and therefore, exacerbates BA symptoms, such as airway hyperreactivity and pulmonary eosinophilic infiltration [64]. The pivotal role of nuocytes in the development of respiratory system pathologies was demonstrated in the model of BA in mice sensitized with ovalbumin [65] and house dust mites (HDM) [63]; it was confirmed that IL-33-treated nuocytes were able to produce significant amounts of IL-5 and IL-13 and elicit airway hyperreactivity.

Other cell types. Apart from the above-mentioned cell types, there are other cells that bear the surface ST2 and can interact with IL-33, such as epithelial and endothelial cells, fibroblasts, glial cells, astrocytes, etc. [66].

Considering that IL-33 induces expression of proinflammatory cytokines (IL-5 and IL-13) and enhances allergen-specific Th2 immune response and that ST2 is found on the majority of cell types involved in allergic inflammation (eosinophils, basophils, mast cells, etc.), it is reasonable to conclude that IL-33 plays an important role in BA pathogenesis and might be a promising target for designing novel therapeutic approaches.

The role of IL-33 in allergic bronchial asthma

Experimental model	Results	Reference
1	2	3
Administration of exogenous IL-33		
BALB/c mice. OVA/adjuvant-induced pulmonary inflammation. IL-33 was administered during allergen sensitization	– upregulated expression of IL-5 and IL-13 in naïve CD4⁺ T cells stimulated with anti-CD3 antibodies in combination with IL-33; – elevated total cell count in the BAL fluid and pronounced infiltration of eosinophils and macrophages into the BAL fluid	[33]
BALB/c RAG2 ^{-/-} mice. IL-33 was administered intranasally (i.n.) for 4 days	– marked bronchial hyperreactivity in IL-33-treated mice; – elevated count of eosinophils and neutrophils in the BAL fluid; – upregulated expression of IL-4, IL-5 and IL-13 mRNAs in the lung tissue several hours after the last administration of IL-33	[79]
BALB/c ST2 ^{-/-} mice. Eosinophils purified from the peritoneal cavity of mice treated with IL-33 for 7 days were applied i.n. in intact mice. After that, the mice received i.n. administration of IL-33 for another 3 days	in mice that received i.n. administration of eosinophils: – high levels of eosinophils, macrophages, lymphocytes and high total cell count in the BAL fluid; – upregulated levels of CCL17, IL-13, CCL11, CCL24, TGF-β	[80]
C57BL/6 mice. OVA-induced pulmonary inflammation. IL-33 was applied i.n. 4 times during BA development and in intact animals	in mice that received i.n. administration of IL-33: – sharply elevated counts of eosinophils, neutrophils, macrophages, and lymphocytes in the lung tissue and the BAL fluid; – induction of pronounced bronchial hyperreactivity; – upregulated expression of the collagen <i>Col1a1</i>, <i>Col3a1</i>, and <i>Col5a1</i> genes and lung tissue remodeling; – elevated level of allergen-specific IgE antibodies; – upregulated IL-4 expression	[81]
IL-33 overexpression		
IL-33-overexpressing transgenic mice	in IL-33-overexpressing transgenic mice: – inflammatory foci in the lung tissue, mainly around blood vessels and alveoli; – goblet cell hyperplasia and mucus accumulation; – elevated counts of eosinophils, lymphocytes, monocytes, and neutrophils in the BAL fluid; – elevated level of serum IgE antibodies; – upregulated expression of IL-33 and soluble ST2 in the BAL fluid; – elevated levels of IL-5, IL-13, IL-8, and IL-10 in the BAL fluid	[82]
Effects of anti-ST2 and anti-IL-33 monoclonal antibodies (mAbs) <i>in vivo</i>		
BALB/c mice. OVA/adjuvant-induced pulmonary inflammation. Anti-ST2 mAbs were administered during allergen sensitization and provocation	in anti-ST2 antibody-treated mice: – decreased count of eosinophils in the BAL fluid; – reduced level of IL-5 in the BAL fluid; – reduced level of OVA-specific IgE antibodies	[84]
C57BL/6 and BALB/c mice. OVA/adjuvant-induced pulmonary inflammation. Anti-ST2 mAbs were administered during allergen provocation	in anti-ST2 antibody-treated mice: – reduced bronchial hyperreactivity; – reduced levels of IL-4 and IL-13 in the BAL fluid	[70]
BALB/c mice. OVA/adjuvant-induced pulmonary inflammation. Polyclonal anti-IL-33 Abs were administered during allergen sensitization and provocation	in anti-IL-33 antibody-treated mice: – lowered total cell count in the BAL fluid; – decreased counts of eosinophils and lymphocytes in the BAL fluid; – lack of infiltrated eosinophils and lymphocytes in peribronchial and alveolar areas in the lung tissue; – reduced levels of IL-4, IL-5, IL-13 in the BAL fluid; – reduced level of total and allergen-specific IgE antibodies in the blood serum	[67]

Table (Contd.)

1	2	3
BALB/c mice. OVA-induced pulmonary inflammation. Anti-IL-33 mAbs were applied once after allergen provocation	in anti-IL-33 antibody-treated mice: – downregulated expression of IL-33 mRNA; – decreased counts of eosinophils and neutrophils in the lung tissue; – suppressed production of proinflammatory cytokines in alveolar macrophages	[78]
BALB/c mice. OVA-induced pulmonary inflammation. Anti-IL-33 mAbs were administered once after allergen provocation	in anti-IL-33 antibody-treated mice: – decreased counts of neutrophils in the lung tissue; – reduced levels of proinflammatory cytokines TNF- α and CXCL1	[85]
BALB/c mice. HDM (house dust mite-induced) pulmonary inflammation. Vaccination by recombinant IL-33 or carrier protein, 3 times at two week-intervals	in mice treated with IL-33+HDM or IL-33+PBS: – markedly suppressed airway hyperreactivity; – decreased eosinophilic infiltration at the site of inflammation; – reduced airway epithelial hyperplasia; – reduced levels of TSLP, IL-25 and IL-17A	[86]
Airway inflammation in <i>ST2</i> -deficient mice		
129xB6 <i>ST2</i> ^{-/-} mice. OVA/adjuvant-induced pulmonary inflammation	in <i>ST2</i> ^{-/-} mice: – unaltered allergen-specific Th2-immune response; – Th2 cells differentiated from Th0 cells; – eosinophilic inflammation in the lung tissue; – high levels of total IgG1 and IgE antibodies in the blood serum	[43]
BALB/c <i>ST2</i> ^{-/-} mice. OVA/adjuvant-induced pulmonary inflammation	in <i>ST2</i> ^{-/-} mice: – unaltered differentiation of Th2 cells from Th0 cells; – unaltered production of Th2 cytokines by stimulated Th2 cells	[87]
BALB/c <i>ST2</i> ^{-/-} mice. OVA/adjuvant-induced pulmonary inflammation	in <i>ST2</i> ^{-/-} mice: – decreased counts of eosinophils and macrophages in the BAL fluid; – reduced levels of IL-5 (but not IL-4 and IL-13) in the BAL fluid; – decreased pulmonary inflammation	[59]
BALB/c <i>ST2</i> ^{-/-} mice. OVA/adjuvant-induced pulmonary inflammation	in <i>ST2</i> ^{-/-} mice: – decreased counts of eosinophils and macrophages in the BAL fluid; – reduced levels of IL-4, IL-5, IL-13, CXCL1 in the BAL fluid; – decreased pulmonary inflammation; – decreased level of serum IgE antibodies	[27]
BALB/c <i>ST2</i> ^{-/-} mice. HDM-induced pulmonary inflammation	in <i>ST2</i> ^{-/-} mice: – decreased methacholine-induced pulmonary hypersensitivity; – reduced infiltration of inflammatory cells into the BAL fluid and lung tissue; – decreased airway epithelial hyperplasia; – decreased level of serum allergen-specific IgE antibodies; – downregulated expression of cytokines involved in the airway inflammation (IL-1b, IL-5, IL-13, IL-33, GM-CSF, TSLP)	[88]
Airway inflammation in <i>IL-33</i> -deficient mice		
Novel <i>IL-33</i> knockout mouse strain. OVA/adjuvant induced pulmonary inflammation	in <i>IL-33</i> -deficient mice: – reduced infiltration of eosinophils and lymphocytes into the BAL fluid; – reduced bronchial hyperreactivity; – decreased pulmonary inflammation; – decreased serum IgE level; – downregulated expression of IL-4, IL-5 in peripheral blood	[89]
Novel <i>IL-33</i> knockout mouse strain. OVA/adjuvant induced pulmonary inflammation	in <i>IL-33</i> -deficient mice: – reduced mucus hypersecretion by bronchial epithelium; – decreased pulmonary inflammation; – lowered eosinophil count in the BAL fluid; – decreased levels of IL-5 and IL-13, but not IL-4, in the lung tissue	[90]

THE ROLE OF IL-33 IN PATHOGENESIS OF BRONCHIAL ASTHMA

Bronchial asthma (BA) is a chronic inflammatory airway disorder characterized by relapsing bronchoconstriction and structural changes in the bronchi [66, 67]. Episodic dyspnea, wheezing, bronchial spasm, and breathing difficulty are among the most typical manifestations of BA. Allergic BA (~70-80% total BA cases) is characterized by the elevated content of total and allergen-specific IgE antibodies in the blood serum and high eosinophil count in the circulation, airway mucosal layers, and bronchoalveolar lavage (BAL) fluid [68-70].

BA is among the most common chronic airway disorders affecting both adults and children. A strikingly high number of children with severe BA is recorded in the USA and Europe. The highest severe BA incidence rates among adults (aged 15-74) are documented in Germany, France, Sweden, Portugal, and Italy [71]. The BA mortality rate varies broadly in different age groups and geographical regions. In 2010, the highest BA mortality rate was recorded in Oceania, Southeast Asia, and North Africa, whereas globally this parameter was 13 and 9 deaths per 100,000 people for males and females, respectively [72]. Apart from the high mortality rate, BA causes significant economic losses due to the deteriorated quality of patients' life and decreased performance.

At present, BA is treated by a symptomatic therapy using antihistamines, antileukotriene agents, and topical corticosteroids. Allergen-specific immunotherapy represents the only pathogen-related therapeutic approach for treating BA [73]. However, based to the ongoing rise in the morbidity rate, the available treatments are apparently insufficient. Because of wide occurrence of BA among different social groups, high mortality rate, and substantial economic costs, development of new safe approaches for treatment and prevention of BA is an urgent task for the healthcare services worldwide. However, the development of new treatments for BA is impossible without unraveling molecular mechanisms underlying pathogenesis of this disease.

According to the current understanding, the molecular mechanism of the development of allergic BA includes two stages: sensitization (primary encounter with an allergen) and effector stage (repeated encounter with an allergen). During the primary encounter, an allergen enters the body through epithelial lesions and undergoes presentation in the complex with the MHC class II molecules expressed on the antigen-presenting cells (APCs). After contacting the allergen, mature APCs migrate into the regional lymph nodes and activate naïve Th0 cells by forming immune synapses with the involvement of the costimulatory molecules CD80, CD86, CD28, CD2, and LFA-3. After activation with certain types of cytokines, Th0 cells differentiate into Th2 cells that produce Th2 cytokines (IL-4, -5, -9, -13) responsible for the develop-

ment of major BA symptoms. However, the mechanism of naïve Th0 cell differentiation into Th2 cells has not been yet fully understood. According to one hypothesis, nucleocytes producing cytokines IL-5 and IL-13 might play a key role in this process; IL-13 shifts the immune response towards the Th2 type. Simultaneously, allergen is recognized in the regional lymph nodes by the B cells via the B cell receptor (BCR), which leads to the activation of B cells and promotes their differentiation into plasma cells producing antigen-specific antibodies. Th2 cytokines produced by the Th2 cells interact with the B cells and switch the antibody production from IgM to IgE that mediate the allergic response *in vivo* [74].

During the second (effector) stage, IgE antibodies bind to the FcεRI and FcεRII receptors on mast cells and basophils. Upon the repeated encounter with the immune system, the allergen interacts with specific IgE antibodies already bound to the surface of mast cells and basophils and promotes degranulation of these cells and release of proinflammatory mediators into the extracellular space. The effects of the mediator release are: (i) chemoattraction, recruitment of other cell types, including eosinophils, neutrophils, lymphocytes and other mononuclear cells, to the site of activation; (ii) activation of inflammation; proinflammatory factors cause blood vessel dilation, edema and (via the platelet activating factor, PAF) microthrombosis and local tissue damage; (iii) spasmogenic effect: spasmogenic factors directly act on smooth muscles, e.g., in the bronchi, causing their contraction and subsequent bronchoconstriction [73].

Simultaneously, the Th2 cells exit from the circulation via the chemokine receptors and enter the site of inflammation, where they are activated by the antigen and secrete IL-4, IL-5, IL-9, and IL-13. By acting on the bronchial epithelium, cytokines IL-4, IL-9, and IL-13 facilitate mucus hypersecretion. IL-5 promotes recruitment of eosinophils to the site of inflammation and their subsequent activation. Activated eosinophils degranulate and release inflammatory mediators that cause the damage of the surrounding tissues [39, 74].

Because IL-33 promotes allergen-specific Th2 immune response and activates various cell types involved in BA pathogenesis (mast cells, basophils, eosinophils, etc.), this cytokine has been extensively investigated, mainly in clinical samples (peripheral blood, BAL fluid, bronchial biopsy) from BA patients and murine asthma models [75, 76]. Thus, it was demonstrated that the IL-33 levels in the lung tissue and epithelial cells from patients with allergic BA were significantly higher than in healthy people [34]. Later, these data were confirmed by Yagami et al., who demonstrated that in the lung tissue, IL-33 is mainly produced by epithelial and endothelial cells rather than smooth muscle cells or fibroblasts [77]. Bunting et al. showed that upregulated IL-33 expression results in the macrophage activation and subsequent cytokine release [78].

The role of IL-33 in the molecular mechanism of BA pathogenesis has been investigated in detail in animal models. Schmitz et al. were the first to discover that eosinophilia might be induced by IL-33 administration in mice [3]. Later, another study reported that treatment of mice with induced allergic asthma with IL-33 exacerbated inflammation in the lung tissue even in the animals with the knocked out *IL-4* gene [33]. Similar study demonstrated that intranasal administration of IL-33 in mice resulted in the development of BA symptoms, such as bronchial hyperreactivity, bronchial goblet cell hyperplasia, lung tissue eosinophilia, and elevated production of proinflammatory cytokines IL-4, -5, -13 in the lungs [79-81]. Moreover, spontaneous development of some BA symptoms, particularly, eosinophilic inflammation in the lungs, bronchial goblet cell hyperplasia and increased amount of proinflammatory cells in BAL fluid, was observed in transgenic mice overexpressing IL-33 in the lungs [82]. Recently, Han et al. demonstrated that intradermal application of the recombinant IL-33 in combination with a model allergen in mice resulted in the manifestation of BA symptoms accompanied by the cell infiltration into the BAL fluid, elevated levels of allergen-specific serum IgE antibodies, and accumulation of mucus-secreting cells in the airways after exposure to the allergen [83].

Experiments on the suppression of IL-33 activity provided a significant insight into the role of IL-33 in BA pathogenesis. Most of these experiments used monoclonal antibodies to block the function of IL-33 in animals. Thus, anti-ST2 antibodies inhibited production of Th2 cytokines, decreased eosinophil count in the lung tissue, and suppressed bronchial hyperreactivity [84]. Inhibition of IL-33 with monoclonal antibodies also resulted in the suppression of pulmonary inflammation [78, 85, 86]. Mice lacking the *ST2* gene displayed less pronounced BA symptoms than the wild-type counterparts, although the deficit of the *ST2* gene did not fully eliminate all BA symptoms [87, 88].

The direct involvement of IL-33 in BA pathogenesis was proven in the studies in *IL-33*-deficient mice. Oboki et al. showed that *IL-33*^{-/-} mice with induced allergic BA had a profoundly reduced count of eosinophils and lymphocytes in the BAL fluid and decreased bronchial hyperreactivity as compared to the mice with intact *IL-33* gene. However, the levels of IL-4 and IL-5 in the BAL fluid and the amount of allergen-specific IgE antibodies in the blood serum were only insignificantly lowered in both the *IL-33*-deficient and wild type mice [89]. Similar results were obtained by Louten et al., who demonstrated that induction of the airway allergic inflammation in *IL-33* knockout animals was characterized by lower levels of eosinophilic inflammation and bronchial mucus secretion, as well as reduced contents of IL-5 and IL-13, but not IL-4, in the BAL fluid [90]. The major studies on the role of IL-33 in BA pathogenesis in murine *in vivo* models are summarized in the table.

IL-33 is one of the most important Th2 cytokines involved in the immune response regulation. It activates both innate and adaptive immune responses. Experiments in murine models, including *IL-33*- and *ST2*-deficient animals, confirmed that this cytokine plays a key role in the development of allergic response in the lungs, as the suppression of these genes resulted in a markedly reduced allergen-specific Th2 response and, consequently, alleviation of major BA symptoms.

However, the results of studies on the role of the ST2/IL-33 signaling axis in the induction of the OVA-mediated airway inflammation in *ST2*-deficient mice remain controversial. In particular, no significant reduction in BA manifestations was observed in the *ST2*-deficient mice in some studies [43, 87], which suggests other mechanisms underlying BA development. At the same time, in a series of studies, the blockade of cytokine IL-33 was more efficient in suppressing the BA symptoms [89, 90] than the blockade of its receptor ST2 or other cytokines, e.g., Th2 cytokines IL-4 and IL-13. This is related to the fact that IL-33 upregulates expression of IL-5 and IL-13 that are involved in the development of major BA symptoms, such as bronchial hyperreactivity, bronchial mucus hypersecretion, and lung tissue eosinophilia. Altogether, these data make IL-33 a promising target for developing new therapeutic approaches to BA therapy.

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