IL-33 induces innate lymphoid cell–mediated airway inflammation by activating mammalian target of rapamycin

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Background: The IL-1 family cytokine IL-33 is involved in the induction of airway inflammation in allergic patients and after viral infection. Several cell types, including $CD4^+$ T_H2 cells and the recently described type 2 innate lymphoid cells (ILCs), are targets for IL-33, yet the mechanisms by which this cytokine modulates their activation are not clear.

Objectives: Our goal was to investigate a role for mammalian target of rapamycin (mTOR) signaling in the activation of T_H2 and ILC responses and the induction of airway inflammation by IL-33.

Methods: We biochemically determined the effect of IL-33 on mTOR activation in T_H2 cells and ILCs and examined the effect of this signaling pathway *in vivo* using a murine model of IL-33–induced lung inflammation.

Results: We found that IL-33 induces mTOR activation through p110δ phosphoinositide 3-kinase and that blockade of the mTOR pathway inhibited IL-33–induced IL-5 and IL-13 production by T_H2 cells and ILCs. Furthermore, use of a ribosomal protein S6 kinase 1 inhibitor implicated a role for ribosomal protein S6 kinase 1 in IL-33–induced mTOR-dependent cytokine production. Intranasal administration of IL-33 to wild-type mice induced airway inflammation, whereas adoptive transfer of wild-type ILCs to IL-33 receptor–deficient *($St2^{-/-}$)* mice recapitulated this response. Importantly, coadministration of the mTOR inhibitor rapamycin reduced IL-33–dependent ILC, macrophage, and eosinophil accumulation; cytokine secretion; and mucus deposition in the airways.

Conclusion: These data reveal a hitherto unrecognized role of mTOR signaling in IL-33–driven, ILC-dependent inflammation *in vivo* and suggest that manipulation of this pathway might represent a target for therapeutic intervention for airway inflammation. (J Allergy Clin Immunol 2012;130:1159-66.)

Key words: *IL-33, T_H2, innate lymphoid cells, asthma, mammalian target of rapamycin, rapamycin*

Abbreviations used

AHR:	Airway hyperresponsiveness
APC:	Allophycocyanin
BAL:	Bronchoalveolar lavage
ICOS:	Inducible costimulator
ILC:	Innate lymphoid cell
Lin:	Lineage-specific marker
mTOR(C1/2):	Mammalian target of rapamycin (complex 1/2)
PE:	Phycoerythrin
PI3K:	Phosphoinositide 3-kinase
rpS6:	Ribosomal protein S6
S6K1:	Ribosomal protein S6 kinase 1
TCR:	T-cell receptor
TSLP:	Thymic stromal lymphopoietin
WT:	Wild-type

The IL-1 family member IL-33 is a pleiotropic cytokine that has been implicated in the induction of airway hyperresponsiveness (AHR) in allergic patients and after viral infection. Administration of IL-33 to mice induces airway inflammation independently of adaptive immune responses,[1] whereas increased levels of expression of IL-33 in bronchial epithelia are associated with increased severity of disease in asthmatic patients.[2-4] An understanding of the mechanisms and cellular targets of IL-33 might therefore lead to therapeutic intervention in patients with asthma and allergic inflammation.[5]

IL-33 promotes $CD4^+$ T-cell differentiation to an atypical T_H2 phenotype characterized by the expression of IL-5 and IL-13 but not IL-4.[6] IL-33 also enhances the differentiation of "alternatively activated" macrophages[7,8] and stimulates mast cell,[9-12] basophil,[13,14] and eosinophil[13,15,16] responses. Interestingly, a number of novel innate lymphoid cell (ILC) populations that are important for the induction of type 2 responses[17-20] and tissue remodeling[21] have recently been described. These cells are of lymphoid origin[22] and are characterized by their rapid production of IL-5 and IL-13 in response to IL-25 and IL-33.[17,19,20] Importantly, IL-33–driven type 2 ILCs were recently shown to contribute to AHR after viral infection and in protease-, ovalbumin-, and glycolipid–induced murine models of airway inflammation.[23-28]

Recently, much work has focused on the requirement for the mammalian target of rapamycin (mTOR) signaling pathway in driving immune responses.[29,30] mTOR is a serine/threonine kinase that links signaling in response to growth factors and nutrients and is important for the regulation of cell growth, metabolism, and differentiation. Interestingly, mTOR activity is important for the induction of AHR by $CD4^+$ T_H2 cells in response to house dust mite allergens.[31] By contrast, the role of mTOR in IL-33 signaling and in type 2 ILC effector responses is unknown.

Here we describe an important role for mTOR signaling in IL-33–dependent T_H2 and ILC effector responses both *in vitro*

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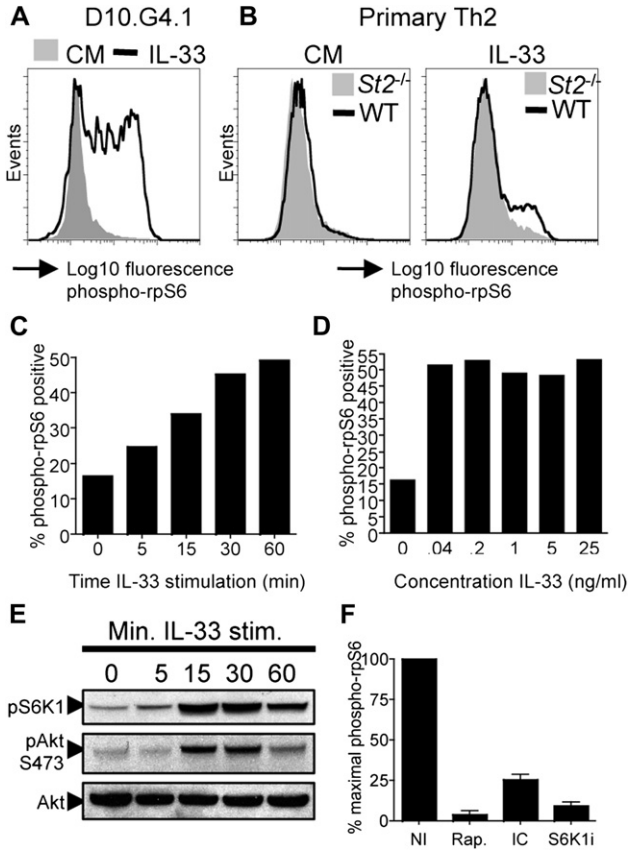

FIG 1. IL-33 induces mTOR activation in T_H2 cells. **A** and **B,** Histograms show phospho-rpS6 in D10.G4.1 or *WT* and *St2*$^{-/-}$ T_H2 cells. *CM,* Complete medium. **C** and **D,** Time course and dose response of IL-33–induced rpS6 phosphorylation in D10.G4.1 cells. **E,** Western blots of phospho-Akt and S6K1. **F,** Relative levels of phospho-rpS6 in D10.G4.1 cells after stimulation with IL-33 in the presence of no inhibitor *(NI),* rapamycin (*Rap.*; 100 nmol/L), IC87114 (*IC*; 5 μmol/L), or PF-4708671 (*S6K1i*; 10 μmol/L). *Error bars* represent SDs (n = 4).

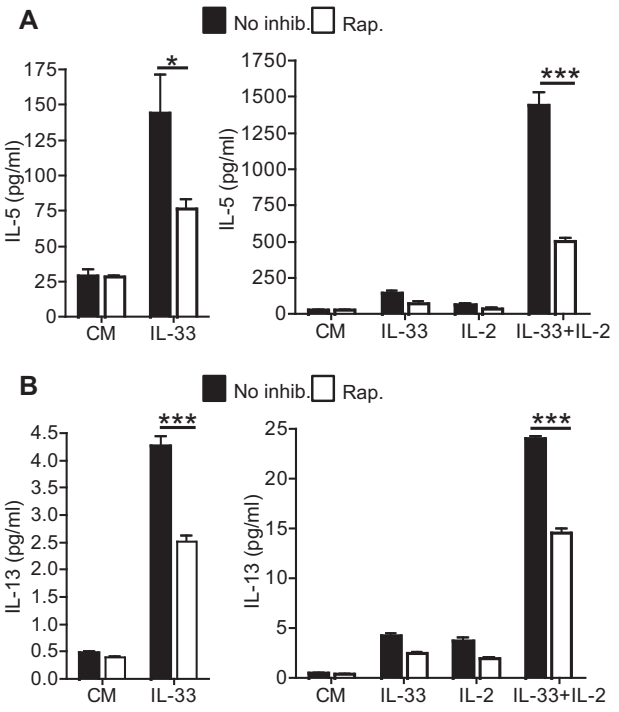

FIG 2. mTOR regulates IL-33–induced cytokine production. Effects of rapamycin (*Rap.*; 100 nmol/L) on levels of IL-5 **(A)** and IL-13 **(B)** induced by IL-33 ± IL-2. *CM,* Complete medium. *$P < .05$ and ***$P < .001$, unpaired Student *t* test).

and *in vivo*. IL-33 directly induced the activation of mTOR in a phosphoinositide 3-kinase (PI3K) p110δ–dependent manner. Furthermore, inhibition of mTOR reduced IL-33–driven IL-5 and IL-13 expression by both T_H2 cells and ILCs *in vitro*. We also show that IL-33–induced airway inflammation was mediated by ILCs and that rapamycin reduced ILC accumulation, macrophage and eosinophil infiltration, cytokine secretion, and mucus deposition in the lung. These data uncover a hitherto unrecognized critical role for mTOR signaling in the biological effects of IL-33 and the effector responses of type 2 ILCs.

METHODS

Mice

BALB/c mice were from Harlan-Olac (Bicester, United Kingdom). BALB/c *St2*$^{-/-}$ mice[32] were bred and maintained at the University of Glasgow. All experiments were performed in accordance with UK Home Office guidelines.

Cell lines

D10.G4.1 cells were maintained in complete RPMI 1640 medium (Gibco, Carlsbad, Calif) containing 20 pg/mL IL-1α (R&D Systems, Minneapolis, Minn) and 10% T-Stim culture supplement (BD Biosciences, San Jose, Calif). For biochemical analysis, D10.G4.1 cells were incubated in complete medium in the absence of IL-1α or T-Stim for 24 hours before stimulation. $CD4^+$ T cells were purified from lymph nodes of BALB/c or *St2*$^{-/-}$ mice by means of negative selection (Miltenyi Biotec, Bergisch Gladbach, Germany) to generate primary T_H2 cells. Cells were stimulated on anti-CD3ε (BD PharMingen, San Jose, Calif)–coated plates in the presence of anti-CD28 (BD PharMingen), anti–IFN-γ (R&D Systems), and 10 ng/mL recombinant IL-4 and IL-2 (R&D Systems) for 4 days. Cells were restimulated for 2 further 4-day rounds of polarization under similar conditions without IL-2. Before restimulation, cells were incubated overnight in complete RPMI in the absence of cytokines and T-cell receptor (TCR) stimulation.

Intranasal IL-33 administration, isolation of ILCs, and adoptive cell transfer

Mice were anesthetized with isoflurane and 30 μL of PBS ± 1 μg of IL-33 ± 1 mg/kg rapamycin inoculated into the nasal passage. For ILC isolation, mice were treated for 5 days with IL-33. Lungs were collected on day 6 and digested in 125 μg/mL Liberase TL and 100 μg/mL DNAse 1 (Roche Diagnostics, Mannheim, Germany). Nonadherent cells were stained with ST2–fluorescein isothiocyanate, lineage markers (B220, FcεRI, CD11b, and CD3ε)–phycoerythrin (PE), CD278-PerCP/Cy5.5, CD45-Alexafluor 700, and UVE/DEAD fixable Aqua Dead cell stain (Life Technologies, Carlsbad, Calif) and sorted with a BD FACS Aria. Cells were rested overnight before *in vitro* analyses. For transfer, 10^6 ILCs in 30 μL of PBS were inoculated intranasally, as described, followed by PBS ± IL-33 ± rapamycin.

Analysis of bronchoalveolar lavage fluid and lungs

Trachea were cannulated, 800 μL of PBS was flushed into the lungs, and the fluid was collected. Bronchoalveolar lavage (BAL) fluid was centrifuged, and supernatants were collected. Cell pellets were resuspended in PBS and counted. Cells (10^5) were used for cytospin preparations and stained by using the Romanovsky method (Raymond A Lamb, Eastborne, United Kingdom). Cell morphology was assessed microscopically under oil immersion.