厚生労働科学研究委託費(難治性疾患等実用化研究事業(免疫アレルギー疾患等実用化研究事業(免疫アレルギー疾患実用化研究分野))

委託業務成果報告（分担）

免疫療法による花粉症治療の新しい展開を目指した研究

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研究要旨
ナチュラルキラーT（NKT）細胞の外因性リガンドとして知られる海綿由来の糖脂質から有機合成によって創生されたα-galactosylceramide（α-GalCer)は、GMP規格のものが国内外での癌患者に対する臨床試験で、直接静脈投与あるいはパルスした樹状細胞投与の形で使用され、高い安全性が確認済である。α-GalCerは溶解性が不良だが、本研究助成でリポソームに封入してアレルギー性鼻炎モデルマウスへの舌下投与はアレルゲン舌下免疫療法の有効な粘膜アジュバントとして抗アレルギー効果が期待できる。標識リポソームを作成して口腔底粘膜の通過性、頸部リンパ節での反応をマウスで確認し、GMP準拠のリポソームとして治験への展開を目指す。癌患者対象の臨床試験で用いられた1日5μgの静脈投与量を基準とした用量設定から、phase1試験を実施する。

A.　研究目的

舌下免疫療法の効果の増強と治療期間の短縮、患者負担の軽減を目的にした粘膜アジュバントとして、NKT細胞のリガンドを含むリポソームの口腔底投与を治験の開始を目標とした検討を進める。

B.　研究方法

米国FDAのIND下で実施中の臨床試験で使用している治験薬の処方を基準に、α-GalCerの各種リポソーム製剤を作成し、マウス舌下投与マウスモデルでスクリーニングする。

（倫理面への配慮）

α-GalCerとリポソーム製剤ともに、臨床試験実績がある化合物を使用する。

C.　研究結果

マウス舌下投与モデルの血中IFN-γ産生誘導能を指標にスクリーニングした結果、最も増強効果が高いリポソーム製剤の処方を見出すことができた。

D.　考察

スクリーニングで見出されたリポソーム製剤の処方は、現在米国の臨床試験で使用中の治験薬の処方と極めて類似していることが明らかとなった。

E.　結論

新規舌下免疫療法の第I相臨床試験のアジュバントとして米国臨床試験の治験薬を使用できると判断した。

G.　研究発表

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2.　学会発表

なし

H.　知的財産権の出願・登録状況

（予定を含む。）

1.　特許取得

特許第4889485号（平成23年12月23非）

2.　実用新案登録　なし

3.　その他　なし

様式第１９

学　会　等　発　表　実　績

委託業務題目「免疫療法による花粉症治療の新しい展開を目指した研究」

機関名　千葉大学大学院医学研究院　耳鼻咽喉科・頭頸部腫瘍学　教授　岡本　美孝

１．学会等における口頭・ポスター発表

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舌下免疫療法の実際.	岡本美孝	横浜 (日本アレルギー学会第1回総合アレルギー講習会)	2014 年 12 月	国内
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2. 学会誌・雑誌等における論文掲載

掲載した論文 (発表題目)	発表者氏名	発表した場所 (学会誌・雑誌等名)	発表した時期	国内・外の別
The IL-33/ST2-p38 axis confers memory Th2 cell pathogenicity in the airway. Immunity.	Endo Y, Hirahara K, Iinuma T, Shinoda K, Tumes DJ, Yamamoto H, Okamoto Y,Nakayama T.	Immunity	in press.	国外

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Measurement of Japanese Cedar Pollen-Specific IgE in Nasal Secretions.	Sakaida H, Masuda S, Takeuchi K.	Allergology International	2014 63:467-473.	国外
ダニアレルゲンワクチン標準化に関する日本アレルギー学会タスクフォース報告	高井敏朗, 岡本美孝, 大久保公裕, 永田真, 阪口雅弘, 福富友馬, 齋藤明美, 安枝浩, 増山敬祐	アレルギー	2014 631229-1240	国内
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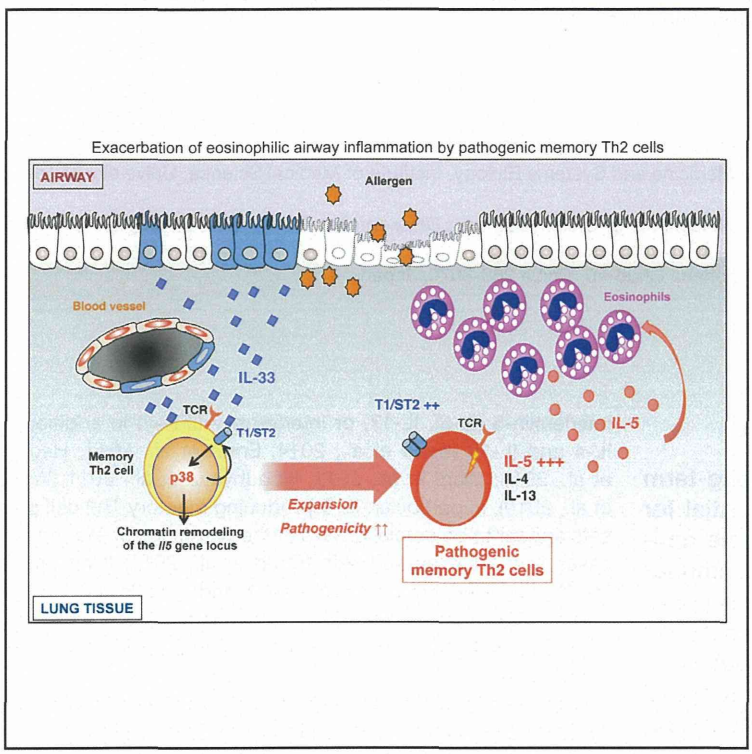
Article

Immunity

The Interleukin-33-p38 Kinase Axis Confers Memory T Helper 2 Cell Pathogenicity in the Airway

Graphical Abstract



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In Brief

IL-33, a IL-1 family member identified as the ligand for the ST2 receptor, is deeply related to allergic inflammation. Nakayama and colleagues demonstrate that the IL-33-ST2-p38 axis is crucial for the induction of pathogenicity of memory Th2 cells in allergic airway inflammation in both mice and humans.

Highlights

- Memory Th2 cells are critical targets of IL-33 in allergic airway inflammation
- IL-33 selectively remodels chromatin of *Il5*, thereby licensing its expression
- Memory-Th2-cell-mediated airway inflammation depends on IL-33 and ST2
- p38 MAPK is a major downstream target of IL-33-ST2 signaling in memory Th2 cells

Endo et al., 2015, Immunity *42*, 294–308
February 17, 2015 ©2015 Elsevier Inc.
http://dx.doi.org/10.1016/j.immuni.2015.01.016

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Immunity
Article

The Interleukin-33-p38 Kinase Axis Confers Memory T Helper 2 Cell Pathogenicity in the Airway

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http://dx.doi.org/10.1016/j.immuni.2015.01.016

SUMMARY

Memory CD4$^+$ T helper (Th) cells provide long-term protection against pathogens and are essential for the development of vaccines; however, some antigen-specific memory Th cells also drive immune-related pathology, including asthma. The mechanisms regulating the pathogenicity of memory Th cells remain poorly understood. We found that interleukin-33 (IL-33)-ST2 signals selectively licensed memory Th2 cells to induce allergic airway inflammation via production of IL-5 and that the p38 MAP kinase pathway was a central downstream target of IL-33-ST2 in memory Th2 cells. In addition, we found that IL-33 induced upregulation of IL-5 by memory CD4$^+$ T cells isolated from nasal polyps of patients with eosinophilic chronic rhinosinusitis. Thus, IL-33-ST2-p38 signaling appears to directly instruct pathogenic memory Th2 cells to produce IL-5 and induce eosinophilic inflammation.

INTRODUCTION

The quality of adaptive immune responses depends on the number and function of antigen-specific memory T cells. Upon antigen recognition via the T cell receptor (TCR), naive CD4$^+$ T cells undergo rapid clonal expansion, followed by differentiation into functionally distinct T helper (Th) cell subsets, such as Th1, Th2, and Th17 cells (O'Shea and Paul, 2010; Reiner, 2007). Some of these effector Th cells are maintained as memory Th cells for long times in vivo (Nakayama and Yamashita, 2008), and it is now becoming clear that these cells display functional heterogeneity (Sallusto and Lanzavecchia, 2009). Recent reports indicate that there are several distinct subsets of memory type Th2 cells that produce large amounts of interleukin-5 (IL-5), IL-17, or interferon-γ (IFN-γ) in addition to IL-4 and IL-13 (Endo et al., 2014; Endo et al., 2011; Hegazy et al., 2010; Islam et al., 2011; Upadhyaya et al., 2011; Wang et al., 2010). In particular, IL-5-producing memory Th2 cell subsets appear to be crucial drivers of the pathology of allergic diseases in the airway and skin (Endo et al., 2011; Islam et al., 2011). However, environmental cues and signals that dictate how memory Th2 cells contribute to the pathogenicity of allergic diseases, including chronic airway inflammation, are poorly understood.

Asthma is a chronic lower-airway inflammatory disease characterized by recurrent airway obstruction and wheezing (Cohn et al., 2004). Allergic asthma is mainly driven by Th2-cell-type cytokines, such as IL-4, IL-5, and IL-13, and is characterized by the presence of elevated numbers of eosinophils in the lungs (Cohn et al., 2004). IL-5 regulates eosinophil development, recruitment to the lungs, and activation (Rosenberg et al., 2013). IL-13 plays an important role in the effector phase of asthma by inducing airway remodeling and airway hyperresponsiveness (AHR) as well as mucus hyperproduction (Ingram and Kraft, 2012). Th2 cells are the major source of IL-4, IL-5, and IL-13 in allergic asthma. Innate Th2 cell counterparts that also produce large amounts of IL-5 and IL-13 (Lin$^-$CD127$^+$ type 2 innate lymphoid cells [ILC2s]) have been identified (Furusawa et al., 2013; Lloyd, 2010; Price et al., 2010; Saenz et al., 2010). Recent research indicates that ILC2s play a critical role in eosinophilic airway inflammation in mice that lack the ability to mount adaptive immune responses (Chang et al., 2011; Halim et al., 2012; Scanlon and McKenzie, 2012).

Chronic rhinosinusitis (CRS) is a common chronic sinus inflammatory disease characterized by distinct cytokine production profiles and tissue-remodeling patterns (Hamilos, 2011; Van Bruaene et al., 2008; Zhang et al., 2008). CRS can be classified into two types of diseases according to the presence of nasal polyps. CRS with nasal polyps (CRSwNP) is often accompanied by Th2-cell-skewed eosinophilic inflammation, whereas CRS without nasal polyps (CRSsNP) is characterized by a predominantly Th1-cell-skewed response (Hamilos, 2011). IL-5 is more

abundant in the nasal mucosal tissues of CRSwNP than in those of CRSsNP (Van Bruaene et al., 2008). CRSwNP is further subdivided into two types of diseases on the basis of the extent of eosinophilic inflammation, particularly for people in East Asia (Zhang et al., 2008): eosinophilic CRS (ECRS) and non-eosinophilic rhinosinusitis (NECRS).

IL-33, a member of the IL-1 family, was newly identified as the ligand for the ST2 receptor (also known as IL-1RL1) (Liew et al., 2010; Schmitz et al., 2005). The major genome-wide association studies have reproducibly found significant associations between *IL33* and *IL1RL1* genetic variants and asthma in humans (Bønnelykke et al., 2014; Grotenboer et al., 2013; Torgerson et al., 2011). IL-33 expression is higher in asthmatic patients and in mouse models of asthma (Lloyd, 2010). Epithelial and airway smooth muscle cells appear to represent two major sources of IL-33 in asthmatics (Préfontaine et al., 2009). Previous reports showed that the depletion of IL-33 or ST2 attenuated murine ovalbumin (OVA)-induced airway inflammation (Kurowska-Stolarska et al., 2008; Oboki et al., 2010). ILC2s are characterized by their rapid production of IL-5 and IL-13 in response to IL-33 exposure (Scanlon and McKenzie, 2012). Therefore, understanding the mechanisms by which IL-33 induces allergic inflammation and differentiating between antigen-specific and antigen-independent functions of IL-33 are crucial for the effective design of therapeutics for patients with allergic inflammatory disorders such as chronic asthma.

We herein investigated the role of IL-33 in allergic airway inflammation induced by memory Th2 cells. We found that IL-33-ST2 signaling was crucial for the induction of pathogenicity of memory Th2 cells in allergic experimental asthma. Moreover, we found that like ILC2s, memory Th2 cells acquired the ability to produce IL-5 directly in response to IL-33; this property was not observed in effector Th2 cells. Genetic deletion of IL-33 or ST2 resulted in impaired memory-Th2-cell-dependent eosinophilic airway inflammation, and we identified p38 mitogen-activated protein kinase (MAPK) as the downstream target of IL-33-ST2 signaling in this cell type. Analysis of nasal polyps from patients with CRS showed that IL-33 could also directly enhance IL-5 production by human memory $CD4^+$ T cells. Thus, we propose that the IL-33-ST2-p38 axis is crucial for the induction of pathogenicity of memory Th2 cells in eosinophilic airway inflammation in both mice and humans.

RESULTS

IL-33 Selectively Enhances IL-5 Production by Memory Th2 Cells

IL-33 is known to induce strong Th2-cell-type immune responses and eosinophilic inflammation in the lung and intestine (Lloyd, 2010). However, the types of cells on which IL-33 acts in these settings are still being defined. To explore the involvement of $CD4^+$ T cells in IL-33-mediated inflammation, we assessed the expression of the IL-33 receptor (ST2) on naive $CD4^+$ T cells, effector Th1 and Th2 cells generated in vitro, and memory Th1 and Th2 cells generated in vivo (Nakayama and Yamashita, 2008) (Figure S1A). Memory Th2 cells showed very high expression of IL-7 receptor-α chain (IL-7Rα), and the majority also showed low expression of CD69 and IL-2Rα (Figure S1B, upper panel). Expression patterns of these surface receptors were quite different from that seen on the in-vitro-generated effector Th2 cells used in this study. We observed that compared to naive $CD4^+$ T cells or memory Th1 cells, memory Th2 cells showed strongly increased expression of *Il1rl1* mRNA (Figure 1A). Compared to naive $CD4^+$ T cells or effector Th1 cells, effector Th2 cells also showed significantly higher expression of *Il1rl1* ($p < 0.01$; Mann-Whitney U test), but this was not as pronounced as the expression observed in memory Th2 cells. The increased *Il1rl1* mRNA was also reflected by higher expression of ST2 on the surface of memory Th2 cells than on the surface of naive $CD4^+$ T cells or effector Th2 cells (Figure 1B). Substantial ST2 expression was detected only on memory Th2 cells, and ST2 was specifically found on those cells with high expression of IL-7Rα and low expression of CD69 and IL-2Rα, strongly indicating that a distinct subset of ST2-expressing cells is induced in memory Th2 cells (Figure S1B, lower panel). In addition, exposure of memory Th2 cells to IL-33 dramatically enhanced ST2 expression (Figure 1C). Importantly, we found that stimulation with IL-33 for 5 days selectively induced IL-5 production by memory Th2 cells but not by effector Th2 cells (Figure 1D). In contrast, IL-33-induced upregulation of IL-4 expression was not observed in response to treatment with IL-33 (Figures 1D and 1F). IL-13 was slightly increased by cultivation of memory Th2 cells with IL-33 (Figures 1E and 1F). IL-33 supported the viability of memory Th2 cells as well as IL-2 and IL-7 (Figure S1C) without inducing significant proliferation (Figure S1D). We reported previously that memory Th2 cells can be subdivided into four distinct subpopulations according to the expression of CXCR3 and CD62L and that IL-5 production is normally restricted to a small number of cells in the $CD62L^{lo}CXCR3^{lo}$ subpopulation (Endo et al., 2011) (Figure S1E). We also examined the effect of IL-33 on the four subpopulations (CXCR3 and CD62L) of memory Th2 cells. ST2 expression was detected on 10%–20% of all four subpopulations of freshly prepared memory Th2 cells (Figure S1F, left). IL-33 treatment enhanced ST2 expression on all four subpopulations (Figure S1F, right). Upon TCR stimulation, IL-5-producing cells were detected only in the $CD62L^{lo}CXCR3^{lo}$ subpopulation of freshly prepared memory Th2 cells (Figure S1G, left), whereas after IL-33 cultivation, IL-5-producing cells were detected in all four subpopulations and showed their highest numbers in the $CD62L^{lo}CXCR3^{lo}$ subpopulation (Figure S1G, right). IL-5 production was also assessed by ELISA, and similar results were obtained (Figure S1H). Thus, these results indicate that IL-33 upregulates ST2 expression and selectively enhances IL-5 expression and production by memory Th2 cells, but not effector Th2 cells.

IL-33 Induces Selective Remodeling of Chromatin at the *Il5* Locus in Memory Th2 Cells

Epigenetic chromatin modifications can control selective expression of genes that function in the immune system (Northrup and Zhao, 2011). We therefore explored whether IL-33 signaling could regulate the chromatin status of the Th2-cell-associated cytokine-encoding genetic loci in memory Th2 cells. We performed chromatin immunoprecipitation (ChIP) assays with antibodies specific to several histone modifications (Figure 2A). At the *Il5* locus, freshly prepared in-vivo-generated memory Th2 cells showed lower modifications associated with active