Importance of IL-18-Induced Super Th1 Cells for the Development of Allergic Inflammation

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ABSTRACT

Th1 cells, which express IL-18R, produce IFN-γ in response to Ag and IL-2 and increase further production of IFN-γ upon additional IL-18 stimulation. They simultaneously produce Th2 cytokines (IL-9 and IL-13), GM-CSF and chemokines (RANTES, MIP-1α). Human Th1 cells also produce IFN-γ and IL-13 in response to anti-CD3 and IL-18. Recently, we demonstrated Th1 cells induce intrinsic type atopic asthma and dermatitis by production of Th1- and Th2-cytokines and chemokines. Here, we review the pathological roles of Th1 cells, stimulated with Ag and IL-18 in vivo, in the pathogenesis of allergic disorders by production of Th1 and Th2 cytokines and chemokines. Based on this unique function of Ag- plus IL-18-stimulated Th1 cells, we proposed to designate them as “super Th1 cells”.

KEY WORDS

allergic inflammation, atopic dermatitis, bronchial asthma, IL-18, super Th1

INTRODUCTION

Bronchial asthma is a complex syndrome characterized by airway hyperresponsiveness (AHR) and reversible airflow obstruction associated with airway inflammation and remodeling and occasional high serum level of IgE.1-7 Th2 cells have been recognized as inducing bronchial asthma by production of Th2 cytokines.1-10 Particularly, IL-13 is suggested to play a critical role in induction of AHR, eosinophilic infiltration, goblet cell metaplasia, and lung fibrosis.9-11 In contrast, Th1 cells had been regarded to inhibit bronchial asthma by production of IFN-γ.12-14 However, several studies have disclosed the disability of Th1 cell to suppress Th2 cell-induced AHR.15-19 On the contrary, a combination of Th1 and Th2 cells or their products rather augment each activity to induce airway inflammation and AHR.15,16,19

We demonstrated recently that OVA (Ag) plus IL-18 acts on adoptively transferred OVA-specific memory type Th1 cells to induce airway inflammation and AHR in a naive host mouse.20 Th1 cells, which express IL-18R, produce IFN-γ in response to OVA and increase further IFN-γ production in response to additional IL-18 stimulation.21 Surprisingly, they simultaneously produce Th2 cytokines (e.g., IL-9 and IL-13), GM-CSF and chemokines (e.g., RANTES and MIP-1) when stimulated with OVA and IL-18.20 Human Th1 cells also produce IFN-γ and IL-13 in response to anti-CD3 plus IL-18.22 Recently, we demonstrated Th1 cells induce intrinsic atopic dermatitis by production of Th1 and Th2 cytokines and chemokines.23 Thus, IL-18 has added its new function to its growing functional list.24-26 Based on this unique function of Ag- plus IL-18-stimulated Th1 cells, we proposed to designate them as “super Th1 cells”.23

THE MOLECULAR MECHANISM FOR IL-18 SECRETION

As IL18, like IL1β, lack leader sequence, IL18 product pro-IL18 cannot be secreted, but is stored intracellularly.24,25,27,28 Many cell types exemplified by macrophages produce pro-IL18 in the steady state.24,27,28 Epithelial cells lining host body, such as respiratory epithelial cells, intestinal epithelial cells and keratinocytes can produce pro-IL18 under normal conditions as well. Pro-IL18 needs appropriate post-translational processing to become biologically active and to be ex-
IL-18 stimulates Th1 cell to produce Th1 cytokine (IFN-γ) and Th2 cytokine (IL-9, IL-13)

**Fig. 1** Mechanisms involved in the processing and releasing IL-18. Macrophages (Mø) and dendritic cells are major cell sources of IL-18. The cells constitutively produce pro-IL-18. After stimulation through Toll-like receptor 4 (TLR4) and Fas, caspases (Casps) are activated for appropriately cleavage of proIL-18, resulting in the release of biologically active mature IL-18. Chymase degranulated from activated human mast cells can process pro-IL-18 into biologically active IL-18 as well.

tracellularly released (Fig.1). Caspase 1 (Casp1) is an authentic processing enzyme for IL-18 and IL-1β. Casp1 is also produced as enzymatically inactive zymogen in the cytoplasm and needs mutual cleavage to become active. Recently, the multiple protein complex named inflammasome is verified to be the platform for Casp1 activation. Inflammasome is composed of Nod-like receptor (NLR), a cytoplasmic sensor, Casp1 activation adaptor ASC, pro-Casp1 and substrates such as pro-IL-18 and pro-IL-1β. Nalp3/NLRP3 is believed to senses extrinsic pathogen-associated molecular patterns (PAMPs). Indeed, after stimulation with LPS, Nalp3 inflammasome is promptly formed, followed by rapid processing of IL-1β and/or IL-18. Thus, microbial infection induces IL-18 and IL-1β release via activation of Nalp3 inflammasome. For example, in response to TLR4 agonist LPS, hepatic tissue macrophages secrete IL-18 and IL-1β in a manner dependent on Casp1, ASC and Nalp3.

IL-18 processing might occur extracellularly as well. Recent report shows that chymase, an enzyme localized in the granules of mast cells, has capacity to cleave pro-IL-18 into biologically active IL-18 (Fig.1). Since mast cells are accumulated into the skin lesion of mice with AD-like dermatitis, chymase from the activated mast cells might exacerbate the skin inflammation by enhancing the local release of biologically active IL-18.

Epithelial cells are major cell source of various proallergic cytokines, such as IL-18, IL-25, TSLP and IL-33. As they are accumulated preferentially in the inflammatory sites of patients with Th2 type allergic diseases, IL-25, TSLP and IL-33 might be involved in the development of Th2 type allergy. By contrast, epithelium-derived IL-18 might trigger infectious type of allergic diseases as described later, although the mechanism for secretion of IL-18 from the epithelial cells are still to be elucidated.

**SUPER TH1 CELLS AND ASTHMA**

IL-18R expression levels determine the intensity of responsiveness to IL-18 and are quite distinct among Th cell subsets. Naïve CD4+ T cells and Th2 cells express little IL-18R, while Th1 cells express high levels of it. Consistently, the amount of IL-4 produced by Th2 cells is not affected by additional stimulation with IL-18. In contrast, Th1 cells produce larger amounts of Th1 cytokines such as IFN-γ and TNF-α when additionally stimulated through their IL-18R (Fig.2). From these early studies IL-18 was regarded as the Th1 response-activating cytokine. Our resent study unveiled a second important property of IL-18 in the adaptive immunity. IL-18 has potential to render Th1 cells to produce Th2 cytokines. Upon simultaneous engagement of TCR and IL-18R, Th1 cells become to produce abundant IL-3, IL-13 and IL-9, but still not IL-4, in addition to the Th1 cytokines. They also produce larger amounts of chemokines that can recruit various pro-atopic cells, including granulocytes, macrophages and lymphocytes. We designate these IL-13/IL-9/chemokine-producing Th1 cells as super Th1 cells. When they are activated with Ag together with IL-18, Th1 cells become to exert their actions as super Th1 cells by producing both Th1 and Th2 cytokines. Among the cytokines, IFN-γ and IL-13 are critical for the development of AHR and airway fibrosis, respectively.

**Fig. 2** Super Th1 cells. When they are activated with Ag together with IL-18, Th1 cells become to exert their actions as super Th1 cells by producing both Th1 and Th2 cytokines. Among the cytokines, IFN-γ and IL-13 are critical for the development of AHR and airway fibrosis, respectively.
cells (Fig.2).

What about in vivo role of super Th1 cells? Naïve mice transferred with OVA-specific Th2 cells that are generated from OVA-specific naïve DO11.10 CD4+ cells by in vitro incubation under Th2 condition, namely “Passive Th2 mice”, expectedly develop asthmatic response upon intranasal OVA challenge.20 They develop AHR, airway eosinophilia and goblet cell metaplasia of airway epithelial cells. Expectedly, IL-13 blockade can protect against the development of all of these manifestations. In contrast, “Passive Th1 mice”, which are generated by the protocol similar to “Passive Th2 mice” except for in vitro incubation of naïve OVA-specific CD4+ cells under Th1 condition, do not show any asthmatic signs and/or symptoms after intranasal challenge with OVA alone. However, whenever challenged with OVA together with IL-18, “Passive Th1 mice” start to succumb to AHR, airway eosinophilia and peribronchial fibrosis, suggesting the possible activation of super Th1 cells. In contrast to Th2 type asthma observed in “Passive Th2 mice”, IL-13 blockade prevents airway eosinophilic inflammation and peribronchial fibrosis, partly and profoundly, but entirely not AHR.37 This AHR can be protected by IFN-γ blockade. Thus, super Th1 cells might be involved in the pathogenesis of certain types of allergic disorders by producing both IFN-γ and IL-13.

INFECTIONOUS TYPE BRONCHIAL ASTHMA

It is well documented that microbial infection aggravates and/or triggers allergic diseases in human. For example, lower respiratory infection with rhinovirus, a common microbe relevant to cold, or with Mycoplasma pneumoniae and Chlamydia pneumoniae, common bacteria causative of community-acquired pneumonia, frequently provokes or exacerbates bronchial asthma in asthmatic patients.38,39 Lesional skin infection with Staphylococcus aureus worsens the disease severity in patients with atopic dermatitis (AD). As microbial infection sometimes evokes IL-18 secretion,25,26 we may assume that microbial products might cause local release of IL-18, which in turn triggers bronchial asthma by activation of super Th1 cells. As expected, murine bronchial epithelial cells can respond to LPS by releasing IL-18. “Passive Th1 mice” or wild-type mice immunized with OVA in Th1 adjuvant (“Active Th1 mice”) show AHR, peribronchial eosinophilic inflammation upon intranasal challenge with OVA in combination with LPS, a cell-wall component of Gram-negative bacteria.37 In sharp contrast, IL-18 blockade can rescue “Active Th1 mice” from these clinical manifestations after intranasal challenge with OVA and LPS. Il18−/− mice immunized with OVA in Th1 adjuvant can evade them after being similarly challenged.37 Thus, endogenously produced IL-18 and exogenously administered OVA both might activate OVA-specific super Th1 cells, leading to the development of asthmatic manifestations in infectious type of asthma.

ATOPIC DERMATITIS INDUCED BY TOPICAL APPLICATION WITH STAPHYLOCOCCAL PRODUCT

Super Th1 cells are also highlighted in infectious type of AD in mice. Consecutive and topical application of protein A (SpA) purified from cell wall of Staphylococcus aureus induces AD-like pruritic dermatitis in mice with genetically impaired skin barrier function, NC/Nga mice.23 CD4+ T cells purified from draining lymph nodes (DLN) of mice prior to the onset show the characteristics of Th1 cells. These cells produce Th1 cytokines (IFN-γ and TNF-α), but not Th2 cytokine (IL-4 and IL-13) upon TCR engagement. However, CD4+ DLN cells prepared from the mice post onset exhibit the feature as super Th1 cells. Keratinocytes freshly isolated from naive mice release IL-18 in response to SpA in vitro,36 suggesting involvement of IL-18 in the in vivo development into super Th1 cells. In fact, IL-18 blockade and deletion of Il18 rescue mice from the development of SpA-induced AD-like dermatitis, concomitant with prevention of their super Th1 cell development. Among cytokines produced by super Th1 cells IFN-γ and TNF-α are important. IFN-γ or TNF-α blockade prevents the development of this skin inflammation. Thus, IL-18-dependent super Th1 cell development is important for the development of this dermatitis.

CLINICAL EVIDENCE FOR IL-18

Accumulating evidence suggests positive relationship between IL-18 levels in the lesion or circulation and allergic diseases, such as asthma, allergic rhinitis and AD.40-42 In particular, after inhalatory challenge test with flour allergens patients with occupational allergic asthma and/or rhinitis show a significant increase in IL-18 levels in nasal lavage fluid. Furthermore, IL18 polymorphism that ensures higher production of IL-18 upon appropriate stimuli is preferentially accumulated in patients with allergic disorders.43-45 Although no polymorphisms differed significantly in frequency between the control and adult asthma groups, functional polymorphism in IL-18 is associated with severity of adult bronchial asthma.46 These results suggest association of IL-18 with allergic disorder in human. However, the molecular mechanism for IL-18 induction of differentiation from Th1 cells into Super Th1 cells is unclear. Nonetheless, possible therapeutics targeting IL-18 might be beneficial for inflammatory type of allergic disorders.

CONCLUDING REMARKS

One may accept that super Th1 cells are activated upon microbial infection of allergic lesion. What is a super Th1 cell subset? Do super Th1 cells, like Th1 cells, require the proper epigenetic regulation? If so,
what is a transcription factor essential for the differentiation into super Th1 cells, like T-bet/STAT4 for Th1 cells (Fig.2)?

Although we need further studies to settle those issues, targeting super Th1 cells and super Th1-associated cytokines might be of value in the therapy of severe, recurrent asthma and perhaps of infectious type allergic diseases. We previously generated human anti-human IL-18 mAb by the gene-manipulating technique.4 This human-derived mAb targeting human IL-18 might be highlighted as a therapeutic agent against infectious type allergic diseases as well.

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