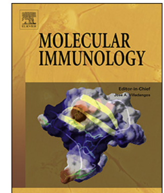
Molecular Immunology 101 (2018) 550–563

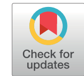
Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm



Interleukin-33 deficiency exacerbated experimental autoimmune encephalomyelitis with an influence on immune cells and glia cells



Yifan Xiao[a], Lin Lai[b], Huoying Chen[c], Junyu Shi[a], FanFan Zeng[a], Jun Li[a], Huiting Feng[a], Jie Mao[a], Feng Zhang[a], Naming Wu[a], Yong Xu[a], Zheng Tan[a,d], Feili Gong[a,d], Fang Zheng[a,d,*]

[a] Department of Immunology, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, PR China
[b] Department of Clinical laboratory, The First Affiliated Hospital of Hainan Medical University, Haikou, Hainan, PR China
[c] Department of Laboratory Medicine, The Second Affiliated Hospital of Guilin Medical University, Guilin, Guangxi, PR China
[d] Key Laboratory of Organ Transplantation, Ministry of Education; NHC Key Laboratory of Organ Transplantation; Key Laboratory of Organ Transplantation, Chinese Academy of Medical Sciences, Wuhan, Hubei, PR China

ARTICLE INFO

Keywords:
Astrocytes
EAE
Effector T cells
Interleukin-33
Microglia/macrophages
Regulatory T cells

ABSTRACT

Interleukin (IL)-33, a member of the IL-1 cytokine family, is highly expressed in central nervous system (CNS), suggesting its potential role in CNS. Although some studies have focused on the role of IL-33 in multiple sclerosis (MS) / experimental autoimmune encephalomyelitis (EAE), an autoimmune disease characterized by demyelination and axonal damage in CNS, the exact role of IL-33 in MS/EAE remains unclear and controversial. Here, we used IL-33 knockout mice to clarify the role of endogenous IL-33 in EAE by simultaneously eliminating its role as a nuclear transcription factor and an extracellular cytokine. We found that the clinical score in IL-33 knockout EAE mice was higher accompanied by more severe demyelination compared with the wild-type (WT) EAE mice. As for the main immune cells participating in EAE in IL-33 knockout mice, pathogenic effector T cells increased both in peripheral immune organs and CNS, while $CD4^+FOXP3^+$ regulatory T cells decreased in spleen and lymph nodes, Th2 cells and natural killer (NK) cells decreased in CNS. Additionally, the populations of microglia/macrophages and $CD11C^+CD11B^+$ dendritic cells (DCs) increased in CNS of IL-33 knockout mice with EAE, among which iNOS-producing microglia/macrophages increased. Moreover, resident astrocytes/microglia were more activated in IL-33 knockout mice with EAE. *In vitro*, after blocking the IL-33, the proliferation of primary astrocytes, the production of MCP-1/CCL2 and TNF-α by astrocytes, and the production of TNF-α by primary microglia stimulated by the homogenate of the peak stage of EAE were increased. Our results indicate that IL-33 plays a protective role in EAE and exerts extensive influences on multiple immune cells and neural cells involved in EAE.

1. Introduction

Interleukin-33 (IL-33), a member of the IL-1 family, has a key role in innate and adaptive immunity, contributing to tissue homeostasis and responses to environmental stress. It is primarily expressed by nuclei showing transcriptional repressor activity under physiological conditions and is released by damaged or necrotic cells, acting as an “alarmin” (Chen et al., 2018, 2015; Liew et al., 2016). After releasing from cells, IL-33 can bind to ST2, which including transmembrane (mST2) and soluble isoforms (sST2) (Mueller and Jaffe, 2015). IL-33 always exerts its cellular functions by binding to a receptor complex composed of mST2 and the IL-1R accessory protein in order to induce MyD88-dependent signaling, while sST2 always functions as a “decoy” receptor to sequester free IL-33 in extracellular (Mueller and Jaffe, 2015; Oshikawa et al., 2002; Xiao and Zheng, 2016).

In recent years, IL-33 has aroused much interest for its involvement in autoimmune diseases, including systemic lupus erythematosus (SLE) (Mok et al., 2010), rheumatoid arthritis (RA) (Chen et al., 2017), as well as multiple sclerosis (MS) and related animal models-experimental autoimmune encephalomyelitis (EAE) (Allan et al., 2016; Finlay et al.,

Abbreviations: BBB, blood-brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; DCs, dendritic cells; EAE, experimental autoimmune encephalomyelitis; GFAP, glial fibrillary acidic protein; IFN, interferon; IL, interleukin; IVIG, intravenous immunoglobulin; ILC2s, type 2 innate lymphoid cells; MS, multiple sclerosis; MCP-1/CCL2, monocyte chemoattractant protein-1; NeuN, neuronal nucleus; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; ST2, suppression of tumor igenicity 2; TNF-α, tumor necrosis factor α; Th, T helper; Tc, cytokine-producing effectors T cells; Tregs, regulator T cells

* Corresponding author.
E-mail address: zhengfangtj@hust.edu.cn (F. Zheng).

https://doi.org/10.1016/j.molimm.2018.08.026
Received 25 May 2018; Received in revised form 7 August 2018; Accepted 23 August 2018
0161-5890/ © 2018 Elsevier Ltd. All rights reserved.

2016; Jiang et al., 2012; Liew et al., 2016). MS/EAE is a chronic inflammatory autoimmune disease of the central nervous system (CNS), characterized by the infiltration of effector T cells and monocytes into CNS, which is further associated with demyelination, axonal damage and local inflammation (Rossi and Constantin, 2016). In MS patients, IL-33 level in serum and cerebrospinal fluid (CSF) was significantly higher (Jafarzadeh et al., 2016). Our previous study also showed that IL-33 was actively released from CNS-resident cells upon inflammatory stimulation (Chen et al., 2015). Presently, although many studies have focused on the role of IL-33/ST2 in MS/EAE, the exact role of IL-33/ST2 in MS/EAE remains unclear and controversial. Bourgeois et al. reported that IL-33 is harmful in EAE (Bourgeois et al., 2009; Jafarzadeh et al., 2014; Li et al., 2012). Pomeshchik et al. hold a different view that IL-33 ameliorates EAE (Jiang et al., 2012; Milovanovic et al., 2012; Pomeshchik et al., 2015). IL-33 blockade or treatment is a routine process for studying its role in diseases. However, IL-33 blockade could not sufficiently abrogate its action in disease because of its dual role. As a nuclear transcription factor, IL-33 can regulate the transcription of other cytokines, thus furtherly regulating the inflammatory response. As a cytokine releasing from cells, IL-33 can regulate many immune cells directly by binding to ST2 or indirectly by complicated network. In this case, the use of IL-33 knockout mice in our research can clearly elucidate the role of endogenous IL-33 in EAE by simultaneously eliminating its role as a nuclear transcription factor and an extracellular cytokine. On the other hand, exogenous IL-33 might be different from endogenous IL-33 with respect to pharmacokinetics and binding properties *in vivo*, and therefore, IL-33 treatment had no effect sometimes (Zhang et al., 2015b), or performed protective functions at other times (Jiang et al., 2012). Therefore, it is necessary to seek for a better method to clarify the role of IL-33 in EAE, and IL-33 knockout seems to be an optimum choice.

Actually, the role of IL-33 is extensive. There are many cells participating in EAE, making the pathogenesis of EAE highly complex. These cells can be divided into two groups, peripheral immune cells and CNS resident cells. Although previous researches partly reveal the possible effect of IL-33 on these cells, the studies were always restricted to a few of peripheral immune cells, let alone that about CNS resident cells are very limited. In fact, IL-33 is highly expressed in brain and spinal cord, indicating its' essential role in CNS, in addition to its role in peripheral immune modulation (Chen et al., 2015; Jiang et al., 2012; Liew et al., 2016). In our research, we particularly study the influence of IL-33 on immune cells in peripheral immune organs and CNS, as well as resident cells in CNS by using IL-33 knockout mice with EAE. The result can provide a comprehensive evidence for exploring the role of IL-33 in EAE.

In this study, IL-33 plays a protective role in EAE by inhibiting pathogenic effector T cells, iNOS producing-microglia/macrophages and DC. Meanwhile, IL-33 increased Tregs in peripheral immune organs, and boosted Th2 and NK populations in CNS. Furthermore, IL-33 can also suppress the activation of microglia and astrocytes as well as the recruitment of macrophages. The role of IL-33 is extensive and far more complicated than we expect. Thus, the present result can provide us a general view of the effect of IL-33 on the main cells participating in EAE, furtherly contributing to the study of IL-33 in future.

2. Materials and methods

2.1. Ethics statements

All animal experiments in this study were performed in strict accordance with the Institutional Animal Care and Use Committee, Tongji Medical College, Huazhong University of Science and Technology. All efforts were made to minimize animal suffering.

2.2. Mice

Wild- type C57BL/6 mice (WT; 6–8-week-old) were purchased from SLAC Laboratory Animal Co., Ltd., (Shanghai, China) and maintained in a specific-pathogen-free (SPF) facility until further use.

IL-33 knockout C57BL/6 mice were custom-made by Cyagen (Guangzhou) Bioscience Inc. Briefly, in the *mIL33* gene, 8 exons have been identified, with the ATG start codon in exon 2 and the TAA stop codon in exon 8. In our experiment, exon 3 was selected for TALEN targeting. The related sequences are as follows: TALEN-L: TTGTTAGG ATCCCAACAGAAGA; TALEN-R: TCTCATGCAGTAGACATG; Spacer: CCAAAGAATTCTGC. As expected, the A and T (bases 200–201) bases of exon3 was knocked out. After construction by the Golden Gate Assembly method and confirmation by sequencing, TALEN mRNA was generated by *in vitro* transcription, which was further microinjected into fertilized eggs for production of knockout mouse. The mRNA transcribed from the targeted allele with a frameshift mutation will undergo nonsense-mediated decay (NMD), leading to the early appearance of the termination codon, thus influencing the translation of the active protein IL-33.

The IL-33 knockout heterozygous mice were crossbred with WT mice to obtain the offspring.The IL-33$^{-/-}$ and WT mice in our experiments were both raised from the offspring to exclude the influence from the differences in genetic background, breeding environment, *etc.*

All mice were housed under standard light- and climate-controlled conditions, with standard chow and water being provided *ad libitum*. All experiments were performed in strict accordance with the Guidelines and Permission of Tongji Medical College Animal Care and Use Committee.

2.3. Polymerase chain reaction (PCR)

The IL-33 knockout heterozygous mice were mated with the WT mice, and the F2 mice were screened for IL-33 knockout by PCR analysis of tail DNA. Tail DNA was prepared by incubating 5 mm of the tail in 100 μL of buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0)-, 0.1% Triton-X-100, and 0.5 mg/mL protease K) for DNA extraction, and the mixture was incubated overnight in a water bath at 56 °C for and then 98°C for 13 min to inactivate protease K. The samples were centrifuged at 12,000 × *g* in a micro-centrifuge for 15 min, and the supernatant contained the total DNA of the mouse. The IL-33 knockout gene was identified by PCR-based analysis using TALEN–specific primers; Talen-F: AGTAGTGCTGTTCCAGCCTCTGTTGG; Talen- R: AGCAAATAGATGC CCATCAGTCTTTCC. After preliminary denaturation at 95 °C for 3 min, the DNA template were subjected to 35 amplification cycles involving denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 1 min. An additional step of extension at 72 °C for 10 min was performed. The 255-bp-longPCR product included the mutated portion. After electrophoresis on agarose gel, the PCR product was sequenced (Qingke Biological Technology Co., LTD., Wuhan, China) to confirm the knockout of the A, and T bases.

2.4. Western blotting

The indicated samples (spinal cord, brain, and cultured cells) were isolated and homogenized on ice in the lysis buffer (50 mM Tris, 150 mM NaCl, 0.02% $NaNO_3$, and 1% Triton X-100) containing the proteinase inhibitor cocktail (Chen et al., 2015). The supernatant was collected after centrifugation at 12,000 × *g* for 15 min at 4°C. To maintain identical volume and quality during sample loading, the protein concentration of different groups were normalized using the BCA Protein Assay Kit (Thermo Fisher) by the addition of different volumes of 1× phosphate-buffered saline (PBS). All samples, degenerated in boilingwater after mixing with 5× sodium dodecyl sulfate (SDS)-loading buffer, were frozen at -80°C.

The protein content was separated by 12% SDS–polyacrylamide gel

Download English Version:

https://daneshyari.com/en/article/9955385

Download Persian Version:

https://daneshyari.com/article/9955385

Daneshyari.com