Biochemical and Biophysical Research Communications 501 (2018) 563–569

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

STAT1 activation represses IL-22 gene expression and psoriasis pathogenesis

Li Bai [a], Huihui Fang [a], Sisi Xia [b], Ruochan Zhang [a], Liwu Li [c], Jordi Ochando [d, e], Jiangnan Xu [a, **], Yaozhong Ding [a, *]

[a] Department of Immunology, Capital Medical University, Beijing, 100069, China
[b] Department of Rheumatology, Chaoyang Hospital, Beijing, 100020, China
[c] Department of Biological Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA
[d] Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY 10129, USA
[e] Immunología de Transplantes, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, Spain

ARTICLE INFO

Article history:
Received 27 April 2018
Accepted 7 May 2018

Keywords:
STAT1
STAT3
IL-22
Inflammation
Psoriasis

ABSTRACT

IL-22 plays an important role in tissue repair and inflammatory responses, and is implicated in the pathogenesis of psoriasis, ulcerative colitis, as well as liver and pancreas damage. The molecular mechanisms of its regulation have been actively studied. Here, we show that the differential regulation of IL-22 expression in $CD4^+$ T cells by IL-6 and IL-27 was detected rapidly after stimulation. Chromatin immunoprecipitation (ChIP) and luciferase reporter assays demonstrated that both STAT1 and STAT3 directly bind to the STAT responsive elements (SRE) of the IL-22 promoter, and the balance between activated STAT3 and STAT1 determines IL-22 promoter activities. We further show that the heterozygous mutation of the STAT1 gene results in elevated levels of IL-22 production and induces much severer skin inflammation in an imiquimod (IMQ)-induced murine psoriasis model. Together, our results reveal a novel regulatory mechanism of IL-22 expression by STAT1 through directly antagonizing STAT3, and the importance of the balance between STAT3 and STAT1 in IL-22 regulation and psoriasis pathogenesis.

1. Introduction

IL-22 was originally identified as a member of the IL-10 family cytokines, designated IL-10-related T cell-derived inducible factor (IL-TIF), in IL-9-stimulated murine T lymphoma cells [1]. T cells were first considered as the source of IL-22, and subsequent studies demonstrated that innate immune cells, including γδ T cells, NK T cells and innate lymphoid cells, can also secret IL-22 [2]. IL-22 functions via a transmembrane receptor complex which consists of two different subunits, IL-22R1 and IL-10R2 [3], and the expression of IL-22R1 determines the cellular response towards IL-22. In the skin, keratinocytes are the main type of IL-22-responsive cells [4]. Strongly increased levels of plasma IL-22 are correlated with the severity of disease in psoriatic patients [5]. Moreover, IL-22 has also been reported to mediate IL-23-induced dermal inflammation and acanthosis [6].

Due to its expression in Th17 cells, IL-22 was considered as a Th17 family cytokine [7]. However, later studies demonstrated that there are also distinct regulatory mechanisms for IL-17 and IL-22 [8–12]. It has also been shown that IL-17 and IL-22 modulate distinct downstream pathways that contribute to the psoriatic phenotype, as IL-17 is more proinflammatory, while IL-22 retards keratinocyte differentiation [13].

Despite being activated downstream of common cytokine and growth factor receptors, the transcription factors STAT1 and STAT3 appear to play opposite roles, and their balanced expression is critical for immunoregulation [14,15]. STAT3 is required for IL-21-induced IL-22 expression in $CD4^+$ T cells [16], STAT1 deficiency abolishes IL-27-mediated suppression of IL-17 and IL-22 [17,18]. Increased levels of STAT3 activation are observed in human psoriatic lesions, and the cooperation between STAT3 activation in keratinocytes and activated T cells is required for the development of psoriasis in a mouse model [19]. However, the precise role of STAT1 in the pathogenesis of psoriasis has not been elucidated.

* Corresponding author. Department of Immunology, Capital Medical University, No.10 Xi Tou Tiao, You An Men Wai, Beijing, 100069, China.
** Corresponding author. Department of Immunology, Capital Medical University, No.10 Xi Tou Tiao, You An Men Wai, Beijing, 100069, China.
E-mail addresses: xujn@ccmu.edu.cn (J. Xu), dingyz@ccmu.edu.cn (Y. Ding).

https://doi.org/10.1016/j.bbrc.2018.05.042
0006-291X/

Studies of the impacts of STAT1 and STAT3 on immune regulation are mostly conducted through the comparison between IL-6 and IL-27 signals due to their common signal-transducing receptor chain, gp130, and their differential abilities of STAT1 and STAT3 activation [20], It does not exclude the possibility that the specific ligand-binding subunits, and the differential cellular distribution and expression of these cytokine receptors may also contribute to, sometimes even play significant roles in, determining their respective signaling and functional characteristics. STAT1 or STAT3 gene deficient cells have also been extensively utilized to dissect their roles in immune regulation, but these all-or-none approaches may not be able to reflect the complexity of that some genes require both STAT1 and STAT3 (heterodimers) to be properly regulated.

In this study, we took advantage of the mice and cells with STAT1 heterozygous mutation and the characteristics of IL-6 and IL-27 to induce differential relative levels of STAT1 and STAT3 activation to dissect the roles of STAT1 and STAT3 in IL-22 regulation and psoriasis pathogenesis. We demonstrate that IL-22 promoter activities and IL-22 gene expression correlate to the balance between STAT3 and STAT1 activation. STAT1 heterozygous mutation results in elevated levels of IL-22 and IL-17 A expression in $CD4^+$ T cells, and severer skin inflammation and higher levels of STAT3 activation in IMQ-induced murine psoriasis model. Together, our results reveal a novel regulatory mechanism of IL-22 expression by STAT1 through directly antagonizing STAT3 and altering the balance between STAT3 and STAT1.

2. Materials and methods

2.1. Mice and regents

STAT1 knockout (129Sv/Ev background) mice were purchased from Taconic Biosciences (Rensselaer, NY, USA), and cross bred with C57BL/6 wild type mice (Charles River Laboratories, Beijing, China) for 10 generations. $STAT1^{+/-}$ mice were selected by PCR. All mice were hosted under specific-pathogen-free conditions, and all the experiments in this study were approved by the Institutional Animal Care and Use Committee of Capital Medical University (Beijing, China). Recombinant mouse IL-6, IL-27, anti-CD3ε and anti-CD28 were purchased from eBioscience (San Diego, CA, USA). Naïve $CD4^+$ T cell isolation kit was purchased from Miltenyi Biotec (Bergisch Gladbach, Germany). Anti-STAT1, anti-STAT3, anti-phosphorylated STAT3 (Tyr705), anti-phosphorylated STAT1 (Tyr701) Abs and ChIP kit were purchased from Cell Signaling Technology (Boston, MA, USA).

2.2. T cell purification and activation

Naïve $CD4^+$ T cells were selected using microbeads and seeded in 96-well round-bottom plates (5×10^4 cells/well). Cells were stimulated with plate-bound anti-CD3ε (5 μg/ml) and soluble anti-CD28 (1 μg/ml), along with various cytokines.

2.3. Real-time RT-PCR

Total RNA was isolated from samples using RNeasy Protect Mini kit (Qiagen, Valencia, CA, USA), and treated with DNAse I (Fisher Scientific, Pittsburgh, PA, USA), and cDNA was reverse transcribed using Omniscript RT kit (Qiagen) with random primers. The primers for quantitative real-time PCR were as follows: *cyclophilin A*, AGG GTG GTG ACT TTA CACGC and ATC CAG CCA TTC AGT CTT GG; *IL-22*, ATG AGT TTT TCC CTT ATG GGG AC and GCT GGA AGT TGG ACA CCT CAA; *IL-17A*, TTT AAC TCC CTT GGC GCA AAA and CTT TCC CTC CGC ATT GAC AC; *IL-23*, ATG CTG GAT TGC AGA GCA GTA and ACG GGG CAC ATT ATT TTT AGT CT; *IL-6*, TAG TCC TTC CTA CCC CAA TTT CC and TTG GTC CTT AGC CAC TCC TTC; *IFN-γ*, ATG AAC GCT ACA CAC TGC ATC and CCA TCC TTT TGC CAG TTC CTC. Reactions were performed using the SYBR Green PCR kit (Qiagen) on the Rotor-Gene Q system (Qiagen). All experiments were performed at least three separate times. Amplification of the *cyclophilin A* gene was used as a housekeeping gene.

2.4. Chromatin immunoprecipitation

ChIP assays were performed according to the manufacture's protocol (Cell Signaling Technology). Briefly, wild type naïve $CD4^+$ T cells were treated with anti-CD3ε and anti-CD28 mAbs in the presence of IL-6 or IL-27 for 24 h, the chromatin (~250bp) was subjected to immunoprecipitation using anti-STAT1 or anti-STAT3. Immunoprecipitated DNA was analyzed by Real-time PCR. The primers used are as follows: IL-22 SRE-FOR, ACG GGA GAT CAA AGG CTG CT and IL-22 SRE-REV, GTG AAT GAT ATG ACA TCA GAC. All experiments were performed at least three separate times.

2.5. Luciferase reporter assay

The wild type IL-22 promoter containing SRE binding motif were amplified from C57BL/6 mouse genomic DNA by PCR with primers carrying restriction sites for *Xho*I and *Hind*III (5′- CCG CTC GAG GGA CAC GGG TCT TTT ATT-3′ and 5′-CCC AAG CTT TCT GAG TGC TTA CCT GTT-3′), and cloned into the PGL 4.18 luciferase reporter vector. EL-4 cells were transfected by electroporation using a Nepa21 pulse generator (Nepa Gene, Chiba, Japan). After transfection, EL-4 cells were stimulated with IL-27 (20 ng/ml) or IL-6 plus IL-27 (20 ng/ml each) or IL-6 (20 ng/ml) immediately, and luciferase activity was measured 24 h later with the luciferase assay kit (Promega) according to the manufacturer's protocol.

2.6. IMQ-induced murine psoriasis model and quantitative analysis of psoriasis-like skin lesions

Mice at 8–12 weeks of age were shaved on the back skin and received daily, for 7 consecutive days, topical applications of IMQ (3.125 mg) from a commercially available cream (5%) (Aldara; 3 M Pharmaceuticals, Saint Paul, MN, USA). Control mice were treated similarly with a vehicle cream (Vaseline Lanette cream; Fagron, Rotterdam, The Netherlands). We used age- and sex-matched littermates, and animals were assigned randomly to experimental groups. A quantitative scoring system based on the Psoriasis Area and Severity Index (PASI) was served to measure the severity of psoriasis-like skin lesions induced by IMQ. Erythema, scale and thickness of the mouse back skin was scored independently on a scale from 0 to 4: 0, none; 1, slight; 2, moderate; 3, marked; 4, very marked. The cumulative score (erythema + scale + thickness) was obtained based on the summation of all the scores (scale 0–12).

2.7. Histopathology and immunohistochemistry (IHC)

For IHC assays, back skin biopsies were collected 24 h after last IMQ treatment, fixed in 4% formaldehyde, embedded in paraffin wax, and cut into 5 μm sections. The tissue sections were probed with rabbit anti-phosphorylated STAT3, followed by HRP-conjugated goat anti-rabbit IgG, and the brown precipitating HRP substrate DAB was used for detection.

2.8. Western blotting

Cells were harvested and lysed in RIPA buffer and homogenized. Protein concentration was determined by Micro BCA Protein Assay kit (Pierce). Proteins were separated by SDS-polyacrylamide,