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TIGIT negatively regulates inflammation by altering macrophage phenotype

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ABSTRACT

Macrophages function as an essential component of innate immune system, contributing to both the initiation and appropriate resolution of inflammation. The exposure of macrophages to the microbial products, such as lipopolysaccharide (LPS), can strongly shift the balance between tissue homeostasis and inflammation in favor of causing systemic damage, in which macrophage M1 polarization play important roles. Strategies aiming at restoring the balance of macrophage polarization remain to be further explored. Herein, we have demonstrated that poliovirus receptor

(PVR), the receptor of TIGIT, was dramatically upregulated on the surface of mouse peritoneal macrophages when exposed to LPS. TIGIT-Fc fusion protein not only inhibited the macrophage activation, but also skewed M1/M2 balance toward an anti-inflammatory profile, especially enhanced the secretion of IL-10. The activation of TIGIT/PVR pathway in macrophages correlated with increased nuclear translocation of c-Maf, which promotes IL-10 transcription. Treatment with fibroblasts stably secreting TIGIT-Fc fusion protein significantly reversed the lethal and sublethal endotoxic shock, which facilitated peritoneal macrophages to switch towards anti-inflammatory M2 cytokine profiles. These findings highlight a novel role of the TIGIT/PVR pathway in macrophage M2 polarization and suggest that TIGIT may have the potential to optimize the treatment of macrophage-involved inflammatory diseases.

1. Introduction

Macrophages play indispensable roles in host homeostasis and defense, which are widely distributed as sentinels for the first line alerts. They are functionally and phenotypically polarized heterogeneous population under different stimulation, such as microbial products, cytokines or other immunomodulatory molecules [1,2]. Polarized macrophages are classified into two major subtypes termed proinflammatory M1 (classically activated) and anti-inflammatory M2 (alternatively activated). M1 macrophages are typically induced by exposure to IFN-γ or TNF-α in the presence bacterial components such as lipopolysacchride (LPS). M2 macrophages could be polarized by a number of stimuli, including IL-4, IL-10 and glucocorticoid [3]. It has been demonstrated that elimination of M2 macrophages precipitates the

Abbreviations: TIGIT, T cell immunoglobulin and ITIM domain; PVR, poliovirus receptor; TLR, toll-like receptor; LPS, lipopolysaccharide; Fc, fragment crystallizable region of immunoglobulin; TNF-α, tumor necrosis factor-α; MCP-1, monocyte chemoattractant protein-1; IL, interleukin; CCL11, C-C motif chemokine 11; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon gamma; CXCL1, C-X-C motif ligand 1; MIP-1α, macrophage inflammatory protein-1α; MIP-1β, macrophage inflammatory protein-1β; RANTES, regulated on activation: normal T cell expressed and secreted; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TGF-β, transforming growth factor β; Fgl2, fibrinogen-like protein 2; Teff, effector T cells; Treg, regulatory T cells; Akt, also known as PKB (protein kinase B); eGFP, enhanced green fluorescent protein; DC, dendritic cells; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte-macrophage colony stimulating factor; ELISA, enzyme-linked immunosorbant assay; c-Maf, V-maf (Avian musculoaponeurotic fibrosarcoma oncogene) homolog; PVDF, polyvinylidene difluoride; Arg1, arginase 1.

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LPS-induced lung inflammation [4]. In contrast, Akt2 ablation resulting in an M2 phenotype promotes the resistance to LPS-induced shock [5]. Delicate equilibrium of M1 and M2 macrophages participates in orchestrating LPS-induced inflammation and its resolution. However, the specific contributions of newly identified molecules on macrophage polarization are not fully understood. It has been demonstrated that macrophages could upregulate poliovirus receptor (PVR, also known as CD155) expression in response to Toll-like receptors (TLRs) activation [6]. Given that PVR serves as a receptor for T cell immunoglobulin and ITIM domain (TIGIT), a newly identified immunosuppressive molecule [7], we suppose that TIGIT may possess the capability to negatively regulate the function of macrophages.

TIGIT has been identified as a new member of CD28 family. It is preferentially expressed on activated effector T cells (Teff), regulatory T cells (Treg), and NK cells [7,8]. The ligand for TIGIT is PVR, an immunoglobulin-like cell adhesion molecule and a member of the nectin-like family, which is expressed on dendritic cells (DCs), endothelial cells and some tumor cells [7,9]. TIGIT/PVR interface forms a conserved specific "lock-and-key" structure and transduces a bidirectional signaling. On one hand, TIGIT acts as a co-inhibitory receptor on T cells and NK cells to inhibit their activation [10,11]. Agonistic anti-TIGIT has been shown to markedly inhibit the activation of T cells through down-regulation of the TCR alpha chain [10]. This novel finding supports the notion that TIGIT acts as a co-inhibitory molecule which acts on the upstream of TCR signaling. Moreover, the ligation of TIGIT on Treg induces a paracrine mechanism that secretion of fibrinogen-like protein 2 (Fgl2) suppress the Teff proliferation [12]. On the other hand, TIGIT acts as a ligand for PVR expressed on DCs. Yu et al. have reported that TIGIT could indirectly inhibit the T cell responses by modulating cytokine profiles of mature DCs via ligation on PVR [7]. In short, TIGIT acts as an immunomodulatory molecule both on adaptive and innate immune cells. But the role(s) of TIGIT on another component of innate immune system, namely macrophages, remains (remain) unknown.

The response to endotoxin, also known as LPS, is an important mechanism for macrophages to defense against Gram-negative bacteria. However, excessive LPS challenge leads to life-threatening endotoxic shock in which M1 macrophages play fundamental roles [4,13]. Herein, we show in a rodent endotoxin shock model that TIGIT leads to a shift from M1 to M2 polarization via ligation on PVR expressed on mature macrophages, resulting in protection against lethal shock.

2. Materials and methods

2.1. Mice

Specific pathogen-free (SPF) male BALB/c mice (6–8 weeks old) were purchased from Hunan SJA Laboratory Animal Company (Changsha, China). Animal were maintained and used according to the NIH Guide for the Care and Use of Laboratory Animals. All animal studies conducted were approved by Animal Research Committee of Tongji Medical College, HUST.

2.2. Cell lines and isolation of mouse peritoneal macrophages

Human embryonic kidney (HEK) 293T cells and NIH/3T3 mouse fibroblasts were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) and maintained in DMEM (HyClone, Logan, UT) supplemented with 10% FBS (GIBCO, Grand Island, NY). Mouse peritoneal macrophages derived from BALB/c mice were prepared as described previously [14]. The cells were cultured in RPMI 1640 (HyClone, Logan, UT) supplemented with 10% FBS. All non-adherent cells were removed before the further experiments.

2.3. Plasmid construction and lentiviral production

Total RNA was isolated from spleens by TRIzol Reagent (Invitrogen, Carlsbad, CA) and cDNA was synthesized by reverse transcription with a ReverTra Ace-α-kit (TOYOBO, Osaka, Japan). The extracellular fragment of mouse TIGIT (amino acids 1–135) excluding stop codon was PCR amplified (primers: 5′AAG CTT ATG CAT GGC TGG CTG CTC CTG GT3′ and 5′GGA TCC GAA CTG AGC CAC TGA GCT TTC TT3′) and cloned into an Igκ-Fc-pSecTag2B vector which contains a human IgG3 Fc domain (generously provided by Prof. Jin Bo-Quan, the Fourth Military Medical University, China). The Fc-fused TIGIT fragment (TIGIT-Fc) was then amplified by PCR using primers (5′GGA AGA TCT CCA CCA TGG AGA CAG ACA CAC T3′ and 5′GGA AGA TCT GAA TTC TCA TTT ACC CGG AGA CA3′) containing the *Bgl*II restriction site and inserted into the lentiviral transfer vector pLOX (generously provided by Dr. Huang Min, Tongji Hospital, HUST, China). The lipofectamine 2000 (Invitrogen, Carlsbad, CA) transfection method was used for lentivirus production. HEK293T cells were seeded at a density of 8×10^6 cells per 15 cm culture dish one day before transfection. Each indicated lentiviral transfer vector (24 μg) (TIGIT-Fc-pLOX or empty vector pLOX) was mixed with pCMVΔR8.2 (18 μg) and pMD.G (9 μg) for co-transfection. Supernatant containing recombinant lentiviral particles was passed through a 0.45 μm filter (Corning, NY, USA) 48 h after transfection and then stored at −80 °C. Multiplicity of infection and viral titer of the supernatant were determined according to the method previously described [15]. Briefly, 5×10^4 293T cells were plated into a 96-well plate and lentivirus was added with dilutions ranging from 10^{-2} to 10^{-1} in the presence of 6 μg/ml of polybrene (Sigma–Aldrich, St. Lous, MO). Four days after transduction, biological titer was calculated by the following equation: transduction unit (TU/ml) = (% of enhanced Green Fluorescent Protein (eGFP) positive cells × number of cells at time of transduction) × dilution factor / (100 × volume of lentivirus added).

2.4. Production of NIH/3T3 cell lines stably secreting TIGIT-Fc

NIH/3T3 cells (2×10^5) were suspended in 0.4 ml RPMI 1640 medium containing 6 μg/ml polybrene in a 1.5 ml tube, and 0.1 ml of viral stock (2×10^7 TU/ml, MOI = 10) was added and incubated at 37 °C for 2 h. Infected cells were then transferred into a 25 cm^2 tissue culture flask with 2 ml of RPMI 1640 medium and incubated at 37 °C with 5% CO_2. The medium was replaced 24 h after infection and transduction efficiencies were assessed on day 4. The percentage of eGFP positive cells was estimated under a fluorescence microscope (Nikon Eclipse TE2000-U, Tokyo, Japan) and determined by Flow cytometry (BD FACS Calibur, Bedford, MA). The cell line stably secreting TIGIT-Fc protein was designated as TIG-Fc-3T3, with the cell line eGFP-3T3 which integrated the empty pLOX vector as control. The concentration of TIGIT-Fc in the supernatant was determined and calculated by automated immunoturbidimetric assay of human IgG3 (Beckman Coulter AU5800, CA, USA).

2.5. Purification of soluble TIGIT-Fc protein

Recombinant TIGIT-Fc was purified by Novoprotein Scientific Inc. (Shanghai, China) for in vitro experiment. In brief, fusion TIGIT-Fc was constructed in a mammalian expression vector using the plasmids containing TIGIT-Fc we provided as a template. Then, the mammalian expression was introduced into CHO cells and secreted Fc-fusion proteins were purified by affinity chromatography on

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