Molecular Immunology 101 (2018) 251–258

Contents lists available at ScienceDirect

Molecular Immunology

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

Myeloid-derived suppressor cells exacerbate Sjögren's syndrome by inhibiting Th2 immune responses

Jingjing Qi[a,1], Dan Li[a,1], Guoping Shi[a], Xuefang Zhang[a], Yuchen Pan[a], Huan Dou[a,b], Genhong Yao[a,c,**], Yayi Hou[a,b,*]

[a] The State Key Laboratory of Pharmaceutical Biotechnology, Division of Immunology, Medical School, Nanjing University, Nanjing, PR China
[b] Jiangsu Key Laboratory of Molecular Medicine, Nanjing, PR China
[c] Department of Rheumatology and Immunology, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, PR China

ARTICLE INFO

Keywords:
Myeloid-derived suppressor cells
Sjögren's syndrome
Th2 cells

ABSTRACT

Myeloid-derived suppressor cells (MDSCs) can regulate various aspects of immune responses based on their potent immune-suppressive activity. Studies reported that MDSCs participated in many autoimmune diseases. However, the role of MDSCs in Sjögren's syndrome (SS) is unknown. In this study, we determined the frequencies and function of MDSCs in non-obese diabetic (NOD) mice and SS patients. The NOD mice were adoptively transferred with MDSCs or treated with anti-Gr1 antibody. Results showed that peripheral MDSCs increased significantly with the development of SS-like syndrome in NOD mice and the percentage of MDSCs was higher in SS patients than healthy controls. The SS-like syndrome aggravated after transfer of MDSCs in NOD mice. The deletion of MDSCs in NOD mice alleviated SS-like syndrome. Mechanistically, MDSCs down-regulated the percentages of Th2 cells in NOD mice and SS patients. In summary, our findings suggested that MDSCs exacerbated Sjögren's syndrome by inhibiting Th2 cells.

1. Introduction

Sjögren's syndrome (SS) is a common chronic systemic autoimmune disorder with lymphocytic infiltration in salivary and lacrimal glands chiefly which results in dry eyes and mouth (Mavragani, 2017; Vakrakou et al., 2018). SS patients have suffered from lacking of effective therapeutic due to its complicated pathogenesis. Therefore, a better understanding of pathogenesis underlying the disease is required for the new therapeutic targets and novel therapeutic strategies. B cell activation, IL-12 and resultant activation of Th1 cells secreting the type II interferon IFN-γ, Th17 cells, and natural killer (NK) cells were reported to related to SS pathogenesis (Nocturne and Mariette, 2013). Thus, targeting to restore the immune cell responses in SS should be explored.

Myeloid-derived suppressor cells (MDSCs) are heterogeneous immature myeloid cells which are derived from the bone marrow and generated in a maturation delay during immunologic stress and characterized by suppressive ability on T cell functions through producing ROS, Arg-1 and NO (Crook and Liu, 2014; Gabrilovich and Nagaraj, 2009; Movahedi et al., 2008; Nagaraj et al., 2009; Vlachou et al., 2016). The function and accumulation of MDSCs are dependent on STAT1 activation by IFN-γ or IL-1β (Hix et al., 2013; Kusmartsev and Gabrilovich, 2005). Initially, MDSCs were reported to relate to tumour pathobiology (Talmadge and Gabrilovich, 2013). While, recently, these cells have been reported to be involved in autoimmunity and pathogenesis of autoimmune diseases (Crook and Liu, 2014; Park et al., 2016; Sica and Massarotti, 2017). Studies have suggested that MDSCs increased and played a pro-inflammatory role in the pathogenesis of systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), type 1 diabetes mellitus (T1D), experimental autoimmune encephalomyelitis (EAE) and other autoimmune diseases (Ji et al., 2016; Whitfield-Larry et al., 2014; Yi et al., 2012; Zhang et al., 2015a). However, the role of MDSCs in SS still remains to be elucidated.

Th2 cell responses are characterized by secreting mediators interleukin-4 (IL-4), IL-5, IL-9, IL-10 and IL-13 (Pulendran and Artis, 2012). And previous study showed that Th1/Th2 imbalance existed in SS (Garcic-Carrasco et al., 2001; Mitsias et al., 2002). According to previous studies, adoptive transfer or DNA vaccination of Th2 cells could

* Corresponding author at: The State Key Laboratory of Immunology, Medical School, Nanjing University, Nanjing 210093, PR China.
** Corresponding author at: Department of Rheumatology and Immunology, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing 210093, PR China.
E-mail addresses: yaogenhong@nju.edu.cn (G. Yao), yayihou@nju.edu.cn (Y. Hou).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.molimm.2018.07.016
Received 18 March 2018; Received in revised form 12 June 2018; Accepted 11 July 2018

protect mice from EAE induction (Garren et al., 2001; Kuchroo et al., 1995). In addition, recent study demonstrated that MDSCs suppressed Th2 cell response to promote chronic infection (Valanparambil et al., 2017). However, the doxorubicin (DOX) treatment promoted lung metastasis by inducing MDSCs to release exosomes miR-126a to increase Th2 cell response (Deng et al., 2017). These studies suggested that the diverse correlation between MDSCs and Th2 cells in different diseases.

In the present study, we found that MDSCs were negatively related to Th2 cell response in SS patients. Of interest, adoptive transfer of MDSCs aggravated SS and reduced Th2 cell response in NOD mice with SS-like disease. While deletion of MDSCs alleviated SS-like symptoms and increased Th2 cell response. Our findings indicated that MDSCs aggravated Sjögren's syndrome by suppressing Th2 cells and targeting MDSCs may be a new treatment strategy for SS patients.

2. Materials and methods

2.1. Study population

The blood was collected from a total of 40 SS patients and 40 healthy controls (HC) from the Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing University. Written informed consents were obtained from all subjects. The study was approved by the ethics committee of our institute.

2.2. Mice

Female NOD mice were obtained from Model Animal Research Center of Nanjing University and kept under pathogen-free conditions in the animal center of the Affiliated Drum Tower Hospital of Nanjing University Medical School.

2.3. Salivary flow rate

After anaesthetization and stimulation by pilocarpina (0.1 mg pilocarpine/kg body weight injected intraperitoneally (i.p.), the whole saliva of the mice was obtained from the oral cavity by micropipette for a 15 min period. Saliva volume was determined gravimetrically.

2.4. Histological analysis

Submandibular glands (SG) of mice were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned at 3 μm, and tissue sections were stained with hematoxylin and eosin (H&E) for histological analysis. Histological scores of SG was determined based on the size and the degree of infiltrates in the organization (Scardina et al., 2007).

2.5. Flow cytometry analysis

Spleen single cell suspensions were prepared after lysing red blood cells. Peripheral blood mononuclear cells (PBMCs) in blood samples were isolated with Ficoll-Hypaque. The appropriate number of cells was pre-incubated with antibodies in optimal concentration (eBioscience).

For analysis of MDSCs, M-MDSCs and G-MDSCs, cells were pre-incubated with surface markers antibodies, anti-mouse CD11b-APC (Clone M1/70), anti-mouse Gr1-PE (Clone RB6-8C5), anti-mouse Ly6G-FITC (Clone RB6-8C5), anti-mouse Ly6C-PE (Clone HK1.4) or anti-human HLA-DR-eFluor450 (Clone L243), anti-human CD11b-APC (Clone ICRF44), anti-human CD14-FITC (Clone 61D3), anti-human CD33-PE (Clone WM-53), anti-human CD66-APC (Clone G10F5).

For analysis of Th2 cells, cells were incubated with 20 ng/ml PMA plus 1 μg/ml ionomycin with 5 μg/ml of brefeldin A (Enzo LifeScience, East Farmingdale, NY, USA) at 37 °C for 4 h. Firstly, surface CD4 with anti-mouse CD4-FITC (Clone GK1.5) were stained. After that, cells were fixed with Cytofix/Cytoperm solution (BD PharMingen), then incubated with anti-mouse IL-4-PE (Clone 11B11) and analyzed on a FACS Calibur flow cytometer (BD Biosciences, Mountain View, CA, USA).

2.6. Adoptive transfer of MDSCs

Splenocyte of 4-week old NOD mice, which were demonstrated to have no SS-like sympotoms, was prepared in single suspension. Pure MDSCs were isolated by using Myeloid-Derived Suppressor Cell Isolation Kit (Miltenyi Biotec) according to the manufacturer's instructions and transfered i.v. 10^6/mouse to 10-week old NOD mice.

2.7. Elimination of MDSCs

10-week old NOD mice were injected intraperitoneally (i.p.) with Rat IgG2b, kappa as control or Ly-6 G (Gr-1) Monoclonal Antibody (RB6-8C5), Functional Grade, eBioscience, 250 μg/mouse every 3d for total 4 times and followed by 200 μg/mouse the last time (Morales et al., 2009).

2.8. ELISA assay

Plasma IL-4, which was collected from mice or HC and SS patients peripheral blood and stored at -80°C immediately, was measured by standard sandwich ELISA kits (R&D Systems) according to the manufacturer's instructions.

2.9. Statistical analysis

Differences in mean ± SEM were evaluated with one-way analysis of variance (ANOVA) followed by Dunnett's test. p values less than 0.05 were considered statistically significant.

3. Results

3.1. MDSCs expansion positively correlated with inflammation during the progression of Sjögren's syndrome in NOD mice

To determine whether the progression of SS-like disease is accompanied by kinetic changes of MDSCs, the frequencies of peripheral MDSCs were detected by flow cytometry in NOD mice. Compared to 4-week old NOD mice (which were demonstrated to have no SS-like symptoms), the percentages of MDSCs increased significantly in PBMC from 8, 10, 12-week old NOD mice (Fig. 1A). The lymphocytes infiltration in SG were also showed in 8, 10 and 12-week old NOD mice, while no lymphocytes infiltration was seen in SG of 4-week old NOD mice (Fig. 1B). Next, we examined the salivary flow rate, which is one of the important indexes for SS, in these mice. The results showed that the volume of saliva decreased significantly by age (Fig. 1C). Next, we detected the expression of immature or undifferentiated phenotype markers CD11c, F4/80, CD80 and MHCII on splenic MDSCs of 4 or 12-week old NOD mice. Results showed that splenic MDSCs from 12-week old NOD mice expressed lower levels of CD11c and F4/80 compared with those in 4-week old NOD mice (Fig. 1D). These data indicated that MDSCs number and suppressive ability related to the severity of SS-like syndrome in NOD mice.

3.2. Adoptive transfer of MDSCs aggravated SS-like syndrome in NOD mice

To define the critical role of MDSCs in SS, the 10-week old NOD mice were adoptively transferred with purified splenic MDSCs from 4-week old NOD mice (Fig. 2A). The flow cytometric analysis results showed the numbers of MDSCs in PBMC and spleen of mice with transfer of MDSCs increased markedly compared with those in control group mice (Fig. 2B). Importantly, the salivary flow rate decreased significantly in MDSC-transferred mice (Fig. 2C). Also, SG of MDSC-