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α-Difluoromethylornithine suppresses inflammatory arthritis by impairing myeloid-derived suppressor cells

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ABSTRACT

Objectives: The chemopreventive drug α-difluoromethylornithine (DFMO) has been shown to have an anti-nociceptive effect on mechanical allodynia in inflammatory arthritis by directly inhibiting ornithine decarboxylase (ODC) and decreasing polyamine production in inflammatory sites. However, little is known about the effect of DFMO on the immune system of inflammatory arthritis. Here, we investigated the effect of DFMO in a well-established collagen-induced arthritis (CIA) mouse model and explored its effect on the immune system.
Methods: The effect of DFMO on the frequency of myeloid-derived suppressor cells (MDSCs) in the spleens of CIA mice and their associations with disease severity, tissue inflammation and the levels of proinflammatory T-helper (Th) 17 cells in lymphoid tissues were investigated. The effects of DFMO on disease severity and Th17 cells were compared with those of antibody depletion of MDSCs. The arthritis severity was also evaluated by adoptive transfer of MDSCs derived from DFMO- or dH_2O-treated mice.
Results: In this study, we showed that both MDSCs and Th17 cells were significantly expanded in CIA mice. Treatment by DFMO at the onset of CIA suppressed the development of arthritis and decreased the frequency of MDSCs and Th17 cells. MDSC depletion by anti-Gr-1 mAb achieved a similar result, while combination treatment of both methods did not achieve a significant difference compared to either of the single treatments. In addition, the adoptive transfer of MDSCs derived from dH_2O-treated mice with CIA restored the arthritis severity of CIA in mice treated with anti-Gr-1 mAb, while the transfer of MDSCs from DFMO-treated mice did not have such an effect.
Conclusions: Our results identified DFMO as a potential therapeutic drug for the treatment of inflammatory arthritis.

1. Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disorder that affects approximately 1% of the population worldwide [1]. Despite the therapeutic revolution made over the past two decades, many issues have not been addressed. For example, although stringent remission (or at least low disease activity) is the current goal for the treatment of RA, many patients do not reach this target [2]. Moreover, the toxicity of conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) and the economic burden of biologic DMARDs (bDMARDs) and targeted synthetic DMARDs (tsDMARDs) also indicate that new therapies are needed.

α-Difluoromethylornithine (DFMO), which irreversibly inactivates ornithine decarboxylase (ODC), the enzyme required for the first stage in polyamine synthesis, was synthesized > 30 years ago. This molecule was proven to have a high degree of safety and tolerability and has been approved by the US Food and Drug Administration (FDA) to treat facial hirsutism [3] and African trypanosomiasis [4]. As the most widely studied example of a polyamine-metabolism inhibitor, this drug has been evaluated not only in animal models but also in clinical trials for suppressing cancer development [5].

The association between polyamines and inflammation has been studied for a long time. A polyamine-deficient diet strongly reduced long-lasting hyperalgesia induced by inflammation or incision in rats [6]. Silva et al. showed that endogenously synthesized polyamines are involved in the development of nociception and edema induced by Complete Freund's Adjuvant (CFA) in rats [7]. Moreover, polyamine production in inflammatory sites may be related to an increase in ODC

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activity stimulated by PKC activation, while subcutaneous administration of the ODC inhibitor DFMO had a long-lasting, antinociceptive effect [7]. Increased polyamine concentrations have been found in urine, synovial fluid, synovial tissue, and peripheral blood mononuclear cells from RA patients as well as animal models [8,9]. Therefore, we hypothesized that DFMO, as a specific irreversible inhibitor of ODC, might be a potential treatment for inflammatory arthritis. Although the chemical mechanism of this drug is known to us, its effect on the immune system of inflammatory arthritis is unclear.

Myeloid-derived suppressor cells (MDSCs) were first described in the late 1970s in patients with cancer [10]. They are a heterogeneous population of cells that expand during cancer, inflammation and infection and have a remarkable ability to suppress T-cell responses [11]. Although ample evidence supports a key role for MDSCs in immune suppression in cancer [12], their role in autoimmune diseases remains controversial. In a collagen-induced arthritis (CIA) mouse model, MDSCs were found to suppress the progression of arthritis by inhibiting the proinflammatory immune response of CD4 + T cells [13]. However, more recent studies have proven that MDSCs have a proinflammatory role in the pathogenesis of autoimmune arthritis in experimental models and in RA patients [14,15]. We showed in previous work that MDSCs were one primary cellular target of DFMO and that DFMO could inhibit tumor growth by impairing the suppressive function of MDSCs in the tumor environment [16]. Therefore, in the present study, we focused on this subset of immune cells, although other groups of immune cells, especially Th17 cells, were also observed.

2. Materials and methods

2.1. Mice

Eight-week-old male DBA/1J mice were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All animal experiments were approved by the Ethical Committee of the Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology. All experiments in this study were carried out in accordance with the National Institute of Health Guide for Care and Use of Animals.

2.2. Induction and assessment of CIA

The CIA model was induced according to the protocol from Chondrex, Inc. Briefly, each DBA/1 mouse received 100 μl emulsion containing 100 mg chicken type II collagen (CII) in 2 mg/ml Freund's complete adjuvant by subcutaneous injection on day 0 as a first immunization. For a booster injection, 100 mg CII in Freund's incomplete adjuvant was given on day 21. Collagen and adjuvants were all purchased from Chondrex, Inc. The severity of arthritis was scored separately by two independent researchers in a blinded manner, and then, the mean score was calculated as follows: 0: normal, no inflammation or redness; 1: red and swelling in one digit; 2: red and swelling in more than one digit or redness and swelling in one digit and ankle and wrist joint; 3: red and swelling present in all digits and joints [17]. The severity scores of each limb were totaled, giving a maximum score of 12 per mouse. For animal model preparation, 8–10 mice were prepared for each group. On day 27, the severity of arthritis of the mice was scored, and any mice scored < 1 would not be chosen. Then the remaining mice were randomly assigned to model or treatment group. Each group was guaranteed to have at least five mice.

2.3. Treatments

DFMO was administered as a 2% solution in drinking distilled H_2O (dH_2O) to mice starting on day 27. The mean DFMO consumption of mice was approximately 1.5 g/kg/d. Mice fed dH_2O without DFMO were used as controls. For MDSC depletion, mice were injected intraperitoneally with 200 μg of anti-Gr-1 monoclonal antibodies (mAb) or with rat IgG2b as an isotype control (Bio x cell) in 500 μl PBS twice a week from day 28 after the first immunization.

2.4. Flow cytometric analysis

Single cell suspensions prepared from spleens or draining lymph nodes (DLN) of mice were incubated with anti-CD16/CD32 antibodies for 20 min on ice, followed by staining with fluorescently labeled antibodies for surface markers. Foxp3 and IL-17 staining was performed according to the manufacturer's instructions (BD Biosciences). Flow cytometry was performed with a FACSVerse flow cytometer (BD Biosciences).

2.5. MDSC isolation, suppression assay and adoptive transfer

MDSCs were isolated from the spleens of CIA mice with clinical scores higher than 6 unless indicated using $CD11b^+$ magnetic beads (Miltenyi Biotec). In some experiments, $CD11b^+Gr\text{-}1^{low}$ and CD11b + $Gr\text{-}1^{high}$ cells were sorted from splenocytes by a FACSAria II cell sorter (BD Biosciences).

For the suppression assay, naïve $CD4^+CD25^-CD62L^+$ T cells selected by magnetic beads were labeled with Proliferation Dye eFluor 450 (eBioscience) according to the manufacturer's instructions. MDSCs were derived from both dH_2O-treated and DFMO-treated CIA mice. A total of 2×10^5 cells were cocultured with or without isolated MDSCs from dH_2O-treated or DFMO-treated CIA mice at different ratios in 96-well culture plates in the presence of 1 μg/ml of anti-CD3/CD28 antibodies (BD Biosciences) for 3 days. Cell proliferation was measured by flow cytometric eFluor450 dye dilution. Supernatants were collected and used to measure IL-17A and IL-1β concentrations.

For adoptive transfer of MDSCs, CIA mice were first treated with anti-Gr-1 mAb on day 28 and day 32 to deplete MDSC. The treated mice were then given MDSCs derived from CIA mice. Splenic MDSCs from CIA mice treated with DFMO or dH_2O purified using CD11b MicroBeads (Miltenyi Biotec) were injected i.v. at 5×10^6 per mouse into anti-Gr-1-pretreated CIA mice at day 33 and day 39 after the initial immunization.

2.6. Cytokine measurement

The concentrations of IL-1β (Dakewe Bioengineering Co., Ltd.) and IL-17A (Life Technologies) in the supernatants or serum were determined by ELISAs according to the manufacturer's instructions.

2.7. Histopathology

For histological analysis, hind paws obtained from mice sacrificed on day 42 were harvested post-mortem, fixed in 4% buffered formaldehyde for 24 h, and decalcified in 10% EDTA solution for 4 weeks. Then, these tissues were paraffin embedded, sectioned, and stained with H&E and Safranin O-fast green. Specimens were assessed semi-quantitatively by two independent researchers for inflammatory cell infiltration, cartilage damage and bone erosion as previously described [18].

2.8. Statistical analysis

Parametric data were analyzed by unpaired Student's *t*-tests, and the clinical and histological CIA scores were analyzed by the Mann–Whitney *U* test. *P* values < 0.05 were considered statistically significant.

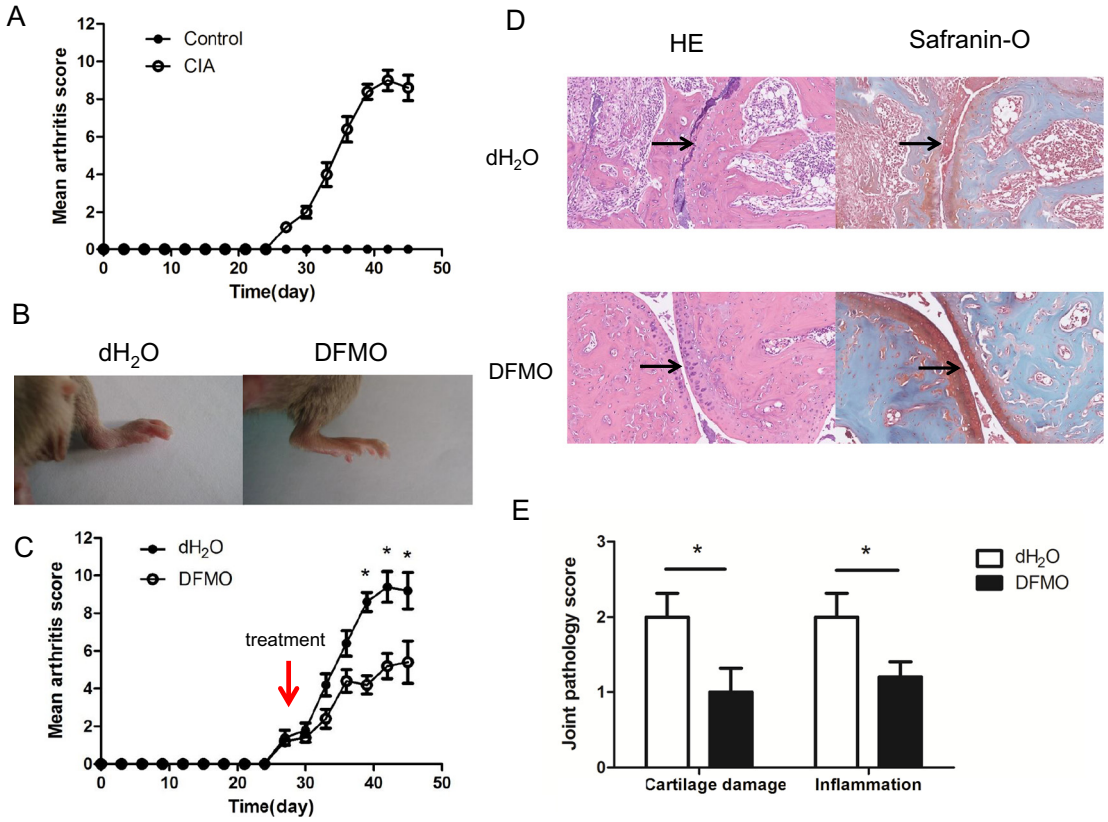

Fig. 1. DFMO treatment suppressed the development of CII-induced arthritis in CIA mice. (A) Clinical arthritis scores of mice after CFA-collagen immunization. (B–C) Representative images of hind paws (B) and clinical arthritis scores (C) of CIA mice treated with 2% DFMO or dH_2O. Arrows in red indicate the time when DFMO or dH_2O was administered (day 27). (D) Representative H&E or Safranin-O/fast green-stained sections of paws of CIA mice 18 days after DFMO or dH_2O treatment. (E) Pathology scores of hind paw sections. Arrows in black indicate the representative joint where the pathology was identified. The results are summarized from the data of 5 mice in each group. Data (mean ± SEM) are representative of three independent experiments. $*p < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

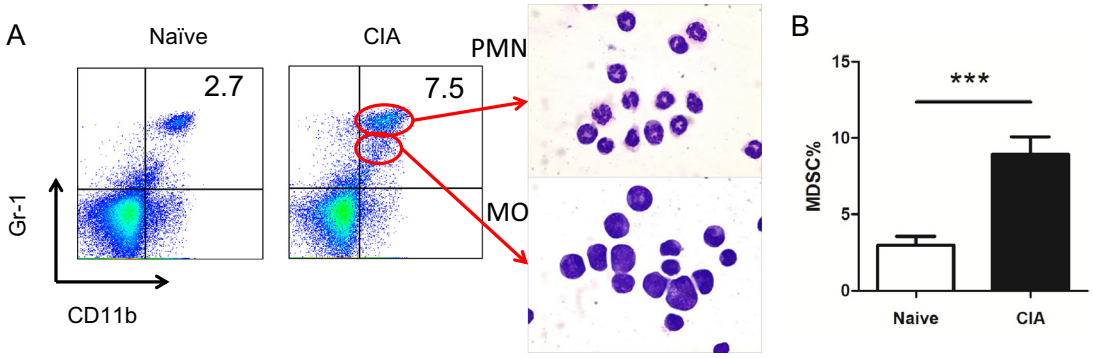

Fig. 2. $CD11b^+Ly6G^+$ MDSCs increased in the spleens of CIA mice. (A) Representative flow cytometric analysis of $CD11b^+Gr\text{-}1^+$ MDSCs from the spleens of naïve or CIA mice (left). The $CD11b^+Gr\text{-}1^{high}$(PMN) and $CD11b^+Gr\text{-}1^{medium}$(MO) cells were sorted by flow cytometry, spun onto a slide and stained with Giemsa (right). (B) Percent of $CD11b^+Gr\text{-}1^+$ MDSCs in the spleen. Data are representative of three independent experiments. $***p < 0.001$.

3. Results

3.1. DFMO treatment suppressed the development of CII-induced arthritis in CIA mice

DBA/1J mice were immunized with CII (Chondrex, Inc. USA) to induce arthritis. The swelling and redness in the paws first appeared around day 27 and peaked on day 42 after immunization (Fig. 1A). To investigate the effects of DFMO consumption on arthritis progression in vivo, we fed DFMO as a 2% solution in drinking dH_2O to CIA mice for two weeks starting on day 27 after the initial immunization. Mice that received DFMO showed a significant reduction of arthritis progression compared with the control mice that were given dH_2O without DFMO (Fig. 1B, C). Histological analysis of the hind paws showed that arthritic lesions, such as cartilage erosion of the articular surface, inflammatory cell infiltration and inflammatory exudation in the articular cavity, were also alleviated by DFMO treatment (Fig. 1D, E).

3.2. DFMO treatment impaired the suppressive effects of MDSCs

To identify an immune mechanism for the DFMO-mediated anti-arthritis effect, we investigated the well-defined immune cell subsets in

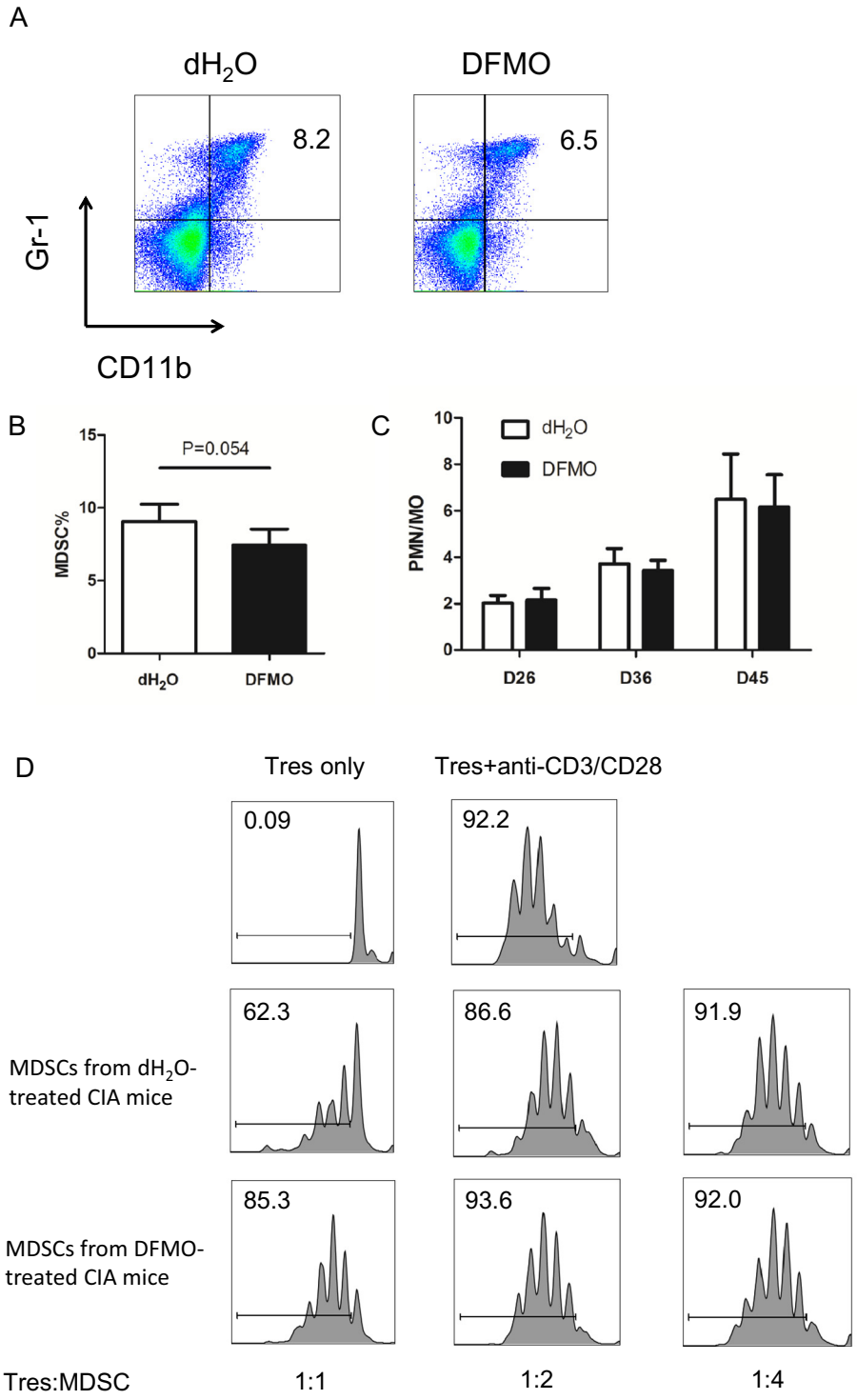

Fig. 3. DFMO treatment impaired the suppressive effects of MDSCs. (A-B) Representative flow cytometric analysis of $CD11b^+Gr\text{-}1^+$ MDSCs (A) and their percent (B) in the spleens of CIA mice 14 days after DFMO or dH_2O treatment. (C) The ratios of $CD11b^+Gr\text{-}1^{high}$(PMN) and $CD11b^+Gr\text{-}1^{medium}$(MO) cells in the spleens of CIA mice treated with DFMO or dH_2O. (D) MDSCs from dH_2O-treated or DFMO-treated CIA mice were added at different ratios to eFluor 450–labeled CD4 + T responder cells (Tres) stimulated with anti-CD3/CD28 for 3 d, and T cell proliferation was measured by flow cytometric eFluor 450 dye dilution (data are representative of three independent experiments).

arthritis. We found that DFMO treatment failed to affect the frequency of splenic $CD4^+Foxp3^+$ Tregs (Supplementary Fig. 1). Because we showed in previous work that MDSCs were one primary cellular target of DFMO, we focused on this subset of immune cells and Th17 cells, which have been reported to play a crucial role in both the CIA model and patients with RA [19,20].

Recent studies have found that MDSCs play an important role in the pathogenesis of autoimmune arthritis, but whether MDSCs play a protective or promoting role remains controversial. Similar to the results in a previous report by Zhang and colleagues, significantly more $CD11b^+Ly6G^+$ MDSCs were found in the spleens of CIA mice compared with naïve mice (Fig. 2A, B) in our study [14]. MDSCs in CIA mice contained two subsets that were characterized by $CD11b^+Gr\text{-}1^{high}$ cells and $CD11b^+Gr\text{-}1^{medium}$ cells, respectively (Fig. 2A). After the cells were sorted by flow cytometry, Wright-Giemsa staining confirmed that $CD11b^+Gr\text{-}1^{high}$ cells were polymorphonuclear and that $CD11b^+Gr\text{-}1^{medium}$ cells were mononuclear (Fig. 2A right). The morphology and lineage surface markers of these MDSCs were analyzed by flow cytometric analysis. As expected, both of these subsets of MDSCs expressed very low levels of CD11c, F4/80 and MHCII and thus showed an immature or undifferentiated phenotype (Supplementary Fig. 2). Compared with the $CD11b^+Gr\text{-}1^{high}$ subset, the CD11b + $Gr\text{-}1^{medium}$ subset

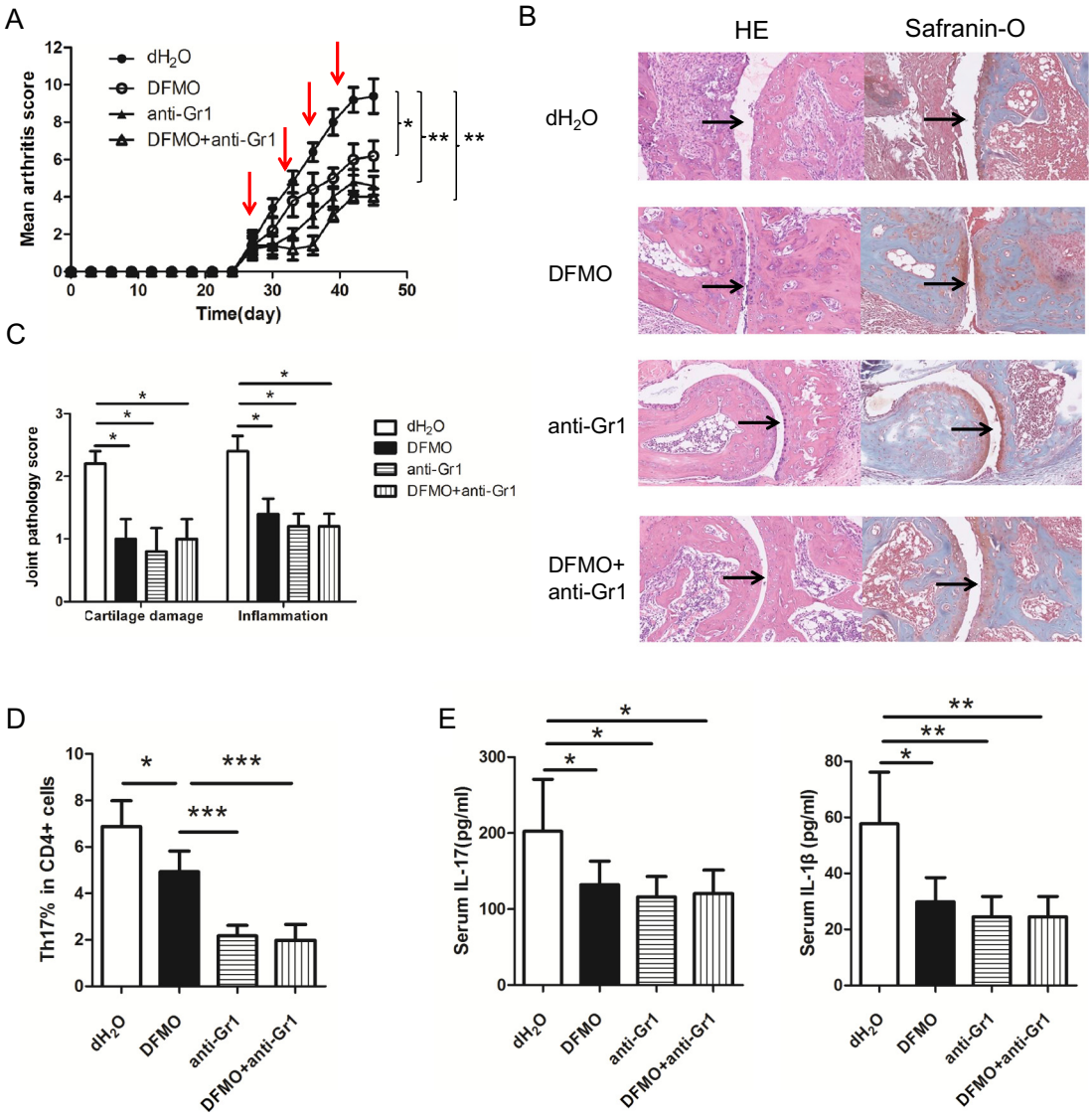

Fig. 4. DFMO alleviated arthritis activity through an MDSC-dependent mechanism. (A-C) The clinical arthritis scores (A), representative H&E or Safranin-O/fast green-stained sections (B) and pathology scores (C) of paws of CIA mice. Arrows in red indicate the time when anti-Gr-1 mAb or control Ab was given. Arrows in black indicate the representative joint where the pathology was identified. MDSC depletion by anti-Gr-1 results in a marked reduction in the severity of arthritis in CIA mice, similar to that of DFMO treatment. The combination of DFMO treatment plus anti-Gr-1 therapy did not have any significant effect compared with any of these two therapies alone (five mice per group). (D-E) The frequency of Th17 cells in $CD4^+$ cells in the DLN (D) and the inflammatory cytokines (IL-17A and IL-1β) in the sera (E). Data (mean ± SEM) are representative of three independent experiments. $*p < 0.05$, $**p < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

expressed higher levels of the monocyte/macrophage marker Ly6C (Supplementary Fig. 2). The ratios of $CD11b^+Gr\text{-}1^{high}$ cells to $CD11b^+Gr\text{-}1^{medium}$ cells increased along with arthritis progression (Fig. 3C white bar).

Although there was no significant difference, there was a trend that DFMO treatment impaired MDSC expansion in CIA mice (Fig. 3A, B). In addition, the ratio of $CD11b^+Gr\text{-}1^{high}$ cells to $CD11b^+Gr\text{-}1^{medium}$ cells (Fig. 3C) and the phenotypes of MDSCs were not changed by DFMO (data not shown).

We next compared the relative T-cell suppressive activities of the MDSCs to examine whether their immunosuppressive activity might be altered after DFMO treatment. MDSCs derived from dH_2O-treated or DFMO-treated CIA mice were added at different ratios to eFluor 450–labeled $CD4^+$ T responder cells (Tres) stimulated with anti-CD3/CD28 for 3 days, and T cell proliferation was measured by flow cytometry. The result showed that the immunosuppressive activity of MDSCs from DFMO-treated CIA mice was impaired compared with that of dH_2O-treated mice (Fig. 3D).

3.3. DFMO alleviated arthritis activity through an MDSC-dependent mechanism

To test whether DFMO suppressed the development of CII-induced arthritis in an MDSC-dependent manner, we performed MDSC depletion in CIA mice using anti-Gr-1 mAb at the initiation of arthritis. MDSC depletion resulted in a marked reduction in the severity of arthritis in CIA mice, similar to that of DFMO treatment (Fig. 4A, B, C), which was correlated with reduced Th17 cells in the DLN (Fig. 4D) and proinflammatory cytokines (IL-17A and IL-1β) in the sera (Fig. 4E). Although anti-Gr-1 therapy or combination therapy resulted in fewer Th17 cells than DFMO treatment, the combination therapy did not have any significant effect on the concentration of cytokines when compared with either therapy alone (Fig. 4D, E). However, it must be admitted that there is another possible explanation for such result: the treatments

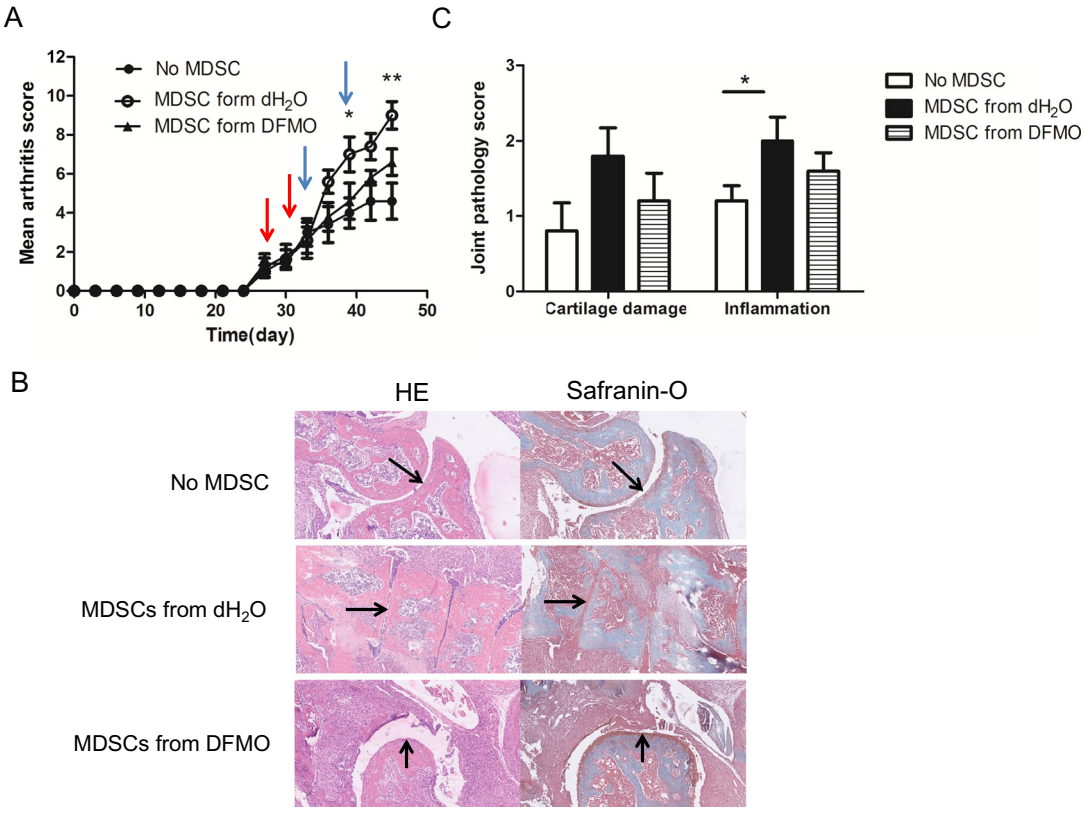

Fig. 5. Adoptive transfer of MDSCs derived from dH2O-treated CIA mice, but not MDSCs derived from DFMO-treated mice, restored the arthritis severity of CIA in mice treated with anti-Gr-1 mAb. (A-C) The clinical arthritis scores (A), representative H&E or Safranin-O/fast green-stained sections (B) and pathology scores on hind paw sections (C) of mice treated with anti-Gr1-mAb, and then administered MDSCs from dH2O- or DFMO-treated CIA mice. Arrows in red indicate the time when anti-Gr-1 mAb was given, and arrows in blue indicate the time when MDSCs were transferred. Arrows in black indicate the representative joint where the pathology was identified. Data (mean ± SEM) are representative of three independent experiments. *p < 0.05, **p < 0.01 and***$p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

individually are prominent, thus using both may not be any better than one. Therefore, we next did an adoptive transfer experiment to further test our hypothesis.

In the adoptive transfer experiment, CIA mice were first treated with anti-Gr-1 mAb on day 28 and day 32 to deplete MDSC. The treated mice were then given MDSCs derived from CIA mice. The mean arthritis score showed that the adoptive transfer of MDSCs derived from dH_2O-treated mice with CIA restored the arthritis severity of CIA in mice treated with anti-Gr-1 mAb, while the transfer of MDSCs from DFMO-treated mice did not have such an effect (Fig. 5A, B, C). Histological analysis also revealed higher inflammatory scores in the joints of mice administered MDSCs from dH_2O-treated CIA mice compared with those without MDSC transfer. In contrast, the inflammatory score in the joints of mice administered MDSCs from DFMO-treated mice was not significantly different from that of mice without MDSC transfer. Although cartilage damage scores did not show significant differences between MDSC from dH_2O group and No MDSC group, they had the same tendency as the inflammatory scores (Fig. 5C).

3.4. DFMO inhibited the Th17 response in CIA mice by impairing MDSCs in an IL-1β-dependent manner

In several previous studies, MDSCs were found to be proinflammatory and regulate CIA by manipulating Th17 cell differentiation [14,15]. Consistent with these published data, we found that the frequency of Th17 cells, similar to MDSCs (Fig. 2B), increased in the DLN of CIA mice compared with naïve mice. However, DFMO treatment reversed this change (Fig. 6A, B). Additionally, DFMO treatment decreased the concentrations of IL-17A and IL-1β in the sera of CIA mice (Fig. 6C).

In an in vitro assay, we found that naïve $CD4^+CD25^-CD62L^+$T cells stimulated with anti-CD3/CD28 mAbs produced little IL-17A, and the production was largely increased in the presence of MDSCs from CIA mice (Fig. 6D). This increase was obviously inhibited in the presence of IL-1Ra (Fig. 6D), suggesting that the induction of the Th17 cell response by MDSCs was at least partly dependent on IL-1β. More intriguingly, the concentration of IL-17 in the supernatant was higher in the presence of dH_2O-treated MDSCs than in the presence of DFMO-treated MDSCs, and the presence of IL-1Ra diminished this difference (Fig. 6D), implying that DFMO might inhibit the Th17 response in CIA mice by impairing MDSCs in an IL-1β-dependent manner.

4. Discussion

Using the well-established CIA mouse model, we showed that oral DFMO administration could be a promising novel therapy for the treatment of inflammatory arthritis. Previous evidence showed that the antinociceptive effect of DFMO on mechanical allodynia in inflammatory arthritis was related to its ability to directly inhibit ODC and thus decrease polyamine production in inflammatory sites [7]. However, we demonstrated here for the first time that DFMO exerts an indirect immune-mediated anti-arthritic effect in an experimental animal model. This effect is at least partly dependent on MDSCs. This novel mechanism has been hypothesized mainly based on the following evidence: first, either DFMO treatment or MDSC depletion at the onset of CIA suppressed the development of arthritis, while combination therapy of both methods did not show significant differences compared to either of the single treatments. Second, the adoptive transfer of

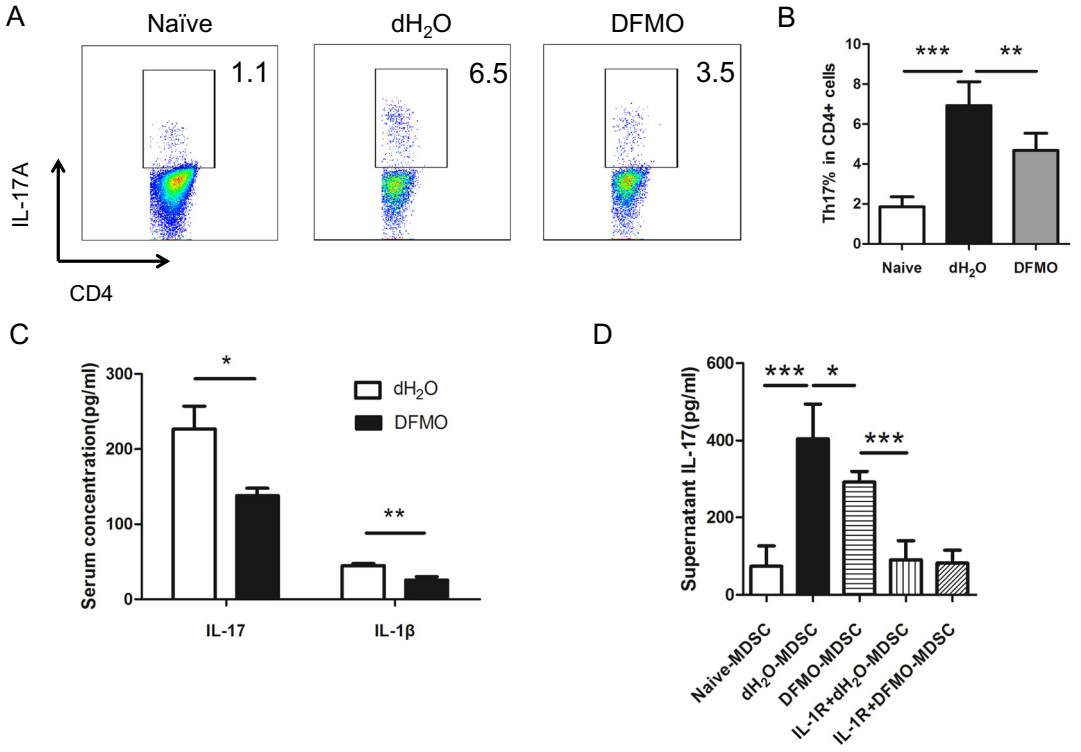

Fig. 6. DFMO inhibited the Th17 response in CIA mice by impairing MDSCs in an IL-1β-dependent manner. (A-B) Representative flow cytometric analysis (A) and the percentage (B) of Th17 cells in CD4+ T cells in the DLN of mice. The frequency of Th17 cells increased in the DLN of CIA mice compared with naïve mice, while DFMO treatment restored this change. (C) DFMO treatment decreased the concentrations of IL-17A and IL-1β in the sera of CIA mice. (D) Naïve CD4 + T cells stimulated with anti-CD3/-CD28 mAbs were cocultured with MDSCs derived from naïve or CIA mice treated with dH_2O or DFMO with or without the presence of IL-1Ra (300 ng/ml) for 3 days. Data (mean ± SEM) are representative of three independent experiments. *$p < 0.05$, **$p < 0.01$ and***$p < 0.001$.

MDSCs derived from dH_2O-treated mice with CIA fully restored the arthritis severity of CIA in mice pretreated with anti-Gr-1 mAb, while the transfer of MDSCs from DFMO-treated mice did not have this effect.

Consistent with the previous observations [14,15], we confirmed that although MDSCs have immunosuppressive functions, they play a critical proinflammatory role in autoimmune arthritis, and the immunosuppressive activity fails to limit the immunopathology. Moreover, the pathogenic effect of MDSCs may at least partly relate to their ability to promote Th17 responses. Th17 cells have been shown to be closely related to autoimmune diseases [21]. Previous data have shown that Th17 cells play a major inflammatory role in the CIA model after the discovery of Th17 cells as a new specialized inflammatory subset in 2006 [22,23]. In RA patients, the receptor activator of nuclear factor kappa-B ligand (RANKL)-dependent induction of osteoclasts via Th17 cells in RA was reported [20]. IL-17 could also maintain the inflammatory cycle via downstream cytokines such as IL-6 [19].

In our earlier study, we showed that MDSCs are a primary cellular target of DFMO and that DFMO could impair the suppressive function of MDSCs in the tumor environment [16]. As expected, the T cell-suppressive activity of MDSCs from DFMO-treated CIA mice was obviously decreased compared with those from dH_2O-treated mice, suggesting that DFMO could impair the immunosuppressive activity of MDSCs in the inflammatory arthritis setting as well. In addition, the frequency of MDSCs as well as Th17 cells increased in the DLN of CIA mice compared with those of naïve mice, while DFMO treatment reversed this change and decreased the elevated concentrations of IL-17A and IL-1β in the sera of CIA mice. In addition, the presence of MDSCs from CIA mice could increase IL-17A production by naïve $CD4^+$ T cells stimulated with anti-CD3/anti-CD28 and this increase was inhibited in the presence of IL-1Ra. Furthermore, the concentration of IL-17 in the supernatant was higher in the presence of dH_2O-treated MDSCs than in the presence of DFMO-treated MDSCs, and the presence of IL-1Ra diminished this difference. The above results implied that DFMO might inhibit the Th17 response in CIA mice by impairing MDSCs in an IL-1β-dependent manner. Therefore, although the immune mechanism of DFMO treatment on inflammatory arthritis has not been fully elucidated, we suggest that there might be at least two possible mechanisms mediating this process: 1. inhibition of the immunosuppressive function of MDSCs and 2. impairment of the Th17-promoting ability of MDSCs.

Compared with current therapeutic drugs used for RA treatment, DFMO has several advantages. First, as a relatively inexpensive chemosynthetic drug, it has a high degree of safety and tolerability. The main side effect of this drug was reversible ototoxicity, which will not be caused by the general treatment dose ($< 1\ g/m^2$) [24]. More than two decades of market observation since its initial launch have also fully demonstrated the safety of DFMO. Second, DFMO is highly soluble in water and therefore can be administered as a solution in drinking water, and we showed that DFMO administered orally could suppress arthritis. The oral delivery will likely improve patient quality of life and compliance. Finally, we previously demonstrated that DFMO could be a potent immunological adjunct in effective cancer therapy, such as that for melanoma, the occurrence of which is one of the concerns for patients using TNFα inhibitors for RA [25]. Therefore, DFMO might have more benefits in combination strategies to enhance the efficacy and minimize the side effects of RA treatment.

5. Conclusions

In conclusion, our observations demonstrated that DFMO is effective in preventing RA-like autoimmune polyarthritis in a murine CIA model and might be a potential therapeutic molecule for the treatment of RA.

Acknowledgements

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Declarations of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.03.040.

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