Polymorphonuclear myeloid-derived suppressor cells play a proinflammatory role via TNF-α + B cells through BAFF/BTK/NF-B signaling pathway in the pathogenesis of collagen-induced arthritis mice

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Abstract

Objectives: Although various studies have been performed on the function of polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) in RA, the results were conflicting. Here we were trying to clarify the role of PMN-MDSCs in the pathogenesis of RA and its specific mechanisms. **Methods**: We detected the frequencies and counts of PMN-MDSCs, TNF- $^{+}$ B cells, and Ki67 $^{+}$ B cells in spleens and inflamed joints of collagen-induced arthritis (CIA) mice using flow cytometry. The pathological role of PMN-MDSCs was examined by anti-Ly6G neutralizing antibodies against PMN-MDSCs or adoptive transfer of PMN-MDSCs. And the modulation of PMN-MDSCs on B cells was conducted by coculture assays, RNA-Seq, RT-qPCR, etc. The mechanism of BAFF regulating B cells was verified through Western Blot and flow cytometry. **Results**: PMN-MDSCs accumulated in the spleens and joints of CIA mice. PMN-MDSCs depletion could alleviate the arthritis severity, which was accompanied by decreased TNF- secretion and proliferation of B cells. And its adoptive transfer also facilitated disease progress. Furthermore, PMN-MDSCs from CIA mice had higher expression level of BAFF, which regulated TNF- expression, proliferation and apoptosis of B cells *in vitro*. What's more, BAFF promoted phosphorylation of BTK/NF-B signaling pathway. And Ibrutinib (BTK inhibitor) could reverse the effect of BAFF on TNF- expression. **Conclusions**: Our study suggested that PMN-MDSCs enhanced disease severity of CIA and manipulated TNF- expression, proliferation and apoptosis of B cells via BAFF, furthermore, BAFF promoted TNF- expression through BTK/NF-B signaling pathway, which demonstrated a novel pathogenesis of PMN-MDSC in CIA.

Introduction

Rheumatoid arthritis (RA) is one of the most prevalent chronic autoimmune diseases, which involves the joints and even cause cartilage and bone damage(1). Autoimmunity and inflammation play important role in the pathogenesis of RA, however the precise mechanism is still unclear(1).

Myeloid-derived suppressor cells (MDSC) are heterogeneous myeloid cell populations that expand in tumors(2), infections (3), autoimmune diseases(4), and other abnormal conditions. The term 'myeloid-derived suppressor cells' was first coined in 2007, indicating its characteristics of myeloid origin and immunosup-

pressive function(5). MDSCs in mice express both CD11b and Gr-1 markers and have two subgroups: polymorphonuclear MDSCs (PMN-MDSCs) ($CD11b^{+}Ly6G^{+}Ly6C^{low}$) and mononuclear MDSCs (M-MDSCs) ($CD11b+Ly6G^{-}Ly6C^{high}$)(6). The role of MDSC in RA pathogenesis is controversial due to their heterogeneity, and some researchers hold the view that MDSCs are protective in RA(7, 8) by inhibiting the proliferation of T cells and B cells, while others believe they are proinflammatory(9, 10). PMN-MDSCs account for the majority of MDSCs in collagen-induced arthritis (CIA), and studies have found that PMN-MDSCs have a therapeutic impact in CIA mice(8, 11). However, we found that PMN-MDSCs play a distinct role in CIA. Therefore, we aimed to explore the mechanism underlying PMN-MDSC-mediated CIA.

The pivotal role of B cells in RA is clearly established by the presence of disease-specific autoantibodies and efficacy of B cell-targeting biological agents(12). A recent study showed that activated B cells also produce cytokines as well as antibodies(12), and B cell-derived TNF could control the development of autoantibodies and regulate the severity of CIA, whereas T cell-derived TNF is dispensable during arthritis(13). Qin et al(14) also found that age-associated B cells induced the activation of fibroblast-like synoviocytes (FLS) via TNF-?, which indicated the important role of TNF-?$^{+}$ B cells in the pathogenesis of RA. Previous studies mainly focused on the PMN-MDSC regulating T cells, less researches have been done on regulating B cells, so we want to add something to this aspect.

Here, we first found that PMN-MDSCs were proinflammatory in CIA model, which could facilitate TNF-? secretion and proliferation of B cells, and inhibit apoptosis of B cells. Thus, our findings explained the pathogenic role of PMN-MDSCs and its regulation of B cells in RA, which provided a novel mechanism of RA.

Results

PMN-MDSCs and B cells accumulated in the joints and spleens of CIA mice

We tested the frequencies of PMN-MDSCs and M-MDSCs in different tissues of CIA mice and found that the PMN-MDSC ratio in $CD11b^{+}$ cells increased significantly in the joints and spleens of CIA mice compared with the control group (DBA/1J mice) (Fig. 1A, 1 B), whereas M-MDSC frequencies were not significantly different between the joints and spleens (Fig. S1A, S1B). PMN-MDSCs accounted for a higher proportion of $CD11b^{+}$ cells than M-MDSCs in the two tissues mentioned above, particularly in the joints. Furthermore, the frequencies of PMN-MDSCs from the joints of CIA mice correlated positively with the clinical arthritis scores of joints (Fig. 1C), indicating that PMN-MDSCs play an important role in arthritis severity. We tested the IgG concentration in plasma and found that it increased significantly in CIA mice (Fig. 1D). In addition, we examined the B cell subsets using flow cytometry, finding that the frequencies and absolute number of Ki67 and TNF-? in B cells increased significantly both in the joints (Fig. 1E) and spleens (Fig. 1F), which indicted that B cells abnormal activation also played an important role in the pathogenesis in CIA.

PMN-MDSCs promoted CIA severity through modulating B cells

To verify that PMN-MDSCs played a proinflammatory or immunosuppressive role in CIA, we depleted PMN-MDSCs in CIA mice from day 32 (disease progression stage) using anti-Ly6G antibodies (1A8), and the control group used the isotype IgG Ab, The Ly6G Ab was effective in reducing the PMN-MDSC ratio and counts in the joints and spleens (Fig. S2A, S2B). PMN-MDSC depletion decreased the severity of arthritis, maintaining an average arthritis score of < 6 (Fig. 2A). Ly6G Ab alleviated joint swelling and decreased inflammatory cell infiltration and synovial hyperplasia compared to control isotype IgG (Fig. 2B). PMN-MDSCs depletion also decreased IgG and TNF-? in the plasma (Fig. 2C). And surprisingly, we found that the frequencies and absolute number of TNF-?$^{+}$B cells and Ki67^{+}B cells performed by flow cytometry also decreased significantly in the joints and spleens after PMN-MDSCs were removed (Fig. 2D-2H). And the TNF-? transcriptional level in the joints and spleens detected by RT-qPCR also declined in Ly6G Ab group (Fig. S2C, S2D).

However, the anti-Ly6G antibody could deplete all cells with the surface marker Ly6G, and its potential

neutralizing effect on neutrophils could not be ruled out. To rule out this possibility, PMN-MDSCs isolated from the spleens of CIA mice (clinical score >6) on day 35 were adoptively transferred to recipient CIA mice on days 21 and 28. The transferred PMN-MDSCs aggravated arthritis severity (Fig. 3A). The swelling of the joints was amplified and the pathology showed increased immune cell infiltration, inflammatory exudation, cartilage damage, and bone erosion (Fig. 3B). The concentration of IgG increased significantly in the plasma after PMN-MDSCs transfer, the TNF-? also increased slightly although had no significant difference (Fig. 3C). In addition, PMN-MDSCs transfer enhanced the frequencies and absolute number of TNF-?$^{+}$B cells and Ki67^{+}B cells in the joints and spleens *in vivo* (Fig 3D-3H). Therefore, the proinflammatory role of PMN-MDSC in the pathogenesis of CIA were validated through selective depletion and adoptive transfer experiments, and B cell responses were modulated by PMN-MDSC.

CIA-derived PMN-MDSCs' effects on TNF-? expression, proliferation and apoptosis of B cells

To verify the regulatory role of PMN-MDSCs on B cells, we cocultured B cells with PMN-MDSCs, finding that TNF-?$^{+}$B cells (Fig. 4A) and Ki67^{+} B cells (Fig. 4B) increased in the presence of PMN-MDSCs. And the enhancing effects increased with increasing ratio of PMN-MDSCs to B cells (Fig. 4E, 4F). The supernatants of the coculture medium had higher IgG (Fig. 4C) and TNF-? (Fig. 4D) concentration compared with B cells alone. In addition, PMN-MDSCs decreased B cells' apoptosis *in vitro* (Fig. 4G, 4H). The coculture assay demonstrated that PMN-MDSCs played supporting role to B cells. We further explored the difference of CIA and DBA/1J mice derived PMN-MDSCs' effects on B cells, finding that PMN-MDSCs derived from DBA/1J mice had weaker or even no modulatory ability compared to the counterparts of CIA mice (Fig. 4I-4K).

PMN-MDSCs modulated B cells via BAFF

We found that PMN-MDSCs from CIA mice had a stronger ability to support B cells than those from DBA/1J mice. To verify the differences in PMN-MDSCs from DBA/1J and CIA mice, we isolated PMN-MDSCs from the spleens of the mice, and PMN-MDSCs were assayed by RNA-Seq. The heatmap of differentially-expressed genes associated with rheumatoid arthritis were shown, revealing that *Tnfsf13b* (*Baff*) was highly expressed in PMN-MDSC from CIA mice (Fig. 5A). BAFF belongs to the TNF family and plays a vital role in the survival of B-lymphocytes (15). We confirmed that PMN-MDSCs from CIA mice expressed higher levels of BAFF than those from DBA/1J mice (Fig. 5B). To confirm that PMN-MDSC-derived BAFF supports B cells, we added an anti-BAFF antibody to block the function of BAFF. After neutralizing the BAFF in the coculture system, the concentration of TNF-? not IgG in the supernatants decreased significantly (Fig. 5C), which indicated that BAFF mainly promoted the secretion of TNF-?, the IgG secretion was probably compensated by other signaling pathway. Furthermore, the PMN-MDSCs mediated increased TNF-? expression and proliferation in B cells weakened (Fig. 5D, 5E), and the decreased apoptosis was also counteracted by anti-BAFF antibody *in vitro* (Fig. 5F, 5G).

BAFF stimulatedTNF-? expression of B cell throughBTK/N F-?B signaling pathway.

Bruton's tyrosine kinase (BTK) belongs to the TEC tyrosine kinase family and plays a major role in B-cell activation, proliferation, maturation, and differentiation(16). NF-?B signaling is known as a downstream target of BTK(17). Activation of canonical NF-?B leads to the expression of proinflammatory cytokines(18). BAFF plays essential role in activation and survival of B cells(19-23), mainly through activating NF-?B pathway(24), however, less is known about its role in the induction of cytokine production of B cells. Therefore, we were here to explore whether BAFF stimulate TNF-? through BTK/NF-?B pathway. We found that TNF-?$^{+}$ B cells increased in the coculture assay after adding BAFF 3 days later (Fig. 6A). And BAFF enhanced the phosphorylation level of BTK, I?B? and p65 after stimulating with BAFF for 5min, 30min and 60min (Fig. 6B). BTK inhibitor Ibrutinib dampened TNF-? increase in a dose dependent manner (Fig. 6C). What's more, the phosphorylation of BTK, I?B? and p65 was also inhibited by Ibrutinib (Fig. 6D). Thus, these results demonstrated that BAFF enhanced TNF-? expression of B cells through BTK/NF-?B signaling pathway.

Discussion

Our study demonstrates that PMN-MDSCs were proinflammatory and pathogenic in CIA and elucidates the supportive role of PMN-MDSCs to B cells through BAFF/ BTK/NF-?B signaling pathway, which suggested a potential pathological mechanism of CIA.

In our study, PMN-MDSCs expanded significantly in the CIA model and correlated positively with the arthritis score, which is consistent with previous studies(9, 10). However, some of our findings are contrary to those of the previous studies. Earlier studies found that PMN-MDSC adoptive transfer reduced the severity of CIA(8, 11), and administration of anti-Gr-1 Abs (MDSC depletion) abrogated the spontaneous improvement of CIA(7), while Guo et al.(9) found that MDSCs depletion could reduce disease severity, and we also found that anti-Ly6G Ab administration (PMN-MDSC depletion) accelerated the recovery of CIA. The different results of these studies could be attributed to the heterogeneity of MDSC.

B-cells are involved in the production of antibodies and cytokines. Andrey et al.(13) found that B-cell-derived TNF mediates the severity of CIA by controlling pathogenic autoantibody production. The success of anti-B cell therapy in RA has confirmed the pathological role of B cells(25). Therefore, it would be interesting to verify whether PMN-MDSCs can modulate B cell activity in autoimmune arthritis. Crook et al.(26) found that M-MDSCs from spleens of CIA mice, but not $Ly6G^+$cells from the BM, inhibited B cell proliferation and antibody production, while Jang et al.(27) found that spleens derived from $CD11b^+Gr\text{-}1^+$ cells directly promoted the survival of plasma cells in a lupus-prone mouse model, and found that $CD11b^+Ly6G^+$ PMN-MDSCs from spleens of CIA could support B cells *in vivo* and *in vitro* . To our knowledge, it is the first demonstration that PMN-MDSCs from CIA mice not only support the B cells' survival, but also promote TNF-? secretion via BAFF, which was probably a potential pathogenesis of RA. What's more, TNF-? was reported to activate BAFF release in RA(28), so the positive feedback between TNF-? and BAFF was probably a significant promotor during the onset and peak of RA.

EAE is a disease model of MS with pathogenesis similar to that of CIA. Benjamin et al.(29) found that PMN-MDSCs from EAE mice during recovery inhibited B-cell proliferation, whereas PMN-MDSCs at disease onset had no such inhibitory effects. In our study, PMN-MDSCs were from CIA mice at peak; therefore, PMN-MDSCs from different stages of disease probably played distinct roles in the pathogenesis. Thus, the evolution of $CD11b^+Ly6G^+$ myeloid cells during disease development is worth exploring. PMN-MDSCs and neutrophils have similar morphological and phenotypic characteristics, but distinct functions. Recent research has reported that mouse PMN-MDSCs express more CD115 and CD244 than neutrophils(30); however, further studies are needed to confirm these markers. MDSC are known to be immunosuppressive; however, accumulating evidence suggests that MDSC are pathogenic in autoimmune diseases and their suppressive function is diminished(9, 31-33), therefore, it is more difficult to distinguish between neutrophils and PMN-MDSCs. Therefore, more studies are needed to identify a set of proper markers to distinguish PMN-MDSCs from neutrophils. In our study, we found a great expansion of PMN-MDSCs, but the potential mechanism is still unknown. PMN-MDSCs were considered to be immunosuppressive, especially in tumors, but we found that PMN-MDSCs could support B cells, suggesting that the microenvironment affects the function of PMN-MDSCs. Therefore, what causes PMN-MDSCs or $CD11b^+Ly6G^+Ly6C^{low}$myeloid cell inflammation in autoimmune diseases and how to reform its suppressive function are also worth further exploration.

The incidence rate of the CIA model is about 90%, the clinical score of arthritic mice usually exceeds 6, and the proinflammatory characteristics of PMN-MDSCs may be covered when the cells are directly transferred to the established CIA mice. We tried to keep the recipient mice in a lower inflammatory state to reveal the inflammatory role of transferred PMN-MDSCs. Therefore, we only administered the recipient mice the first immunization; the PMN-MDSCs from established CIA mice (score > 6) were adoptively transferred to the recipient mice on days 21 and 28, and we found that the onset of the disease was accelerated and the swelling of the joints was also exacerbated. Thus, the inflammatory role of PMN-MDSCs is important in CIA pathogenesis.

This study had some limitations. BAFF is crucial for B cell survival and development(20-23), however, in our study, we only focused on BAFF expression in PMN-MDSCs, while other immune cells or FLS expression

were not detected. Whether PMN-MDSCs were the main source of BAFF in the joints and spleens is not yet known. Apart from TNF-?, B cell produced a variety of cytokines, such as IL-10, IL-6, GM-CSF and so on, which play important role in the pathogenesis of RA(34, 35), and B cell also had subtypes, such as plasma B cell(36), marginal zone B cell(37), germinal center B cell(38) and so on, however, in this work we did not explore the regulation of PMN-MDSC to B cell differentiation and other cytokines production. Further studies were needed to understand how PMN-MDSC regulates B cells.

In our study, we found that PMN-MDSCs increased B cells' TNF-? secretion via BAFF/BTK/NF-?B signaling pathway, which indicated the importance of BAFF and BTK in the pathogenesis of CIA and provided additional evidence for the application anti-BAFF monoclonal antibody and BTK inhibitor in the treatment of RA.

Methods

Mice

Male DBA/1J mice, 8-10 weeks old) were purchased from Changzhou Cavens Laboratory Animal Co., Ltd. Mice were housed in specific pathogen–free conditions under controlled conditions of 12-hour light/12-hour dark and with food and water ad libitum at 25°C. All experimental protocols were approved by the Ethics Committee for Animal Research of the Affiliated Drum Tower Hospital of the Nanjing University Medical School.

CIA induction and evaluations

The mice were injected intradermally with 100 ?g bovine CII (Chondrex) in 0.05 M acetic acid emulsified in complete Freund's adjuvant for the first immunization on day 0. On day 21, the secondary immunization was performed with 100 ?g CII in incomplete Freund's adjuvant. Severity of inflammation was evaluated using a blind method by an independent observer who did not know the grouping of the mice. Arthritis score was evaluated as follows: 0 = normal; 1 = erythema and mild swelling confined to the midfoot; 2 = erythema and mild swelling extending from the ankle to the midfoot; 3 = erythema and mild swelling extending from the ankle to metatarsal joints; 4 = erythema and severe swelling encompassing the ankle, foot, and digits. Scores for four limbs were summed for each mouse.

Preparation of mononuclear cells

The spleens and ankle/wrist joints were dissected from the CIA mice. Spleens were passed through 40 ?m cell strainers, then the erythrocytes were lysed with red blood cell lysis buffer. The skins of joints were peeled off, then the joints were digested in the RPMI 1640 (MULTICELL) containing 1 mg/ml collagenase D (Sigma-Aldrich) and 0.2 mg/ml DNase I (Sigma-Aldrich) at 37 for 45 min. The joints cells were passed through 40 ?m cell strainers, and red blood cell lysis buffer was performed afterwards. The cell suspension was washed and resuspended in the culture medium for further analysis.

Flow cytometric analysis

Single-cell suspensions were prepared from the spleens and joints of mice. After incubation with anti-CD16/CD32 (93, Biolegend) for 20 min at 4, dead cells were excluded with eBioscience^{TM}Fixable Viability Dye eFluor^{TM} 506, and then anti-mouse mAbs against Gr-1(RB6-8C5), Ly6G(1A8), CD11b(M1/70), Ly6C(HK1.4), CD19(6D5) were used to stain specific surface antigens for 20 min at 4. For intracellular cytokine and nuclear factor staining, single cell suspensions were cultured at 37 for 4h in RPMI 1640 media containing 100 ng/ml phorbol-12-myristate-13-acetate (Enzo), 1 ?g/ml ionomycin (Enzo) and 5 ?g/ml brefeldin A (Enzo). Cells were fixed, permeabilized, and stained with TNF-? (MP6-XT22) and Ki67 (16A8) after staining with CD19. Abs were conjugated with APC, FITC, PE, PE-cy7, or PE/Dazzle^{TM} 594 from Biolegend. Data were acquired using BD LSRFortessa (BD Biosciences) and analyzed with FlowJo software (Tree Star).

PMN-MDSCs and B cells isolation and culture assay

Splenocytes from CIA and DBA/1J mice were stained with anti-CD11b, anti-Ly6G, anti-Ly6C, and anti-CD19 antibodies. $CD11b^{+}Ly6G^{+}Ly6C^{low}$PMN-MDSCs and $CD19^{+}$B cells were sorted using FACS Aria II I sorter (BD Biosciences). The purity of the sorted PMN-MDSCs and B cells was >95%.

1x10ˆ5 $CD19^{+}$ B cells per well were plated in 96-well U-bottom plates, stimulated in RPMI 1640 medium containing 10% FBS, 50?g/ml anti-CD40 (FGK4.5, Bio X Cell) and 10ng/ml IL-4 (GenScript) at 37 and 5% CO_2 for 48h (for apoptosis test) or 72h (for TNF-? and Ki67 test), and cocultured with PMN-MDSCs in different ratios or alone. To block function of BAFF, 5?g/ml anti-mouse BAFF (R&D) were added. To test function of BAFF, 100ng/ml mBAFF (R&D) were added in the B cell culture assay.

In vivo **depletion of PMN-MDSCs**

To assess the effect of depleting PMN-MDSCs, mice were administered intraperitoneally with 400?g anti-Ly6G antibodies per mouse each time (1A8, Bio X cell) or rat IgG2a isotype (Bio X cell) control antibodies on day 32, 34, 36, 38, 40 after first immunization. And mice were sacrificed on day 42.

PMN-MDSCs adoptive transfer

For adoptive transfer, $CD11b^{+}Ly6G^{+}Ly6C^{low}$PMN-MDSCs were isolated from the spleens of CIA mice. Then, PMN-MDSCs (3 x 10ˆ6 per mouse) were transferred into mice on days 21 and 28, which were immunized with CII only once on day 0. PBS was used as the control.

RNA Sequencing (RNA-Seq) and Transcriptome Analysis

Total RNA was extracted from $CD11b^{+}Ly6G^{+}Ly6C^{low}$PMN-MDSCs from the spleens of CIA and DBA1/J mice using Trizol Reagent (Vazyme Biotech, China). RNA sequencing was conducted using BGI Genomics (BGI, Shenzhen, China). The sequencing data were filtered with SOAPnuke (v1.5.2), and clean reads were obtained and stored in the FASTQ format. Clean reads were mapped to the reference genome using HISAT2 (v2.0.4). Bowtie2 (v2.2.5) was used to align the clean reads to the reference coding gene set, and the expression level of the gene was calculated using RSEM (v1.2.12). A heatmap was drawn using pheatmap (v1.0.8) according to gene expression in different samples. Differential expression analysis was performed using DESeq (v1.4.5) with a Q-value [?] 0.05. To gain insight into the changes in phenotype, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of annotated differentially expressed genes were performed using Phyper based on the hypergeometric test. The significance levels of terms and pathways were corrected by the Q value with a rigorous threshold (Q value [?]0.05) by Bonferroni correction.

RNA isolation and real-time qPCR

Total RNA was isolated using TRIzol Reagent (Vazyme Biotech, China), based on the chloroform method. cDNA was synthesized according to the manufacturer's instructions using the HiScript III RT SuperMix for qPCR (+gDNA wiper) (Vazyme Biotech, China). Quantitative PCR was performed using the AceQ qPCR SYBR Green Master Mix (High ROX Premixed, Vazyme). The relative expression of each gene was determined and normalized to the expression of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and calculated using the $2^{-\Delta\Delta^{\circ}T}$ method.

Primer sequences for gene expression were as follows:

Tnf-? : Forward primer: CCTGTAGCCCACGTCGTAG; reverse primer: GGGAGTAGACAAGGTACAACCC.

Baff : Forward primer: ACACTGCCCAACAATTCCTG; reverse primer: TCGTCTCCGTTGCGTGAAATC.

Gapdh : Forward primer: AGGTCGGTGTGAACGGATTTG; reverse primer: GGGGTCGTTGATGGCAACA.

Western Blot

Denatured protein samples were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Beyotime). Proteins were then transferred onto 0.2 μm PVDF membranes (Merck KGaA) and blocked with 5% skim milk for at least 1 h at room temperature. The membranes were then incubated overnight at 4°C with primary antibodies. Anti-p-BTK was from Cell Signaling Technology, anti-BTK were from ABclonal, ant-p-I?B?, anti-I?B?, anti-p-p65, anti-p65, anti-GAPDH were from AiFang biological, which were diluted in TBST with 5% BSA at 1:1000. Diluted HRP-conjugated secondary antibodies (anti-rabbit/anti-mouse IgG, 1:10000, Beyotime catalog A0208/catalog A0216) were added to appropriate immunoblots and incubated at room temperature for 1 hour. Finally, immunoblots were visualized by ECL reagents (Beyotime). Immunoblot images were quantified and analyzed using ImageJ (v1.8.0).

Histopathology

CIA mice were sacrificed, and the joints were removed. The specimens were then fixed in 10% phosphate-buffered formalin and decalcified for 30 days in 10% EDTA or 5% formic acid. After decalcification, the joints were embedded in paraffin and histological examination was performed using hematoxylin and eosin (HE) staining (Servicebio, Wuhan, China).

Enzyme-linked immunosorbent assay

Co-culture supernatant and plasma were collected, and IgG and TNF-? level were determined by ELISA using the Mouse IgG or TNF-? ELISA Kit according to the manufacturer's instructions (Multisciences).

Statistical analysis

GraphPad Prism software (v.8.0, San Diego, CA) was used to analyze. Student's t-test and one-way analysis of variance were used for comparisons between two groups or multiple groups, respectively. For clinical assessment, two-way ANOVA was used to determine significant differences in arthritis scores. $P < 0.05$ was considered statistically significant. P -values are depicted as follows: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Data availability statement

Data supporting the results of this study are available from the corresponding authors upon reasonable request. The raw sequencing data supporting this study have been deposited in the 'NCBI Sequence Read Archive' under accession code PRJNA922970.

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Conflict of interest

The authors declare no conflict of interest.

Ethics statement

This animal study was approved by the Ethics Committee of the Affiliated Drum Tower Hospital of Nanjing University Medical School. Animal experiments were carried out under project license (no. 2020AE01061).

Author contributions

ML performed the experiments, analyzed the data, and wrote the manuscript. ZCT, HLW, and YW performed pilot experiments. ZYC, ZXC, XYY, NZ analyzed the data. XJT conceptualized the idea, provided technical support, and revised the manuscript. HYZ conceptualized the idea, revised the manuscript, granted project funding, and supervised the project. LYS provided administrative support. All the authors approved the final manuscript.

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Figure legend

Figure1. PMN-MDSCs and B cells expanded abnormally in CIA models.

Joint and spleen cells were stained for CD11b, Ly6G, and Ly6C 7 weeks after the induction of CIA. Naïve DBA/1J mice not treated with CFA-collagen were used as controls. (**A, B**) Representative contour plots (**A**) and percentages (**B**) of PMN-MDSCs ($CD11b^+$ $Ly6G^+Ly6C^-$) in control (n=4) and CIA (n=4) mice. (**C**) Correlation between the frequency of PMN-MDSCs in the Joints and the clinical score (n=15) of the corresponding joints. (Spearman's r analysis.) (**D**) Comparison of plasma IgG concentrations between control (n=4) and CIA (n=4) groups. (**E, F**) The percentage and counts of TNF-?$^+$ B and $Ki67^+$ B cells in the joints (**E**) and spleens (**F**) of control and CIA group. *, $P < 0.05$; **, $P < 0.01$; ***,$P < 0.001$; ****, $P < 0.0001$; ns, not significant

Figure 2. PMN-MDSCs depletion alleviated the severity of CIA.

(**A, B**)Control and Ly6G Ab group were CIA mice treated with isotype IgG(n=4) or Ly6G antibody (n=3) i.p. every other day (highlighted by arrows). The clinical arthritis score of joints (**A**) and the representative image of joints and HE (**B**) between Control and Ly6G Ab group. Scale bar, 10?m. (Two-way ANOVA with Bonferroni's.) (**C**) The plasma IgG and TNF-? were assayed by ELISA in control (n=3) and Ly6G Ab (n=3) group. (**D-H**) Flow-cytometry analysis of frequency and absolute number of TNF-? and Ki67 in $CD19^+$ B cells from the joints and spleens. *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

Figure 3. PMN-MDSCs administration enhanced disease severity.

Control (n=4) and PMN-MDSC transfer groups (n=4) were treated with PBS and PMN-MDSCs (5*10^6/mouse) respectively (highlighted by arrows). (**A**) The clinical arthritis score of CIA mice increased after adoptive transfer of PMN-MDSCs. (Two-way ANOVA with Bonferroni's correction). (**B**) Representative joints and HE staining of the control and PMN-MDSC transfer groups. Scale bar, 10?m. (**C**) The IgG concentration in the plasma increased after PMN-MDSCs transfer. (**D-H**) The representative flow chart (**D**) depicting the frequency and count of TNF-?$^+$ B cells and $Ki67^+$ B cells from the joints and spleens between control and PMN-MDSCs transfer group. *, $P < 0.05$; **,$P < 0.01$; ***, $P < 0.001$; ****,$P < 0.0001$; ns, not significant.

Figure 4. PMN-MDSCs facilitated TNF-? expression of B cells and proliferation, and hindered apoptosis *in vitro*.

(**A, B**) The expression of TNF-? (**A**) and Ki67(**B**) in $CD19^+$ B cells upon coculture with PMN-MDSCs (1:1) from CIA spleen. (**C, D**) The concentrations of IgG (**C**) and TNF-? (**D**) in the supernatants were assayed by ELISA. (**E, F**) The percentage of TNF-?$^+$(**E**) and $Ki67^+$ (**F**) in B cells when cocultured with PMN-MDSC from the CIA spleen at different ratio. (**G-H**) The apoptosis of $CD19^+$ B cells was assessed

when coculture with PMN-MDSC (1:1) from spleen of CIA mice. (**I-K**) The analysis of different effects on B cells of PMN-MDSCs from CIA and DBA/1J mice spleen. (ANOVA with Tukey's post-hoc.) . **, $P < 0.01$; ***,$P < 0.001$; ****, $P < 0.0001$; ns, not significant.

Figure 5. BAFF derived from PMN-MDSC-CIA mediated the enhancement of B cells' function.

(**A**) PMN-MDSCs isolated from the spleens of control DBA/1J and CIA mice were assayed by RNA-seq. The differentially expressed genes are shown in the heatmap, indicating that *Tnfsf13b* (also known as*Baff*) was up-regulated in the CIA group. (**B**) The mRNA expression of *Baff* increased in PMN-MDSCs from CIA mice (n=7) compared to control DBA/1J mice (n=7). (**C**) anti-BAFF antibody decreased the concentration of TNF-? not IgG in the supernatant. (ANOVA with Tukey's post-hoc.) (**D**) The flow chart depicting TNF-? and Ki67 expression in B cell when coculture with PMN-MDSCs from CIA mice with or without anti-BAFF antibody. (**E**) The PMN-MDSC mediated enhancement of TNF-? and Ki67 in B cells decreased in the presence of anti-BAFF antibody. (ANOVA with Tukey's post-hoc.) (**F-G**) The PMN-MDSCs reduced B cells' apoptosis, and anti-BAFF antibody counteracted PMN-MDSCs' effect on B cells. The representative flow chart and statistical analysis were shown. (ANOVA with Tukey's post-hoc.). *, $P < 0.05$; **,$P < 0.01$; ***, $P < 0.001$; ns, not significant.

Figure 6. BAFF promoted TNF-? secretion of B cell through BTK/NF-?B signaling pathway.

(**A**) BAFF promoted the TNF-? expression in B cells. (**B**) The phosphorylation level of BTK, I?B?, p65 in B cells were shown in 5, 30 and 60 min after stimulation with BAFF. (**C**) Ibrutinib inhibited the effects of BAFF on TNF-?$^+$ B cells in a dose dependent way. (ANOVA with Tukey's post-hoc.) (**D**) The phosphorylation of BTK, I?B?, p65 in B cells were reduced by Ibrutinib. *, $P < 0.05$; **, $P < 0.01$; ***,$P < 0.001$; ns, not significant.

Figure S1 . The frequency of M-MDSC in the joint (**A**) and spleen (**B**) (gated in CD11b$^+$ cells) in control (DBA/1J mice) and CIA group. ns, not significant.

Figure S2 . (**A-B**) The frequency of PMN-MDSC decreased after administrating Ly6G Ab in the joint and spleen (gated in CD11b$^+$ cells). The relative expression of TNF-?in SP (**C**)and joint (**D)** in control and Ly6G Ab groups. *,$P < 0.05$. ***, $P < 0.001$.