LUPUS SCIENCE & MEDICINE

Systemic blockade of proBDNF inhibited the expansion and altered the transcriptomic expression in CD3⁺B220⁺ cells in MRL/lpr lupus mice

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ABSTRACT

Objectives The overexpansion of CD3$^+$B220$^+$ cells is the hallmark and main pathological mechanism of clinical manifestations of spontaneously developed MRL/lpr mice, which are primarily used as a mouse model of SLE. Our recent report demonstrated that blocking brain-derived neurotrophic factor precursor (proBDNF) suppressed the antibody-secreting cell differentiation and proliferation and inhibited the progression of SLE; however, the effect of proBDNF blockade on these CD3$^+$B220$^+$ cells in MRL/lpr mice is unclear.

Methods To explore the effect of proBDNF on CD3$^+$B220$^+$ cells, MRL/lpr mice at 12 weeks old were intraperitoneally injected with monoclonal anti-proBDNF antibody (McAb-proB) or control IgG continuously for 8 weeks. The manifestations in mice were observed, and peripheral blood and splenocytes were collected and analysed via flow cytometry at 20 weeks old. In addition, splenic CD3$^+$B220$^+$ cells were subjected to RNA sequencing (RNA-seq) analysis to identify transcriptomic alterations.

Results CD3$^+$B220$^+$ cells in peripheral blood ($p=0.0101$) and spleen ($p<0.0001$) were expanded in MRL/lpr mice. Meanwhile, inhibition of proBDNF signalling reduced the percentage of CD3$^+$B220$^+$ cells in peripheral blood ($p=0.0036$) and spleen ($p=0.0280$), alleviated lymphadenopathy, reduced urine protein level ($p<0.0001$) and increased the body weight ($p=0.0493$). RNA-seq revealed 501 upregulated and 206 downregulated genes in splenic CD3$^+$B220$^+$ cells in McAb-proB-treated MRL/lpr mice compared with IgG-treated mice. The differentially expressed genes were found to be involved in apoptosis, tumour necrosis factor signalling, and T cell differentiation and proliferation.

Conclusion Systemic blockade of proBDNF inhibited the overexpansion of CD3$^+$B220$^+$ cells and altered their signals related to cell cycle, cell apoptosis and the immune response, which may contribute to the attenuation of disease symptoms in murine lupus.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ CD3$^+$B220$^+$ cells are abnormally expanded and are associated with disease activity in MRL/lpr spontaneous lupus mice.

⇒ Brain-derived neurotrophic factor precursor (proBDNF) has been shown to drive the differentiation and proliferation of antibody-secreting cells and promote disease progression in SLE; however, its role in CD3$^+$B220$^+$ cells in MRL/lpr mice is unknown.

WHAT THIS STUDY ADDS

⇒ Blockade of proBDNF inhibited the abnormal expansion of CD3$^+$B220$^+$ cells, altered the transcriptomic expression of splenic CD3$^+$B220$^+$ cells, and alleviated proteinuria and lymphadenectasis in MRL/lpr mice.

⇒ Neutralising proBDNF might be critical for eliminating pathogenic CD3$^+$B220$^+$ cells and ameliorating disease progression in MRL/lpr lupus mice.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The findings of the present study further provide evidence of the regulatory action of proBDNF on immune responses and its possible role in the treatment of autoimmune diseases, such as SLE.

INTRODUCTION

SLE is a heterogeneous autoimmune disease characterised by the excessive production of autoantibodies that lead to multiple organ dysfunctions, of which nephritis is the most common and lethal manifestation.[1] Plenty of factors, including genetic and infectious exposures, are involved in the pathogenesis of the disease.[2] Despite B cell overactivation and proliferation being considered as the main shared mechanism of SLE,[3] other immune cells, such as monocytes[4] and T cells,[5] have also been demonstrated to play important roles in the progression of SLE. However, the aetiology of SLE is largely not understood yet.

MRL/lpr mice are useful models for studying the pathology and therapeutic effect of drugs in SLE. MRL/lpr mice are deficient in Fas signalling, which failed to eliminate the normal proliferation of CD3$^+$B220$^+$ lymphocytes, and spontaneously develop lymphoproliferation, splenomegaly and many autoimmune manifestations similarly observed

in patients with SLE, including glomerulonephritis, autoimmunity and a very short life span.[6] $CD3^+B220^+$ cells are pathological as inhibition of their differentiation via irradiation significantly attenuates progression of the disease in MRL/lpr mice.[7] In addition, suppressing the functions of these cells alleviates the progression of kidney damage in MRL/lpr mice by reducing the secretion of inflammatory factors, such as interleukin (IL) 17 and interferon gamma (IFN-γ).[8] Therefore, excessive proliferation and differentiation of the $CD3^+B220^+$ cells is closely correlated with disease progression in MRL/lpr mice.

Brain-derived neurotrophic factor precursor (proBDNF) is a precursor of mature BDNF, which is highly expressed and has multiple functions in the central nervous system. ProBDNF is also expressed in immune cells; however, its functions in immune cells remain elusive. Our studies have proven that proBDNF derived from immune cells contributes to the progression of immune-mediated inflammatory diseases.[9–12] For instance, the expression of proBDNF in $CD4^+$ T cells is associated with the proinflammatory environment in the meninges, which promotes the development of cognitive dysfunction in septic mice.[10] We also found that proBDNF is significantly upregulated in lymphocytes and monocytes in the spleen of lipopolysaccharide (LPS)-induced septic mice.[12] Recently, we have reported a pathological role of proBDNF in SLE by promoting the differentiation of antibody-secreting cells (ASCs) and the production of autoantibodies, indicating its potential as a therapeutic candidate for SLE treatment.[13] However, whether proBDNF specifically affects $CD3^+B220^+$ cells in MRL/lpr mice is unknown.

In the present study, we investigated the effect of a monoclonal antibody that neutralises proBDNF function on the expansion and RNA transcriptomic alterations of $CD3^+B220^+$ cells in MRL/lpr mice.

METHODS

Mice

Twenty-two female MRL/lpr mice and six C57BL/6J mice were purchased from Shanghai Slack Laboratory. All mice were maintained in specific-pathogen-free conditions at Central South University Animal Services (Changsha, China).

Monoclonal anti-proBDNF antibody injection

To determine the role of monoclonal anti-proBDNF antibody (McAb-proB) in MRL/lpr mice, 16 female MRL/lpr mice were intraperitoneally administered with McAb-proB (100 µg) or control IgG starting at 12 weeks of age and followed by once a week of 30 µg McAb-proB or control IgG injection, for a total of 2 months. At the age of 20 weeks, urine was collected in the morning, then mice were euthanised. The spleen, peripheral blood and axillary lymph node were harvested for further tests.

Flow cytometry

To observe the changes in the percentage of $CD3^+B220^+$ in MRL/lpr mice, whole blood was collected from the retro-orbital vein of the mice, and splenocytes were separated by gently crushing the spleens from the mice in a 70 µm strainer. Whole-blood cells and splenocytes were then stained with combinations of antibodies, including anti-CD45 (BioLegend, cat 103132), anti-CD3 (BD Biosciences, lot 1228718) and anti-CD45R (BD Biosciences, lot 1319820) for 30 min at 4°C in the dark. Cells were then washed and resuspended in phosphate buffer saline (PBS). Erythrocytes were lysed with lysing buffer (Biosharp, lot BL503B) for 10 min at room temperature in the dark. Cells were then washed and resuspended in PBS again. Stained cells were read on a flow cytometer (Cytek, Fremont, California, USA). Data were analysed using FlowJo V.10.4 software.

$CD3^+B220^+$ cell isolation

To isolate mice splenic $CD3^+B220^+$ cells, the spleen was collected to prepare a single-cell suspension. Erythrocytes were lysed with lysing buffer (Biosharp, lot BL503B) for 10 min at room temperature. Cells were then washed and resuspended in PBS. $CD3^+B220^+$ cells were isolated from splenic lymphocytes using mouse CD45R(B220) microbeads (Miltenyi Biotec, catalogue 130-049-501) and CD3 microbeads (Miltenyi Biotec, catalogue 130-094-973) according to the manufacturer's instructions. The purity of enriched $CD3^+B220^+$ cells was determined by flow cytometry and was higher than 90%.

RNA sequencing analysis

Splenic $CD3^+B220^+$ cells from IgG-treated or McAb-proB-treated MRL/lpr mice at 20 weeks were isolated with microbeads as stated above and subjected to RNA sequencing (RNA-seq) analysis. RNA-seq was performed by Gene Denovo (Guangzhou, China) Biotechnology using the Illumina NovaSeq 6000 platform.

Measurement of mouse urine

Urine samples from individual mice were collected at 20 weeks as described previously, and the concentration of urinary microalbumin was analysed via an automatic chemistry analyser (SYSMEX UC3500).

Statistical analysis

Statistical analysis was performed in GraphPad Prism V.9.0. Data are presented as mean±SD. Statistical differences between the two groups were assessed using a two-tailed Student's t-test. P value <0.05 was considered statistically significant.

RESULTS

Expansion of $CD3^+B220^+$ cells in peripheral blood and spleen in MRL/lpr mice

We first examined the expression of $CD3^+B220^+$ cells in peripheral blood and spleen in MRL/lpr mice at 20 weeks old. As shown in figure 1, $CD3^+B220^+$ cells were significantly expanded in MRL/lpr mice. The percentage of $CD3^+B220^+$ cells in $CD45^+$ lymphocytes in peripheral blood was 39.38%±13.45% in MRL/lpr mice compared

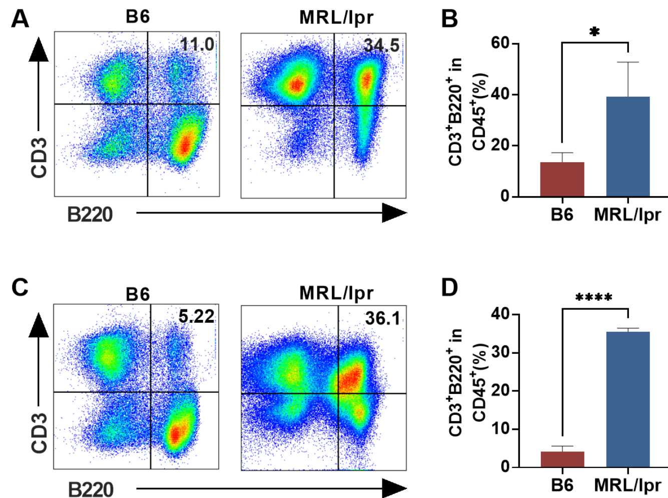

Figure 1 Expansion of $CD3^+B220^+$ cells in $CD45^+$ cells in MRL/lpr mice. The expression of $CD3^+B220^+$ cells in peripheral blood and spleen of MRL/lpr and control C57BL/6J mice at 20 weeks old was tested by flow cytometry. Representative flow cytometry (A) and statistical comparison (B) of $CD3^+B220^+$ cells in peripheral blood between MRL/lpr and C57BL/6J mice at 20 weeks old. Representative flow cytometry (C) and statistical comparison (D) of the percentage of $CD3^+B220^+$ cells in the spleen between MRL/lpr and C57BL/6J mice at 20 weeks old. Data are expressed as mean±SD. Two-tailed Student's t-tests were performed; n=6 in each group.*$P<0.05$ and ****$P<0.0001$.

with 13.63%±3.65% in B6 mice (p=0.0101; figure 1A,B). Meanwhile, the percentage of $CD3^+B220^+$ cells in $CD45^+$ lymphocytes in the spleen was 35.55%±0.926% in MRL/lpr mice relative to 4.188%±1.432% in B6 mice (p<0.0001; figure 1C,D). These results are consistent with previous reports.

Blockade of proBDNF reduced the percentage of $CD3^+B220^+$ cells in MRL/lpr mice

We next explored the effect of proBDNF on the percentage of $CD3^+B220^+$ cells. To this end, MRL/lpr mice aged 12 weeks old were intraperitoneally injected with McAb-proB, which was previously verified,[11] or control IgG continuously for 8 weeks. The percentage of $CD3^+B220^+$ cells in peripheral blood and spleen of MRL/lpr mice treated with McAb-proB or IgG at 20 weeks old was tested by flow cytometry. Compared with that of the IgG-treated MRL/lpr mice, the percentage of $CD3^+B220^+$ cells in peripheral blood and spleen of McAb-proB-treated mice was significantly reduced. The percentage of $CD3^+B220^+$ cells in $CD45^+$ lymphocytes in peripheral blood of IgG-treated mice was 47.82%±4.772%, and this percentage was reduced to 29.87%±11.05% in McAb-proB-treated mice (p=0.0036; figure 2A,B). Similarly, the percentage of $CD3^+B220^+$ cells in $CD45^+$ lymphocytes in the spleen was also reduced to 29.38%±9.261% in McAb-proB-treated MRL/lpr mice (p=0.0280; figure 2C,D).

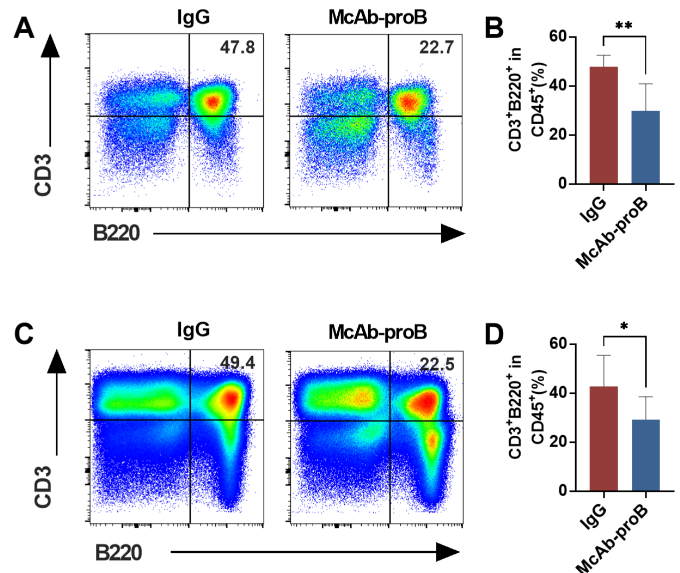

Figure 2 Blockade of proBDNF reduced the percentage of $CD3^+B220^+$ cells in peripheral blood and spleen of MRL/lpr mice. MRL/lpr mice aged 12 weeks were intraperitoneally injected with McAb-proB or IgG continuously for 8 weeks. The expression of $CD3^+B220^+$ cells in peripheral blood and spleen of MRL/lpr mice at 20 weeks old was tested by flow cytometry. Representative flow cytometry images (A) and statistical comparison (B) of the percentage of $CD3^+B220^+$ cells in $CD45^+$ cells in peripheral blood in MRL/lpr mice aged 20 weeks old treated with McAb-proB or IgG. Representative flow cytometry images (C) and statistical comparison (D) of the percentage of $CD3^+B220^+$ cells in $CD45^+$ cells in the spleen in MRL/lpr mice aged 20 weeks treated with McAb-proB or IgG. Data are expressed as mean±SD. Two-tailed Student's t-tests were performed; n=8 in each group. *$P<0.05$ and **$P<0.01$. McAb-proB, monoclonal anti-proBDNF antibody; proBDNF, brain-derived neurotrophic factor precursor.

Blockade of proBDNF alleviated proteinuria and lymphadenopathies in MRL/lpr mice

We next investigated the effect of proBDNF on the disease progression in MRL/lpr mice. McAb-proB treatment did not significantly affect the body weight (p=0.5598; figure 3A), urine microalbumin level (p=0.1740; figure 3B) and urine creatinine level (p=0.1863; figure 3C) in MRL/lpr mice when compared with the IgG treatment at 8 weeks old. However, at 20 weeks old, McAb-proB treatment significantly increased the body weight (p=0.0493; figure 3D) and reduced the level of urinary microalbumin (p<0.0001; figure 3E) in MRL/lpr mice relative to IgG treatment, while there were no significant changes in urinary creatinine level (p=0.2763; figure 3F). In addition, McAb-proB treatment also alleviated lymphadenopathy. The sizes of the axillary lymph nodes of McAb-proB-injected MRL/lpr mice were smaller than those of IgG-injected mice (figure 3G). Taken together, these data suggest that inhibition of proBDNF reduced the percentage of $CD3^+B220^+$ cells, maintained good growth, and alleviated proteinuria and lymphadenopathies in MRL/lpr mice.

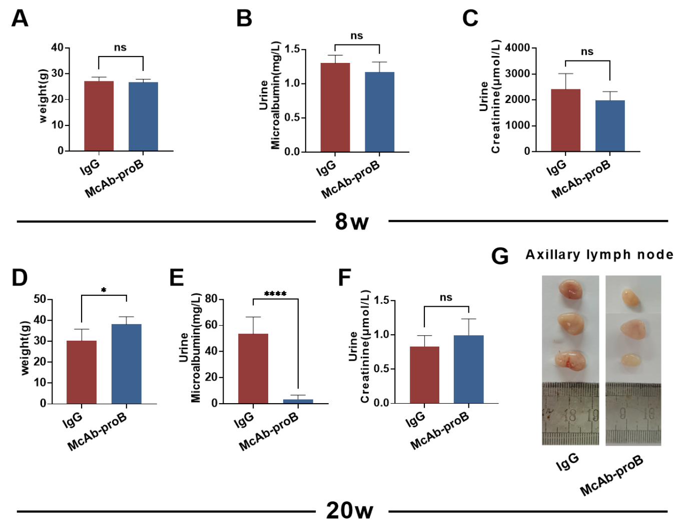

Figure 3 Blockade of proBDNF alleviated proteinuria and lymphadenopathies in MRL/lpr mice. MRL/lpr mice aged 12 weeks were intraperitoneally injected with McAb-proB or IgG continuously for 8 weeks. The weight of the mice was recorded, urinary microalbumin and creatinine levels were tested using a urine automatic chemistry analyser, and the axillary lymph nodes were obtained from the mice at 20 weeks old. No significant differences in body weight (A), urinary microalbumin levels (B) and urinary creatinine levels (C) were observed between IgG-treated and McAb-proB-treated MRL/lpr mice at 8 weeks old. McAb-proB-treated MRL/lpr mice showed increased body weight (D) and reduced levels of urinary microalbumin relative (E) to IgG-treated mice. No significant differences in urinary creatinine levels (F) were observed between MRL/lpr mice treated with IgG or McAb-proB at 20 weeks old. (G) Representative images showing the reduced size of the axillary lymph nodes of McAb-proB-treated MRL/lpr mice at 20 weeks old compared with IgG-treated MRL/lpr mice. Data are expressed as mean±SD. Two-tailed Student's t-tests were performed; n=8 in each group. $^{*}P<0.05$ and $^{****}P<0.0001$. 8w, 8 weeks; 20w, 20 weeks; McAb-proB, monoclonal anti-proBDNF antibody; proBDNF, brain-derived neurotrophic factor precursor.

Blockade of proBDNF altered transcriptomic expression in splenic CD3$^+$B220$^+$ cells in MRL/lpr mice

We further examined the effect of proBDNF on transcriptome expression in CD3$^+$B220$^+$ cells in MRL/lpr mice. RNA-seq analysis of isolated splenic CD3$^+$B220$^+$ cells showed 706 differentially expressed genes (DEGs; false discovery rate <0.05 and fold change >2) between mice treated with McAb-proB and those treated with IgG. Of the 706 DEGs, 205 were downregulated and 501 were upregulated (figure 4A,B). The representative DEGs, including GZMB, Ikbkb, IL-2R, CD52 and STAT4, were involved in apoptosis, tumour necrosis factor (TNF) signalling, and T cell differentiation and proliferation (figure 4C). Further Gene Ontology (GO) classification analysis showed that the DEGs related to biological processes mainly clustered in cell proliferation, immune response, metabolic regulation, biosynthesis and replication, and signal transduction. DEGs related to molecular functions are primarily

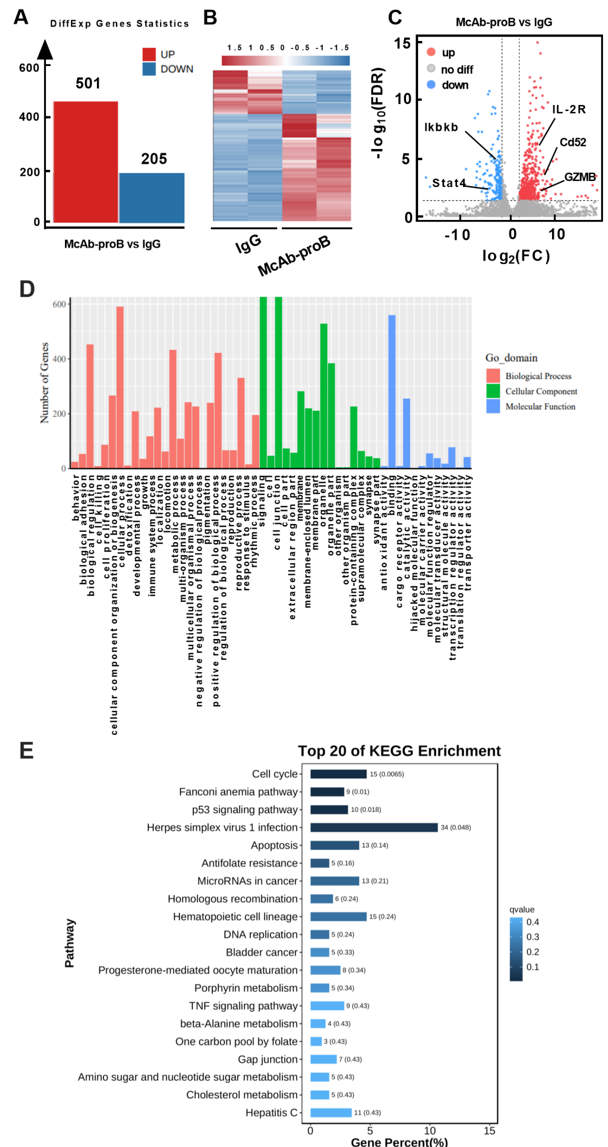

Figure 4 Blockade of proBDNF function altered the transcriptomic expression of splenic CD3$^+$B220$^+$ cells in MRL/lpr mice. MRL/lpr mice aged 12 weeks old were intraperitoneally injected with McAb-proB or IgG continuously for 8 weeks. The CD3$^+$B220$^+$ cells in the spleen were isolated using microbeads and subjected to RNA sequencing analysis at 20 weeks old. The total DEGs (A) and the heat map of DEGs (B) in the splenic CD3$^+$B220$^+$ cells in MRL/lpr mice treated with McAb-proB or IgG. (C) Volcano map of DEGs showing upregulated and downregulated genes in the splenic CD3$^+$B220$^+$ cells in MRL/lpr mice treated with McAb-proB or IgG. Blue represents downregulated genes, red represents upregulated genes, and grey represents genes with potentially 'no difference' (below) or insignificant (in the middle) differences in expression. (D) GO classification analysis of DEGs in splenic CD3$^+$B220$^+$ cells in MRL/lpr mice treated with McAb-proB or IgG. (E) The top 20 KEGG enrichment terms of DEGs in splenic CD3$^+$B220$^+$ cells in MRL/lpr mice treated with McAb-proB or IgG. DEGs, differentially expressed genes; FC, fold change; FDR, false discovery rate; GO, Gene Ontology; IL, interleukin; KEGG, Kyoto Encyclopedia of Genes and Genomes; McAb-proB, monoclonal anti-proBDNF antibody; proBDNF, brain-derived neurotrophic factor precursor; TNF, tumour necrosis factor.

concentrated in antioxidant response, transcription and translation regulation, molecular binding, and catalytic activity. DEGs related to cellular component are mainly clustered in cell components, cell membranes, organelles, nuclear division, nuclear chromosome segregation, nuclear mitosis and cell cycle progression (figure 4D). In addition, Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis showed that the DEGs in the splenic $CD3^+B220^+$ cells of the two groups were mainly enriched in the cell cycle, apoptosis-related signalling pathways, such as p53 signalling, and TNF signalling pathways for immune regulation and inflammatory responses, among others (figure 4E). Therefore, the transcriptomic alterations indicate a role of proBDNF signalling in $CD3^+B220^+$ cells in MRL/lpr mice, and proBDNF may promote $CD3^+B220^+$ cell proliferation and survival and contribute to the development of lupus-like manifestations in MRL/lpr mice.

DISCUSSION

In the present study, we demonstrated that $CD3^+B220^+$ cells were extensively expanded in MRL/lpr mice and that blockade of proBDNF via intraperitoneal injection of McAb-proB reduced the percentages of $CD3^+B220^+$ cells in peripheral blood and spleen, alleviated proteinuria and lymphadenectasis, and altered the transcriptomic expression, which may be involved in $CD3^+B220^+$ cell proliferation and survival in MRL/lpr lupus mice.

SLE is a complex autoimmune disease with multiple pathogenic factors and diverse clinical manifestations. Animal models are useful tools for studying the mechanisms of SLE development. Therefore, identification of animal models for lupus is important. Currently, the animal models for studying SLE mainly fall into two types: drug-induced lupus mice and spontaneously developed lupus mice. For the former, pristane-induced lupus is widely used as it is low cost and non-sensitive to normal environmental breeding.[14] However, it has many issues as an animal model for SLE. It takes up to 5–8 months from modelling to the appearance of typical lupus nephritis. In addition, it cannot fully represent the clinical manifestations of SLE. Many other diseases, such as mouse plasmacytoma, which is the experimental model of human multiple myeloma, are also induced by intraperitoneal injection of pristane.[15] Meanwhile, spontaneously developed MRL/lpr mice are an extensively used lupus mice model for SLE studies and are characterised by an early onset and clinical features that are very similar to clinical SLE manifestations. The average age of onset in female MRL/lpr mice is 15 weeks. In addition, MRL/lpr mice produce an excess of various autoantibodies, prominent by lymphadenopathy and splenomegaly, and display multiple organ dysfunction that is observed in patients with clinical SLE.[16] Thus, MRL/lpr mice are more valuable and suitable for the transformation study of SLE.

$CD3^+CD220^+$ cell expansion is a specific manifestation in MRL/lpr mice. $CD3^+B220^+$ cells are T lymphocytes that are highly expressed by the B cell marker B220 and are also negative for CD4 and CD8 expression. The proliferation of this cell population has been reported to be mainly related to the deletion of the Fas gene in MRL/lpr mice, which can lead to defects in apoptosis of T cells, B cells and monocytes, among others.[17] Indeed, in the present study, we observed a significantly increased percentage of $CD3^+B220^+$ cells in peripheral blood and spleen of MRL/lpr mice at 20 weeks old.

As the precursor of extensively studied mature BDNF, in general, related studies of proBDNF have mainly explored its role in the nervous system. In contrast to the mature products, proBDNF predominantly induces neuronal apoptosis and long-term inhibition.[18] To extend, our teamwork found and proved that proBDNF signalling is expressed in the immune system and has an important immunoregulatory role in many immune cells and is involved in a series of inflammatory diseases.[9–12] Recently, by using the lupus mice model and human blood samples, our latest study further proved and suggested a pathological role of proBDNF in SLE development by inhibiting the proliferation and differentiation of ASCs. We found that proBDNF was preferentially expressed in ASCs in patients with SLE, as well as both the pristane-injected and MRL/lpr lupus mice. Blockade of proBDNF by monoclonal antibody suppressed the activity of ASCs and ameliorated pristane-induced lupus progression.[13] Thus, these data strongly imply an important role of B cell-derived proBDNF in the pathology of SLE disease.

Considering the pathological role of these $CD3^+CD220^+$ cells in the development of lupus, it is interesting to further identify whether and how proBDNF modulates $CD3^+B220^+$ cells in the MRL/lpr mice. Our results showed that McAb-proB treatment significantly inhibited the proportion of $CD3^+B220^+$ cells in both peripheral blood and spleen of MRL/lpr mice. As a result, the level of proteinuria and lymphadenectasis was also significantly alleviated by McAb-proB treatment in MRL/lpr mice. Interestingly, through RNA-seq analysis, we also found that blockade of the proBDNF signal significantly altered the transcriptomic expression profile of the splenic $CD3^+B220^+$ cells. Of the DEGs, STAT4 genes were downregulated, while CD52, GZMB and IL-2R were upregulated in $CD3^+B220^+$ splenic cells in McAb-proB-treated mice. According to a previous study, STAT4 is the transcription factor through which the functional role of IL-12 is carried out, which leads to the production of IFN-γ. Additionally, STAT4 is a susceptible gene that increases the risk of SLE.[19] Meanwhile, T cells with high expression of the lymphocyte surface marker CD52 may suppress other T cells by binding to the inhibitory receptor Siglec-10 and impairing phosphorylation of the T cell receptor.[20] GZMB is a serine protease that is known for its proapoptotic function.[21] Several studies have also demonstrated that the IL-2 signalling via the high-affinity IL-2R is critical for Treg cell homeostasis, survival and immunosuppressive functions of FoxP3 and CD25 expression.[22] In addition, GO and KEGG enrichment

analyses of the DEGs between McAb-proB-treated and IgG-treated MRL/lpr mice revealed significant alterations in apoptosis, cell cycle, survival, immune regulation and inflammatory responses. Therefore, our results suggest a possible role of proBDNF signalling in cell proliferation and the apoptosis balance of the $CD3^{+}B220^{+}$ cells in MRL/lpr mice. However, further research is required to identify how proBDNF regulates the proliferation and function of the $CD3^{+}B220^{+}$ cell in MRL/lpr mice, as well as its significance in SLE disease.

Taken together, our data showed that systemic blockade of proBDNF inhibited the overexpansion of $CD3^{+}B220^{+}$ cells and altered their signal related to cell cycle, cell apoptosis and immune response, which contributed to the attenuation of disease symptoms in murine lupus. The present study further provides evidence of the regulatory action of proBDNF on immune responses and its possible role in the treatment of autoimmune diseases, such as SLE.

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Contributors A-HZ: experimentation, data curation, writing—original draft. CL: data curation, formal analysis, writing—review and editing. WY-S: methodology, supervision, formal analysis. R-PD: supervision, formal analysis, review and editing. DF: conceptualisation, investigation, visualisation, writing—review and editing, and DF is responsible for the overall content as guarantor.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval Animal procedures were approved by the Animal Research Ethics Committee of Xiangya Hospital, Central South University (no: 2020147, Changsha, China) and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

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