Inappropriate expansion of antibody-secreting cells (ASCs) is typical of systemic lupus erythematosus (SLE), but the regulatory signaling of pathogenic ASCs is unclear. The present study shows that brain-derived neurotrophic factor precursor (proBDNF) and its high-affinity pan-75 neurotrophin receptor (p75NTR) are highly expressed in CD19⁺CD27hiCD38hi ASCs in patients with SLE and in CD19⁺CD44hiCD138⁺ ASCs in lupus-like mice. The increased proBDNF⁺ ASCs were positively correlated with clinical symptoms and higher titers of autoantibodies in SLE. Administration of monoclonal antibodies against proBDNF or specific knockout of p75NTR signaling plays a critical pathogenic role in SLE through promoting ASC dysfunction.

Brain-derived neurotrophic factor precursor (proBDNF), the intermediate during the synthesis of mature BDNF, binds to its high-affinity receptor, pan-p75 neurotrophin receptor (p75NTR), and exerts various biological functions (20, 21). Although highly expressed in the nervous system, proBDNF signaling is also expressed in immune cells and plays an important role in immune-mediated inflammatory diseases. Our previous studies have shown that proBDNF derived from monocytes/macrophages is involved in the pathogenesis of spinal cord injury (22), inflammatory pain (23), and aortic dissection disease (24). Up-regulation of proBDNF in CD4⁺ T cells regulates sepsis-associated encephalopathy (25). Recently, we reported that proBDNF-p75NTR signaling is up-regulated in the lymphocytes of both patients with multiple sclerosis (MS) and in experimental autoimmune encephalomyelitis (EAE) mouse models (26); a monoclonal antibody against proBDNF (McAb-proB) was found to attenuate clinical scores, limit demyelination, and inhibit pro-inflammatory cytokines in EAE mice (26). These results suggest that proBDNF signaling released from B cells may implicate into the pathogenesis of autoimmune diseases.

In the present study, we characterized the expression of proBDNF signaling in the specific cluster of ASCs and investigated its pathogenic roles in SLE. The results show that proBDNF/p75NTR signaling is up-regulated in the CD19⁺CD27hiCD38hi ASCs and correlates with the disease activity in patients with SLE. Blocking the up-regulated proBDNF by monoclonal antibody or genetic deletion of p75NTR in B cells inhibits ASC differentiation and antibody production and attenuates disease activity in lupus-like mice. Thus, proBDNF⁺ ASCs might represent a potential therapeutic target for SLE.

## RESULTS

**Prominent up-regulation of proBDNF in ASCs in patients with SLE**

We explored proBDNF expression in B cells in peripheral blood mononuclear cells (PBMCs) from 52 healthy donors (HDs) and 67 patients with SLE (table S1). Similar to previous reports, the
percentages of CD19⁺ B cells (P = 0.0032; Fig. 1A) and CD27⁺CD38⁺ ASCs (P = 0.0048; Fig. 1B) were increased in patients with SLE relative to HDs. We then screened proBDNF expression in ASCs (CD19⁺CD27⁺CD38⁺), as well as in memory (CD19⁺CD27⁺CD38⁻) and naïve B cells (CD19⁺CD27⁻) (fig. S1). The percentages of proBDNF⁺ cells were 15.0 ± 12.26% and 27.7 ± 21.1% in circulating ASCs in HDs and patients with SLE, respectively (P = 0.0003; Fig. 1C). Similarly, proBDNF mean fluorescence intensity (MFI) of circulating ASCs in patients with SLE was approximately twofold higher than that in HDs (P < 0.0001; Fig. 1, D and F) but was not significantly different compared with that in other B cell subsets (Fig. 1F). Notably, in patients with SLE, circulating ASCs displayed the highest average proBDNF level relative to other subsets (P < 0.0001; Fig. 1, E and F). We then conducted unbiased data analysis of flow cytometry by applying the dimensionality reduction algorithm, t-distributed stochastic neighbor embedding (tSNE), and the clustering algorithm, PhenoGraph. As shown, the tSNE plot visualizing proBDNF⁺ cells (Fig. 1G, Left) and cell-subset distributions (Fig. 1G, right) demonstrates that proBDNF⁺ cells were highly coincident with ASCs in patients with SLE (Fig. 1G).

In HDs, p75NTR was predominantly expressed in ASCs (Fig. 1, H and J) and was further increased in patients with SLE (P = 0.0072; Fig. 1, I and J). In patients with SLE, p75NTR expression in ASCs was higher than that in other B cell subsets (P < 0.001; Fig. 1, I and J).

Correlation of proBDNF levels in ASCs with disease activity and prognosis in patients with SLE

We next investigated the correlations of proBDNF MFI in ASCs with clinical manifestations in patients with SLE. Remarkably, higher proBDNF expression in ASCs was correlated with apparent symptoms, including joint symptoms (P = 0.0001; Fig. 2A), hematological symptoms (P < 0.0001; Fig. 2B), and leukopenia (P < 0.0001; Fig. 2C) in patients with SLE. Patients with SLE with positive symptoms showed higher proBDNF levels in ASCs than in patients with SLE with nonapparent symptoms (P = 0.0135, Fig. 2A; P = 0.0216, Fig. 2B; P = 0.0364, Fig. 2C). In addition, elevation of proBDNF MFI in ASCs was observed in patients with anti–double-stranded DNA (dsDNA; P = 0.0361; Fig. 2D), anti–ribonucleoprotein (RNP) (P = 0.0009; Fig. 2E), or autoantibodies (P = 0.0003; Fig. 2F).

The proBDNF expression in ASCs was positively correlated with erythrocyte sedimentation rate (ESR) (P = 0.003; Fig. 2G) but negatively correlated with serum complement (C) 3 levels (P = 0.005; Fig. 2H) in patients with SLE. The proBDNF level in ASCs was also positively correlated with SLE Disease Activity Index 2000 (SLEDAI) scores (P = 0.0001; Fig. 2I and fig. S2A). After in-hospital treatment (table S2), the proportion of ASCs was reduced from 13.3 ± 7.9% to 5.01 ± 4.8% (P = 0.0037; Fig. 2J). In addition, the percentage of proBDNF⁺ cells in ASCs decreased from 28.5 ± 13.7% to 8.9% (P = 0.0263; Fig. 2K), and proBDNF MFI in ASCs decreased by approximately 33% (P = 0.0217; fig. S2B) in treated patients with SLE compared to these parameters at pretreatment. In parallel, p75NTR levels in ASCs were also down-regulated in newly diagnosed patients with SLE who were treated (P = 0.0371; Fig. 2L).

Last, we compared the association of the entire ASCs and proBDNF⁺ ASCs with SLE disease activity. The percentage of ASCs in CD19⁺ B cells was associated with lupus activity (R = 0.440, P = 0.001; Table 1 and Fig. 3A) and ESR (R = 0.391, P = 0.005; Table 1 and Fig. 3B). However, the percentages of proBDNF⁺ ASCs in B cells displayed a closer correlation with lupus activity (R = 0.493, P < 0.001; Table 1 and Fig. 3C) and ESR (R = 0.461, P = 0.001; Table 1 and Fig. 3D) than the whole ASCs. In contrast, proBDNF ASCs were not correlated with disease activity (R = 0.232, P = 0.089; Table 1 and Fig. 3E) or ESR (R = 0.094, P = 0.512; Table 1 and Fig. 3F). These data indicate that the proBDNF⁺ ASCs subset was a specific cluster of ASCs more closely associated with SLE disease activity than the entire ASCs.

Elevated proBDNF levels in ASCs in two mouse models of lupus

We next measured proBDNF levels in lupus-like mice. As reported previously (27), 8-week-old MRL/MpJ-Fas-/-/J group (MRL/lpr) mice showed apparent expansion of ASCs (CD19⁺CD27⁺CD38⁺) in the spleen when compared to the control (fig. S3, A and B). The percentage of proBDNF⁺ cells and proBDNF MFI levels in splenic total B cells (P = 0.0002; Fig. 4, A and B, left; P < 0.0001; Fig. 4, C and D, left) and in ASCs (P = 0.0016; Fig. 4, A and B, right; P = 0.0003; Fig. 4, C and D, right) in MRL/lpr mice were greatly elevated.

In pristane-injected mice, splenomegaly was developed at 8 weeks postinjection (fig. S3C). The percentages of proBDNF⁺ cells in total splenic B cells and ASCs were increased by approximately 45.8% (P = 0.0173; Fig. 4, E and F, left) and 37.8% (P = 0.0362; Fig. 4, E and F, right) in pristane-injected mice, respectively. ProBDNF MFI levels were higher in total splenic B cells (P = 0.0005; Fig. 4, G and H, left) and ASCs (P = 0.0055; Fig. 4, G and H, right) in pristane-injected mice. Furthermore, the increased proBDNF expression was remarkably localized in splenic B220⁺ cells in MRL/lpr mice (Fig. 4I) and in pristane-injected lupus mice (Fig. 4J). Last, we also confirmed the elevation of proBDNF protein levels in the spleens of two types of lupus-like mice by Western blot (fig. S4).

Correspondingly, p75NTR levels were up-regulated in splenic B cells (P = 0.0031; Fig. 4, K and L, left) and in ASCs (P = 0.0105; Fig. 4, K and L, right) in 8-week-old MRL/lpr mice. The p75NTR levels in splenic ASCs were also elevated in pristane-injected mice (P = 0.0297; Fig. 4, M and N). Furthermore, p75NTR was broadly colocalized with B220 in the spleen in lupus-like mice (fig. S5). In addition, proBDNF and p75NTR were up-regulated and expressed in B220⁺ cells in lymph node in the two types of lupus-like mice (fig. S6).

McAb-proB treatment attenuates disease progression and inhibits ASC populations in lupus-like mice

We next evaluated the effect of McAb-proB on disease progression in lupus-like mice. At 8 weeks after pristane injections, systemic injection of McAb-proB (100 μg) was performed once, followed by a weekly dose of 30 μg for 3 months. These treatments reduced the spleen weight in pristane-injected mice by approximately 25% as compared with immunoglobulin G (IgG) treatment (P = 0.0446; Fig. 5A). Furthermore, McAb-proB treatment inhibited the pristane injection–induced increased numbers of leukocytes (P = 0.0026; Fig. 5B, left), total B cells (P = 0.0035; Fig. 5B, right), and ASCs (P = 0.0034; Fig. 5C) in the spleens. McAb-proB treatment also inhibited pristane-induced increased serum levels of anti–ANCA (P = 0.0021; Fig. 5D) and anti–dsDNA (P = 0.0267; Fig. 5E) as compared with those following IgG treatments. After pristane injection, nephritis and IgG deposition in the kidney were obvious and were subsequently ameliorated by McAb-proB treatment (Fig. 5F). Similarly, McAb-proB treatment reduced the proteinuria level to 54.67 ± 23.6 mg/dl, as compared to 96.92 ± 47.2 mg/dl in the IgG
Fig. 1. Up-regulation of proBDNF in ASCs in patients with SLE. PBMCs were isolated from patients with SLE and HDs and were analyzed by flow cytometry. (A) Percentages of CD19+ cells in HDs and patients with SLE. (B and C) Flow cytometric analysis showing frequencies of CD27hiCD38hi ASCs in CD19+ B cells (B), as well as proBDNF+ cells in ASCs (C), in HDs and patients with SLE. Data are presented as a representative flow plot (upper panel) and summary graph (lower panel). (D and F) The expression levels of proBDNF MFI in ASCs in HDs and patients with SLE were analyzed by flow cytometry. (E and F) The expression of proBDNF in each subpopulation of B cells in patients with SLE was calculated by flow cytometry. (G) t-Distributed stochastic neighbor embedding (tSNE) plot of flow cytometry data showing proBDNF+ cells (left) and cell-subset distributions (right) in patients with SLE. (H and J) Analysis of p75NTR expression in ASCs in HDs and patients with SLE. Data are shown as the means ± SD. Two-tailed Student’s t tests (A to C) and two-way ANOVA followed by Tukey’s post hoc tests (F and J) were performed. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. MFI, mean fluorescent intensity.
Fig. 2. ProBDNF levels in ASCs are positively correlated with disease activity of patients with SLE. PBMCs isolated from patients with SLE or HDs were analyzed by flow cytometry. (A to C) Association of proBDNF MFI in ASCs with apparent and nonapparent clinical manifestations, including joint symptoms (A), hemopoietic symptoms (B), and leukopenia (C) in patients with SLE. ns, not significant. (D to F) Association of proBDNF MFI in ASCs with negative and positive autoantibody levels, including anti-dsDNA (D), anti–U1-nRNP (U1-nuclear ribonucleoprotein) (E), and anti-Sm antibody (F) in patients with SLE. (G to I) Correlation analysis of proBDNF MFI in ASCs with ESR (G), C3 (H), and SLEDAI (I) in patients with SLE. DAPI, 4′,6-diamidino-2-phenylindole. (J to L) Representative flow cytometry images and statistical analysis showing changes in the percentages of ASCs in CD19+ B cells (J), proBDNF+ cells in ASCs (K), and p75NTR MFI in ASCs (L) before and after conventional clinical treatment in patients with SLE. Data are shown as the means ± SD. One-way ANOVA (A to F) and Spearman’s rank correlation analysis (G to I) and two-tailed Student’s t tests (J to L) were performed. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. Anti-Sm, anti-Smith; C3, complement component 3.
group ($P = 0.0174$; Fig. 5G). McAb-proB injection also greatly suppressed serum cytokine levels including interleukin-6 (IL-6; $P = 0.0407$; Fig. 5H) and tumor necrosis factor–α ($P = 0.0348$; Fig. 5I) in lupus-like mice.

**B cell depletion of p75$^\text{NTR}$ attenuates disease progression in lupus-like mice**

To better understand the role of p75$^\text{NTR}$ in B cells in the pathogenesis of SLE. We crossed floxp-flanked p75$^\text{NTR}$ mice with gene-targeted animals expressing Cre under the CD19 promoter (CD19$^{\text{cre}}$-p75$^{\text{fl/fl}}$) (fig. S7, A to C). CD19$^{\text{cre}}$-p75$^{\text{fl/fl}}$ mice did not express p75$^\text{NTR}$ in CD19$^+$ B cells (Fig. 6A) and did not show the altered number of CD45$^+$ and CD19$^+$ splenocytes or percentage of CD19$^+$ cells in CD45$^+$ splenocytes at adulthood (fig. S7, D to E). However, as compared with CD19-p75$^{\text{fl/fl}}$ mice, CD19$^{\text{cre}}$-p75$^{\text{fl/fl}}$ mice exhibited significantly fewer leukocytes ($P = 0.0053$; Fig. 6B), CD19$^+$ B cells ($P = 0.0122$; Fig. 6B), and ASCs ($P = 0.0340$; Fig. 6B) in the spleen.

### Table 1. Statistical relation between the frequency of B cell subsets with SLEDAI and ESR levels.

| Correlation | ASCs in B cells (%) | ProBDNF$^+$ASCs in B cells (%) | ProBDNF$^-$/ASCs in B cells (%) |
|-------------|---------------------|-------------------------------|-------------------------------|
|             | $r$ | $P$  | $n$  | $r$ | $P$  | $n$  | $r$ | $P$  | $n$  |
| SLEDAI      | 0.440 | 0.001 | 55   | 0.493 | <0.001 | 55   | 0.232 | 0.089 | 55   |
| ESR         | 0.391 | 0.005 | 51   | 0.461 | 0.001  | 51   | 0.094 | 0.512 | 51   |

**Fig. 3. ProBDNF$^+$ cells in ASCs are positively correlated with disease activity in patients with SLE.** PBMCs isolated from patients with SLE or HDs were analyzed by flow cytometry. Correlation analysis of ([A and B] ASCs$^+$%), ([C and D]) proBDNF$^+$ASCs$^+$%, and ([E and F]) proBDNF$^-$ASCs$^+$% with SLEDAI and ESR. Spearman’s correlation analysis was performed.
Fig. 4. ProBDNF expression is increased in ASCs in different mouse models of lupus. Spleens were collected from 8-week-old MRL/lpr mice, 16-week-old pristane-induced mice (8 weeks after pristane injections), and control mice. (A and B) Representative flow cytometric images (left) and statistical analysis (right) indicate the percentage of proBDNF+ cells in B cells and ASCs in 8-week-old MRL/lpr mice. (C and D) Representative flow cytometry images (left) and statistical analysis (right) showing proBDNF MFI in CD19+B cells and CD44hiCD138+ASCs in MRL/lpr mice and their corresponding controls. (E and F) Representative flow cytometric images (left) and statistical analysis (right) indicated the percentage of proBDNF+ cells in B cells and ASCs in 16-week-old pristane-injected mice. (G and H) Representative flow cytometry images (left) and statistical analysis (right) showing proBDNF MFI in CD19+B cells and CD44hiCD138+ASCs in pristane-injected mice and the controls. (I and J) Immunofluorescence staining was performed in splenic sections from MRL/lpr mice (I) and pristane-injected mice (J) and their controls. Scale bar, 20 μM. (K to N) Representative flow cytometry images (left) and statistical data (right) showing p75NTR MFI in B cells and ASCs in MRL/lpr mice (K to L), as well as in pristane-induced lupus-like mice (M and N) and their controls. Data are shown as the means ± SD. Each experiment was performed at least three times. Two-tailed Student’s t tests were performed. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. Con, control group; proB, brain-derived neurotrophic factor precursor.
Fig. 5. Systemic blockade of proBDNF ameliorates disease severity in pristane-injected lupus-like mice. C57BL/6 mice were injected with pristane at 8 weeks old and were treated intraperitoneally with either McAb-proBDNF or isotype IgG at a loading dose of 100 μg at 16 weeks old. Then, a weekly maintenance dose of 30 μg was followed, and mice were euthanized at 28 weeks old. (A) Representative images of the size of the spleen (left) and statistical data (right) showing the weights of the spleens in mice. (B and C) The absolute counts of CD45+ and B220+ cells (B) and ASCs (C) in each group were assessed by flow cytometry. (D and E) Enzyme-linked immunosorbent assays (ELISAs) of ANA (D) and dsDNA IgG (E) in the sera from each group of mice. (F) Paraffin-embedded kidney sections from each group of mice were stained for hematoxylin and eosin (H&E) and IgG deposits. Scale bar, 20 μM. (G) Statistical data showing changes of proteinuria in each group of mice. (H and I) LEGENDplex multianalyte flow assays showing IL-6 (H) and TNF-α (I) expression in the sera from each group for mice. Data are shown as the means ± SD. Each experiment was repeated at least three times. One-way ANOVA (A to I) was performed. *P < 0.05, **P < 0.01, and ***P < 0.001. ProBDNF/proB, brain-derived neurotrophic factor precursor; Con, naïve control; NS, normal saline; IgG, control IgG; α-proB, anti-proBDNF antibody; OD, optical density; TNF-α, tumor necrosis factor–α.
Fig. 6. CD19+ B cell–specific p75NTR depletion ameliorates pristane-induced pathologies in lupus-like mice. (A) Expression profile of splenic p75NTR tested by flow cytometry in CD19cre-p75fl/fl mice. (B) Flow cytometric analysis indicating the absolute counts of splenic leukocytes and the subpopulation in pristane-immunized CD19cre-p75fl/fl mice and their controls at 2 months after pristane injection. (C to G) Serum and kidneys were obtained at 5 months after pristane immunization in CD19cre-p75fl/fl and control mice. (C and D) ELISAs of ANA (C) and autoantibodies against dsDNA (D) in the serum. (E) Proteinuria was assessed by Coomassie brilliant blue. (F and G) Paraffin-embedded kidney sections were stained with hematoxylin and eosin (H&E) (F) and indirect immunofluorescence of anti-IgG (G). Scale bar, 20 μM. (H) Heatmap of RNA-seq indicating differentially expressed genes in the mesenteric lymph nodes of the two groups of mice at 16 weeks old. (I to N) Real-time quantitative PCR confirming differentially expressed genes indicated by RNA-seq. Data are shown as the means ± SD. Each experiment was repeated at least three times except RNA-seq. Two-tailed Student’s t tests (A, B, and D to G) were performed. *P < 0.05, **P < 0.01. CD19-p75fl/fl, control littermates; CD19cre-p75fl/fl, CD19 conditioning knockout of p75NTR mice.
after pristane injection. Consistently, serum levels of anti-ANA (P = 0.0253; Fig. 6C) and anti-dsDNA (P = 0.0132; Fig. 6D) were reduced by approximately twofold in CD19cre-p75fl/fl mice. Immunized CD19cre-p75fl/fl mice displayed less-severe proteinuria (P = 0.0169; Fig. 6E), ameliorated glomerulus pathologies (Fig. 6F), and IgG deposition compared to those in p75fl/fl mice (Fig. 6G).

We then conducted RNA sequencing (RNA-seq) in mesenteric lymph nodes after pristane injections in CD19cre-p75fl/fl mice and in littermate controls. There were 24 differentially expressed genes, among which 17 were down-regulated and 7 were up-regulated, that were associated with ASC functions in CD19cre-p75fl/fl mice relative to those in control mice (Fig. 6H and table S3). The down-regulated genes (as confirmed by polymerase chain reaction (PCR))—including Igkv1-22, Igv8-12, Igkv1-110, Igkv8-24, Igkv10-96, and Igkv14-162—were associated with antigen binding, immune responses, and immunoglobulin production (Fig. 6, I to N). Gene ontology (GO) enrichment annotations were further analyzed, and we obtained 24 highly enriched GO terms (padj < 0.05), including immune system responses, positive regulation of biological processes, and responses to stimuli and binding (fig. S8).

**Blocking proBDNF inhibits ASC proliferation and function in vitro**

The above results suggested an important role of proBDNF/p75NTR signaling in ASC activity. To test this hypothesis, we cultured splenic B cells from wild-type mice and treated them with the Toll-like receptor 7 (TLR7) agonist, R848. R848 treatment dose-dependently increased the percentage of CD44hiCD138−ASCs in B220+B cells, suggesting the induction of ASCs (Fig. 7A and fig. S9, A and B). Concomitantly, proBDNF and p75NTR expression levels were significantly up-regulated in the induced ASCs (fig. S9, C to F). Exogenous proBDNF protein further induced ASC proliferation (P = 0.0112; Fig. 7A), while McAb-proB reduced the percentage of ASCs (P < 0.001; Fig. 7A).

Upon R848 stimulation, IgG1, IgG2a, and IgM were elevated (Fig. 7, B to D). Although exogenous proBDNF promoted release of IgG1 (P = 0.0006; Fig. 7B), it had no effect on IgM levels. By contrast, McAb-proB reduced the supernatant levels of IgG1 (P = 0.0002; Fig. 7B), IgG2a (P < 0.001; Fig. 7C), and IgM (P = 0.0006; Fig. 7D). Similarly, in cultured CD19+B cells from CD19cre-p75fl/fl mice, R848 stimulation induced less ASC differentiation (P = 0.0022; Fig. 7E), as well as less IgG1 (P = 0.0228; Fig. 7, F and G) and IgM (P = 0.0198; Fig. 7, F and H) secretion, when compared to those in CD19-p75fl/fl control mice.

Next, we verified the role of proBDNF signaling on ASC activity in human PBMCs. In PBMCs from HDs, the TLR9 agonist, class B CpG oligonucleotide (CpG-B), significantly induced CD19+B cell proliferation, and this proliferation was inhibited by McAb-proB, as compared with that in the IgG group (P = 0.0324; fig. S10A). Concomitantly, the percentage of ASCs in PBMCs, as presented by CD27+CD38hi cells, increased to 23.9 ± 3.2% after CpG-B stimulation, while McAb-proB reduced the percentage of ASCs to 12.1 ± 2.4% (P = 0.0011; fig. S10B). Furthermore, blockade of proBDNF inhibited the production of IgA (P = 0.0345; fig. S10C), IgG (P = 0.0222; fig. S10D), and IgM (P = 0.0006; fig. S10E) in B cells relative to that from IgG treatment.

We then used the p75NTR extracellular domain (p75ECD-Fc) to block the functions of p75NTR. The results showed that p75ECD-Fc suppressed CD40 (P = 0.0035; fig. S10F) and CD86 (P = 0.0257; fig. S10G) expression in B cells and reduced B cell proliferation upon CpG-B treatment (P = 0.0123; fig. S10, H and I). In PBMCs from patients with SLE, proBDNF and p75NTR were elevated in CD19+B cells after CpG-B treatment (Fig. 7, I and J). The percentage of ASCs in McAb-proB treatment group was approximately half of that in the IgG group after CpG-B treatment (P = 0.0137; Fig. 7K). These findings suggest that proBDNF/p75NTR signaling in B cells is a key regulatory signaling pathway involved in ASC proliferation and differentiation in patients with SLE.

**DISCUSSION**

In the present study, we found that proBDNF signaling was markedly increased in the ASC subgroup in patients with SLE and lupus-like mice. The increased level of proBDNF in ASCs was correlated with SLE disease activity and severity, and it was lower after effective treatment. Blocking the increased proBDNF or deletion of p75NTR in CD19+B cells limited ASC proliferation, reducing autoantibody production and alleviating lupus nephritis. Last, blocking the increased proBDNF inhibited the proliferation and antibody production in B cells in vitro both in rodents and humans.

The role of biological targets in autoimmune disease has drawn great interest over the past several decades. Exploring controllable molecules in ASCs may meet the unmet need for clinical SLE therapy (8–11). However, it is always difficult to discern SLE activity before autoantibody expansion and activity of nephritis. Studies have attempted to find diagnostic markers for better recognition of SLE flares. For example, high-serum interferon-α (IFN-α) levels are associated with higher risk of relapse in patients with SLE in remission (28). HLA-DR11CD27+CD20+CD19dim plasmablasts were proposed as a means of more precisely representing lupus activity (18). In the present study, we defined high expression of proBDNF in ASCs in patients with SLE with the following characteristics. First, proBDNF levels in ASCs in recrudescent patients with SLE were higher compared to those in HDs or other subsets in B cells in patients with SLE. Second, proBDNF levels in ASCs correlated with clinical symptoms and experimental indicators. Third, proBDNF+ASCs showed a closer correlation with SLEDAI and ESR in patients with SLE than whole ASCs did. Last, clinical conventional therapies including corticosteroids and immunosuppressors normalized the increased proBDNF expression in ASCs in the newly diagnosed patients with SLE. These findings indicate that proBDNF+ASCs are likely a subset of highly pathogenic B cells, and they likely promote SLE development and may be usable to predict disease activity in patients with SLE.

Our recent study has shown up-regulation of proBDNF signaling in the lymphocytes in another autoimmune disease, patients with MS, and EAE mice model (26). In addition, McAb-proB treatment ameliorated the clinical score and limited spinal cord injury and production of proinflammatory cytokines of EAE mice (26). However, it was not shown which cells are the main source and targets of proBDNF in the patients with MS. In the present study, we showed that ASCs are the main source and target of proBDNF in the pathogenesis of SLE. We observed that systemic administration of McAb-proB lowered spleen weight, inhibited ASC proliferation, reduced the level of autoantibodies and proinflammatory cytokines, and alleviated nephritis in lupus-like mice. In this way, McAb-proB may be a suitable intervention for treatment of SLE by inhibiting the dysfunctions of ASCs.

ProBDNF can bind to several receptors in the central nervous system, although it is still in investigation; previous studies have...
proved its high affinity for receptor p75NTR, especially in the peripheral system (20, 21). Although little study from other researchers has addressed proBDNF’s regulatory role on B cells, the importance of p75NTR in B cells function has drawn attention in recent years. In B cell malignancies, p75NTR has been broadly detected in both resting, active, and memory B cells, and it participates in long-lasting survival of B cells after nerve growth factor stimulation (29, 30). One study mapped the expression profile of p75NTR in EAE mice and indicated elevated p75NTR in B lymphocytes in the spinal cord (26). TLR4-activated plasmacytoid dendritic cells showed inducible p75NTR expression and facilitated the connection between neurotrophin signaling and regulation of immune response in asthma.

Fig. 7. Blocking proBDNF-p75NTR signaling inhibits TLR7-mediated B cell differentiation and autoantibody production. Normal murine splenic B cells were cultured for 72 hours with IL-4, followed by stimulation of R848 and inhibition of proBDNF signaling. (A) Flow cytometric analysis showing changes in percentages of CD44hiCD138+ cells (ASCs) in splenic B cells. (B to D) Flow cytometric bead-based immunoassays detecting levels of IgG1, IgG2a, and IgM in the supernatants of splenic B cells in each group. (E) Flow cytometric analysis showing the percentages of CD44hiCD138+ cells (ASCs) in splenic B cells from CD19cre-p75fl/fl and control mice in the presence of IL-4 and R848 stimulation. (F to H) Flow cytometric bead-based immunoassays showing representative images (F) and statistical data (G and H) of IgG1 and IgM levels in the supernatants of splenic B cells from CD19cre-p75fl/fl and control mice in the presence of IL-4 and R848 stimulation. Data are shown as the means ± SD. Each experiment was performed at least three times. (I to K) PBMCs from patients with SLE were cultured for 72 hours with or without CpG-B stimulation. (I and J) Flow cytometric analysis showing the expression of proBDNF (I) and p75NTR (J) in CD19+ B cells in stimulated PBMCs from patients with SLE. (K) Flow cytometric analysis showing the percentage of ASCs (CD19+CD27hiCD38hi) in PBMCs from patients with SLE upon different stimulations. Two-tailed Student’s t tests (E to J) and one-way ANOVA (A to D and K) were performed. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. NT, no treatment; α-proB, anti-proBDNF antibody; CD19cre-p75fl/fl, control littermates; R848, TRL7 agonist; CpGB, TRL9 agonist.
and graft-versus-host response (31). In the present study, we also found selectively elevated p75NTR levels in ASCs in B cells both in patients with SLE and in the mice with lupus. The CD19Cre-p75fl/fl mice had phenotypes similar to those of control littermates and did not show altered immune cell counts, suggesting that p75NTR has little effect on the development of B cells. As with the results from McAb-proB, selective ablation of p75NTR in B cells also ameliorated lupus and limited the expansion of ASCs, production of autoantibodies, and severity of nephritis. p75NTR-Fc treatment inhibited the ASC proliferation and antibodies in vitro experiments. These results provide evidence for the role of proBDNF-p75NTR signaling in regulating ASC function in SLE. Supporting these assumptions, RNA-seq analysis showed markedly changed genes such as Ighv and Igkv in immunized CD19 Cre-p75 fl/fl mice relative to their controls, which are associated with antigen binding, immune response, and immunoglobulin production. The concurrent expression of p75NTR and proBDNF in ASCs suggests that proBDNF alters the function of ASCs with autocrine functions.

In summary, we have characterized a subset of proBDNF+ ASCs that highly correlated with SLE disease activity. The proBDNF-p75NTR signaling played an important pathogenic role in SLE through promoting the expansion of ASCs. Thus, the proBDNF+ ASCs are a potential therapeutic biological target for SLE.

MATERIALS AND METHODS

Patients

Patients with SLE (n = 67) and healthy gender- and age-matched donors (n = 52) were recruited at the Second Xiangya Hospital, Central South University in China. Patients with SLE met the American College of Rheumatology revised criteria for diagnosis of SLE (32). The exclusion criteria were as follows: (i) more than 10 mg/day of prednisolone at the time of enrollment; (ii) previously B cell-depleting therapy; and (iii) the use of cyclophosphamide, methylprednisolone, or other immunosuppressants. All human participants signed the informed consent forms and donated their blood samples for research. The written informed consent forms were received from participants before inclusion in the study. The study was approved by the Institutional Medical Ethics Review Board of the Second Xiangya Hospital and enrolled in Chinese Clinical Trial Registry (no. ChiCTR1900021328). The clinical characteristics of patients with SLE are shown in table S1.

Clinical data assessment

Disease activity was measured by the SLEDAI (33). The clinical manifestations and the results of laboratory tests were recorded for analysis of clinical features. Leukopenia was defined as white blood cell count less than 4 x 10^9/liter. Hematological symptoms described previously, and the concentration of urinary protein was analyzed using an automatic chemistry analyzer (FAITH-1000). For renal histopathology assessment, left kidneys were embedded in paraffin, and 5-μm sections were stained with hematoxylin and eosin using a standard staining technique. IgG deposits were performed by indirect immunofluorescence on 5-μm paraffin-embedded kidney sections using goat anti-mouse IgG and FITC-conjugated anti-goat IgG.

Flow cytometry

Human PBMCs were isolated from HDs and SLE patients’ fresh blood by lymphoprep separation liquid. Splenocytes were separated by gently crushing spleens from mice in a 70-μm strainer. To determine human B cell subsets and p75NTR expression, human PBMCs and splenocytes were stained with antibodies against different cell surface markers by incubation for 30 min at 4°C. The absolute cell count of splenic B cells was determined by precision count beads as introduced before (35). The antibodies used are listed in table S4. To measure the intracellular staining of proBDNF, fixation and permeabilization were conducted after surface staining. Biotin anti-human proBDNF monoclonal antibody (1:200) as introduced by our previous studies (25) or isotype IgG control (1:200) was stained for 30 min at room temperature, followed by recognition by fluorescein isothiocyanate (FITC) anti-biotin IgG secondary antibody for 30 min at room temperature. Stained cells were read on a flow cytometer (Cystek, Fremont, CA, USA), and data were analyzed using FlowJo vX0.7 software.

Evaluation of renal injury

Urine samples from individual mice were collected at 28 weeks as described previously, and the concentration of urinary protein was analyzed using a standard staining technique. IgG deposits were performed by indirect immunofluorescence on 5-μm paraffin-embedded kidney sections using goat anti-mouse IgG and FITC-conjugated anti-goat IgG.

Cytometric bead–based immunoassays of multiple soluble analytes

Mouse serum and supernatant of cultured splenocytes were collected and stored at −80°C for detection. Cytokines in serum were measured
using an LEGENDplex multianalyte flow assay kit according to the manufacturer’s protocol, and the concentration of cytokines was determined by using LEGENDplex software (BioLegend). Immunoglobulin level in cultured splenic B cell supernatant was detected by cytometric bead array (CBA) assay according to the manufacturer’s protocol and read by flow cytometry (Cystek, Fremont, CA, USA).

**RNA-seq analysis**

Mesenteric lymph nodes from pristane-immunized CD19cre-p75fl/fl mice and their littermate controls at 16 weeks of age were subjected to RNA-sequencing analysis at Songon Biotech (Shanghai, China). Gene expression levels were quantified using StringTie software. Differentially expressed genes were identified using DESeq software with fold change >2 and q-value <0.05. GO analyses were performed using topGo software. GO categories were selected by P value.

**Quantitative PCR**

Total RNA from mesenteric lymph nodes was extracted with TRIzol reagent and was reverse transcribed using revertAid first-strand complementary DNA (cDNA) synthesis kits. Diluted CDNAs were used as templates for qPCR with Fast SYBR Green Master Mix in the CFX96 Touch Deep-Well Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). The primer sequences used are shown in Table 2.

**Enzyme-linked immunosorbent assay**

Immunoglobulin levels in human PBMC culture supernatant were measured by enzyme-linked immunosorbent assay (ELISA) using human IgA, IgG, and IgM ELISA kits. The supernatant was diluted in 1:20 for testing. Serum ANAs and anti-dsDNA antibodies of mice were detected by using the Mouse ANA ELISA Kit and the Mouse anti-dsDNA ELISA Kit, respectively. The mouse serum was diluted 1:5 for testing.

**Culture of splenic B cell**

Primary B cells were isolated from the splenocytes using anti-CD45R conjugated magnetic beads. Purified B cells were suspended in Dulbecco’s modified Eagle’s medium complete medium [10% fetal bovine serum, 1% streptomycin/penicillin, and 55 μM 2-mercaptoethanol (2-ME)] with the presence of IL-4 (30 ng/ml) and contained in 96-well round-bottom plates in which each well contained 2 × 10^5 cells in 200 μl of medium and cultured in 37°C and 5% CO₂. McAb-proB (2 μg/ml), McAb-proB (2 μg/ml), or isotype IgG (2 μg/ml) was added at the initiation of culturing. After 24 hours, the cells and supernatant were collected for assessment of B cell differentiation and antibody production by flow cytometry after 72 hours.

**Culture of human PBMCs**

PBMCs were isolated from heparinized blood from HDs and patients with SLE using Ficoll density gradient centrifugation. PBMCs were resuspended in Iscove’s modified Dulbecco’s media (IMDM) complete medium (10% fetal bovine serum and 1% streptomycin/penicillin) in round 96-well plates, in which each well contains 4 × 10^5 cells in 200 μl of medium and cultured in 37°C and 5% CO₂. CpG-B (3 μg/ml) was used for stimulation and activation. For proBDNF signaling intervention, exogenous protease–resistant proBDNF protein (100 ng/ml), McAb-proB (2 μg/ml), isotype IgG (2 μg/ml), p75ECD-Fc (500 ng/ml), or Fc control was added at the initiation of culturing, respectively. For assessment of the expression of CD40, CD86, and proBDNF signaling expression, cells were cultured for 72 hours. For assessment of proliferation and antibody production, cells were cultured for 7 days.

**CFSE staining**

CFSE (carboxyfluorescein diacetate succinimidyl ester) was used to assess proliferation of in vitro stimulated human B cells with a CFSE staining kit. Briefly, PBMCs were washed to remove any protein and stained with 5 μM CFSE at 37°C for 8 min. After one round of washing with IMDM media containing 10% fetal calf serum to stop CFSE staining, cells were further incubated with fresh complete IMDM media for another 1 hour at 37°C. Then, PBMCs were washed three times and resuspended with complete IMDM medium at concentration of 1 × 10^6 cells/ml and for further culturing and flow cytometric analysis.

**Immunofluorescence**

Paraffin-embedded sections were heated at 60°C for 2 hours and dewaxed by xylene, followed by serial rehydration steps (100% ethanol, 80% ethanol, and 50% ethanol; 5 min each). Antigen retrieval was done by microwave boiling in 10 mM citrate buffer for 20 min. Then, slides were blocked with 10% BSA with 0.5% Triton X-100 in PBS for 1 hour. For staining of proBDNF, slides were incubated with rat anti-CD45R antibody (1:500) and human anti-proBDNF antibody (1:500) at 4°C overnight, followed by incubation with Alexa Flour 647 Donkey anti-rat IgG (1:1000) and FITC Shp pAb (1:1000) to Hu IgG for 1 hour at room temperature. For staining of p75NTR, slides were incubated with rat anti-CD45R antibody (1:500) and rabbit anti-p75NTR antibody (1:500) at 4°C overnight, followed by incubation with Alexa Flour 647 Donkey anti-Rat IgG and Alexa Fluor 488 Anti-Mouse IgG H&L (Alexa Fluor 488, 1:1000) for 1 hour at room temperature.

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**Table 2. Primer sequences used in quantitative PCR.** GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

| Gene   | Sense (5’-3’)          | Antisense (5’-3’)          |
|--------|------------------------|---------------------------|
| lghv1-22 | TGCAACAGCTGGACCTGAG    | CCGTGGCGCTAAGGTCTTGG     |
| lghv8-12 | TCTGGTAGGGTGGAGCTG     | GGCTTCTCAGGGGATGGAATA    |
| lgkv1-110 | CAGTCTGCAAAGCTCTGAG   | AAACCTCCCCAGATCCTACGCC   |
| lgkv8-24 | GCAAGTCCAGTCAGACGCTT  | TCCACTGCTAATGAGCGAT      |
| lgkv10-96 | GGGCAAGTCAGGACATTAGC  | TCTTCTCTCCAGTTGCT        |
| lgkv14-162 | TATGCATCGCTGGGAGAGAG | CCGACAGCTGAGAGGGAGAG    |
| GAPDH   | TGGCCTCCATGGCCCAAG     | CTGCCAGAACCACCATCACCCT   |

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Flour 488 goat anti-Rabbit IgG (H + L) for 1 hour. After rinsing, slides were further stained with 4',6-diamidino-2-phenylindole (DAPI). Images were collected by a scanning microscope (Pannoramic DESK, P-MIDI, P250, P10000) and analyzed using Panoramic Scanner software.

Western blotting
For protein extraction, tissues and cells were homogenized in ice-cold radioimmunoprecipitation assay buffer containing proteinase inhibitor. Total protein was quantified using a Coomassie protein stain. Western blotting was conducted to examine the association between proBDNF MFI in ASCs and related clinical variables in patients with SLE. P < 0.05 was considered statistically significant.

SUPPLEMENTAL MATERIALS
Supplemental material for this article is available at https://science.org/doi/10.1126/sciadv.8abj2797

View/request a protocol for this paper from Bio-protocol.

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Acknowledgments: We would like to thank S. Yile Biotechnology Corp. for providing the humanized monoclonal anti-proBDNF antibodies. Funding: This work was funded by the...
National Natural Science Foundation of China (81771354 and 82071347 to R.-P.D., 81873770 to H.L., and 81901231 to Z.-L.H.) and Hunan Province Science Foundation for Young Scientists of China (2020JJ5820 to C.L. and 2020JJ5809 to Z.-L.H.). **Author contributions:** Conceptualization: R.-P.D., W.-Y.S., and C.L. Methodology: W.-Y.S., C.L., X.-J.L., R.-Y.L., Z.-L.H., and E.J.C. Investigation: W.-Y.S., C.L., X.-J.L., H.L., J.-M.X., and R.-P.D. Visualization: W.-Y.S., C.L., H.L., M.Z., and R.-P.D. Supervision: R.-P.D., P.R.H., Z.-L.H., X.-F.Z., and M.Z. Writing (original draft): W.-Y.S., C.L., and R.-P.D. Writing (review and editing): W.-Y.S., C.L., X.-F.Z., and R.-P.D. **Competing interests:** R.-P.D. and X.-F.Z. are inventors on a pending patent related to this work filed by Shanghai Yile Biotechnology Co. Ltd. (no. US15/627,305, filed 19 June 2017). R.-P.D. is an inventor of a pending patent related to this work filed by Shanghai Yile Biotechnology Co. Ltd. (no. 201980022274, filed 26 March 2019). The authors declare that they have no other competing interests. **Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials.

Submitted 4 May 2021
Accepted 24 November 2021
Published 19 January 2022
10.1126/sciadv.abj2797