

# Brain-derived neurotrophic factor modulates immune reaction in mice with peripheral nerve xenotransplantation

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**Background:** Brain-derived neurotrophic factor (BDNF) has been demonstrated to play an important role in survival, differentiation, and neurite outgrowth for many types of neurons. This study was designed to identify the role of BDNF during peripheral nerve xenotransplantation.

**Materials and methods:** A peripheral nerve xenotransplantation from rats to mice was performed. Intracellular cytokines were stained for natural killer (NK) cells, natural killer T (NKT) cells, T cells, and B cells and analyzed by flow cytometry in the spleen of the recipient mouse. Serum levels of related cytokines were quantified by cytometric bead array.

**Results:** Splenic NK cells significantly increased in the xenotransplanted mice ( $8.47 \pm 0.88 \times 10^7$  cells/mL) compared to that in the control mice ( $4.66 \pm 0.78 \times 10^7$  cells/mL,  $P=0.0003$ ), which significantly reduced in the presence of BDNF ( $4.85 \pm 0.87 \times 10^7$  cells/mL,  $P=0.0004$ ). In contrast, splenic NKT cell number was significantly increased in the mice with xenotransplantation plus BDNF (XT + BDNF) compared to that of control group or of mice receiving xenotransplantation only (XT only). Furthermore, the number of CD3+ T cells, CD3+CD4+ T cells, CD3+CD4- T cells, interferon- $\gamma$ -producing CD3+CD4+ T cells, and interleukin (IL)-17-producing CD3+CD4+ T cells, as well as CD3-CD19+ B cells, was significantly higher in the spleen of XT only mice compared to the control mice ( $P<0.05$ ), which was significantly reduced by BDNF ( $P<0.05$ ). The number of IL-4-producing CD3+CD4+ T cells and CD3+CD4+CD25+Foxp3+ T cells was significantly higher in the spleen of XT + BDNF mice than that in the spleen of XT only mice ( $P<0.05$ ). Serum levels of IL-6, TNF- $\alpha$ , interferon- $\gamma$ , and IL-17 were decreased, while IL-4 and IL-10 were stimulated by BDNF following xenotransplantation.

**Conclusion:** BDNF reduced NK cells but increased NKT cell accumulation in the spleen of xenotransplanted mice. BDNF modulated the number of splenic T cells and its subtype cells in the mice following xenotransplantation. These findings suggest that BDNF inhibits rejection of peripheral nerve following xenotransplantation by regulating innate as well as adaptive immune reaction.

**Keywords:** peripheral nerve, xenotransplantation, brain-derived neurotrophic factor

## Introduction

Neurotrophins are a family of secreted proteins, including brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3, and neurotrophin-4, which have been recognized as important regulators of synaptic plasticity.<sup>1-4</sup> These molecules have been shown to control the generation, survival, differentiation, and regeneration of neurons in the peripheral and central nervous systems.<sup>5</sup> Induction of BDNF in the peripheral nerve and muscle would be important to stimulate Schwann cell differentiation and proliferation, a prerequisite for axonal regeneration. However,

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neurotrophins are now considered to be growth factors with a wide spectrum of functions outside the nervous system, including modulation and regulation of the immune function.<sup>6</sup> NGF has been demonstrated to act as a cytokine-like factor in the development and function of the immune system, and the function of NGF on B cells, T cells, and macrophages has been well described.<sup>7</sup> However, limited studies on the role of BDNF in the immune system, especially in transplant immunity, are available.

Two distinct classes of cell surface receptors mediate the biological effects of BDNF, the high affinity receptor tyrosine kinase B (TrkB) and the low affinity p75 neurotrophin receptor. In adult central nervous system, the most prominent expression of BDNF and its receptor, TrkB, is found in structures associated with epilepsy and synaptic plasticity, such as the hippocampus, cerebellum, and neocortex, suggesting that BDNF may modify neuronal plasticity in adult animals. Indeed, accumulating evidence indicates that BDNF is involved in synaptic transmission, neuronal excitability, and the development and maintenance of neuronal plasticity in adult central nervous system. It has been shown that both T and B lymphocytes express TrkB receptors on their surface.<sup>8–10</sup> T cells, B cells, and other cells, including innate immune cells, have been demonstrated to play an essential role in the pathogenesis of rejection following organ transplantation. However, the role of BDNF in modulating the function of the immune cells following nerve transplantation has not been investigated.

Therefore, in order to identify whether BDNF could prevent immune rejection after peripheral nerve xenotransplantation and facilitate the nerve regeneration, we performed nerve xenotransplantation from rats to mice and found a dramatic reduction of natural killer (NK) cells, B cells, type 1 T helper (Th1) cells, and type 17 T helper (Th17) cells, while a remarkable increase of natural killer T (NKT) lymphocytes, type 2 T helper (Th2) cells, and regulatory T cells (Tregs) was observed in the spleen of xenotransplanted mice treated with BDNF compared to that in mice receiving xenotransplantation only. In addition, serum concentrations of interleukin (IL)-6, IL-17, interferon  $\gamma$  (IFN- $\gamma$ ), and TNF- $\alpha$  were significantly decreased, while IL-4 and IL-10 were significantly increased in the presence of BDNF following xenotransplantation.

## Materials and methods

### Animals

Adult male Sprague Dawley rats (6.0 $\pm$ 0.1 weeks old and 190 $\pm$ 5 g weight in average) and adult male BALB/c mice (70.1 $\pm$ 0.2 weeks old and 20.2 $\pm$ 0.5 g weight in average) were

used as donors and recipients, respectively (at least five mice in each group). All animals were purchased from Jilin University (Changchun, People's Republic of China). All surgical procedures and postoperative care of the animals were approved by the Institutional Animal Ethics Committee of Jilin University.

### Peripheral nerve xenotransplantation

Rats were euthanized followed by exposing the sciatic nerve of the donor rat through an incision splitting the dorsal gluteal muscle. A segment (0.5 cm) of sciatic nerve was then harvested and used immediately. The recipient mouse was given 3.5% chloral hydrate (0.1 mL/kg) intraperitoneally. The skin over the mouse's right hind limb was incised, and the underneath muscle was bluntly dissected to expose the sciatic nerve. A 0.5 cm gap on the sciatic nerve was created. The rat sciatic nerve graft (0.5 cm) was interposed to the transected nerve and repaired with 11-0 nylon epineurial sutures. Mice in which the sciatic nerve was exposed without xenotransplantation were used as the sham control group. All mice were sacrificed on the third day after transplantation.

### BDNF application

BDNF (eBioscience, San Diego, CA, USA) was diluted with physiological saline (500  $\mu$ g/50  $\mu$ L), and 10  $\mu$ L (total 100  $\mu$ g) was topically applied at the place of the transplanted nerve graft only once during the operation.

### Flow cytometric analysis of intracellular cytokine staining

Whole spleen was homogenized in PBS, and the cell suspension was filtered to remove the debris. Cells were collected by centrifugation at 1,500 $\times$  *g* for 10 minutes at 4°C. The cells were then washed and resuspended with FACS buffer. After processing, 1–2 $\times$ 10<sup>6</sup> cells/mL were stimulated with phorbol 12-myristate 13-acetate (PMA; Sigma Chemicals, Perth, Australia) (50 ng/mL) plus 2 mg/mL of ionomycin for 6 hours. Brefeldin A (10 mg/mL) was then added 2 hours before the cells were collected and stained with antibodies. The cells were stained with antibodies against surface markers (antimouse CD3, CD4, CD25, and NK; eBioscience). Cells were then permeabilized with membrane-permeabilizing reagents (Caltag, Burlingame, CA, USA) and fixed using a fixing solution (Caltag), following the manufacturer's instructions, followed by incubation with anti-IL-4, anti-IFN- $\gamma$ , anti-IL-17, and anti-Foxp3 (eBioscience), or isotope-matched control antibodies for 30 minutes. Flow cytometry (FACSCalibur™; Beckton

Dickinson, Franklin Lakes, NJ, USA) was performed and data were analyzed using FlowJo software.

## Cytometric bead array of serum cytokines

Blood was collected from the orbital sinus of the recipient mice. The concentration of serum cytokines (IFN- $\gamma$ , TNF- $\alpha$ , IL-4, IL-6, IL-10, and IL-17) was determined by cytometric bead array (CBA), following the manufacturer's protocol (BD Biosciences, San Jose, CA, USA) with minor modifications. Briefly, 25 mL of individual serum was used in duplicate for analysis. The concentration of serum cytokines was quantified using the CellQuestPro and CBA software (Becton Dickinson) on a FACSCalibur cytometry (BD Biosciences).

## Statistical analysis

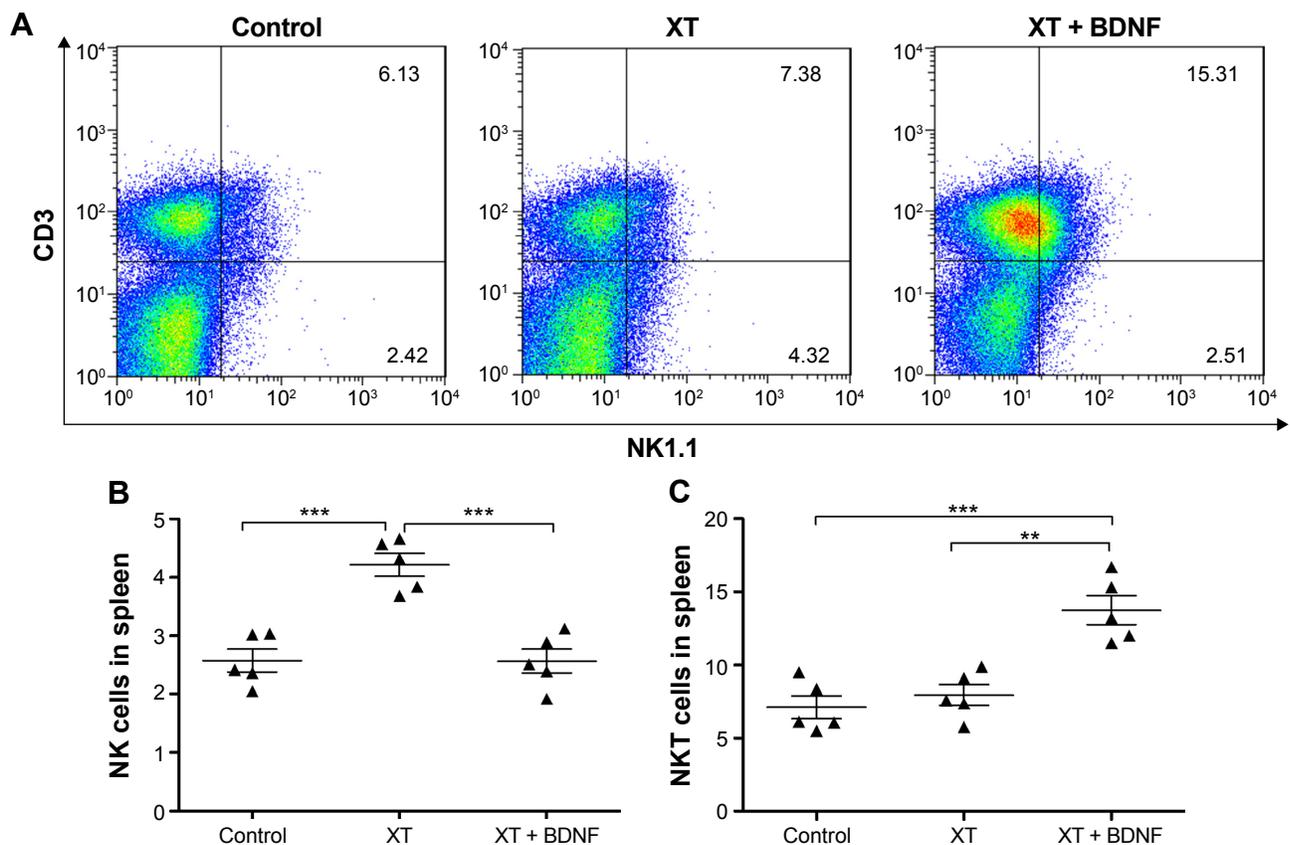
Difference of paired or unpaired groups was determined by the Student's *t*-test for parametric data sets. All statistical analyses were performed using Prism 4 (GraphPad Software,

Inc., La Jolla, CA, USA).  $P \leq 0.05$  was considered statistically significant for all analyses in this study.

## Results

### Effect of BDNF on the number of splenic NK cells in the xenotransplanted mice

All mice survived post-transplantation regardless of BDNF treatment. The total cell number of splenic mononuclear cells was highest in the mice of xenotransplantation without BDNF treatment (XT only) group ( $2.68 \pm 0.32 \times 10^9$  cells/mL), mildly high in the group of transplantation plus BDNF treatment (XT + BDNF,  $2.52 \pm 0.41 \times 10^9$  cells/mL), and least in the sham control group ( $2.41 \pm 0.38 \times 10^9$  cells/mL). However, there was no statistical difference among the three groups. The number of splenic NK cells was further counted and characterized, and it was found to be significantly increased in the mice of XT only group ( $8.47 \pm 0.88 \times 10^7$  cells/mL) than that in the sham control group ( $4.68 \pm 0.78 \times 10^7$  cells/mL,  $P = 0.0003$ , Figure 1A). In contrast, the number of splenic NK cells in the mice of



**Figure 1** Flow cytometric analysis of splenic NK and NKT cells.

**Notes:** Mice spleens were collected from three groups on day 3 and used for flow cytometry analysis as described in the method. **(A)** One representative result of two separate experiments on NK1.1 and CD3 expression in splenic lymphocytes on day 3. **(B)** Comparison of CD3+NK1.1+ NK cells between three groups ( $n=5$  in each group). Vertical axis: percentage of NK1.1 cells; horizontal axis: treatment groups ( $n=5$  each group). **(C)** Comparison of CD3+NK1.1+ NKT cells. Vertical axis: percentage of NKT cells.  $**P < 0.01$ ;  $***P < 0.001$ .

**Abbreviations:** NK, natural killer; NKT, natural killer T; XT, xenotransplantation; BDNF, brain-derived neurotrophic factor.

XT + BDNF treatment group ( $4.85 \pm 0.87 \times 10^7$  cells/mL) was remarkably reduced, and it was significantly less than that in the group of XT only group ( $8.47 \pm 0.88 \times 10^7$  cells/mL,  $P=0.0004$ ). In addition, there was no significant difference between the XT + BDNF group and the sham control group (Figure 1B,  $P=0.9728$ ).

### Effect of BDNF on the number of splenic NKT cells in the xenotransplanted mice

The number of NKT cells was significantly increased in the spleen of mice receiving XT + BDNF ( $2.60 \pm 0.42 \times 10^8$  cells/mL) compared to that in the sham control group ( $1.29 \pm 0.31 \times 10^8$  cells/mL,  $P=0.0007$ ) or the XT only mice ( $1.60 \pm 0.32 \times 10^8$  cells/mL,  $P=0.0015$ , Figure 1C). However, there was no significant difference in the number of NKT cells between the XT only group and the sham control mice ( $P=0.4551$ ).

### Effect of BDNF on the number of splenic T cells in the xenotransplanted mice

As shown in Figures 2 and 3, the percentage of T cell and its sub-group was analyzed in the splenic mononuclear cells. The number of CD3+ T cells ( $12.56 \pm 0.94 \times 10^8$  cells/mL), CD3+CD4+ T cells ( $6.56 \pm 0.74 \times 10^8$  cells/mL), CD3+CD4- T cells ( $3.15 \pm 0.39 \times 10^8$  cells/mL), IFN- $\gamma$ -producing CD3+CD4+ T cells ( $7.51 \pm 0.96 \times 10^7$  cells/mL), and IL-17-producing CD3+CD4+ T cells ( $3.18 \pm 0.20 \times 10^7$  cells/mL) was significantly increased in the spleen of XT only mice compared to that in the sham control group (CD3+ T cells:  $6.0 \pm 0.54 \times 10^8$  cells/mL, CD3+CD4+ T cells:  $3.46 \pm 0.42 \times 10^8$  cells/mL, CD3+CD4- T cells:  $1.55 \pm 0.16 \times 10^8$  cells/mL, IFN- $\gamma$ -producing CD3+CD4+ T cells:  $1.76 \pm 0.09 \times 10^7$  cells/mL, and IL-17-producing CD3+CD4+ T cells:  $0.51 \pm 0.09 \times 10^7$  cells/mL, respectively,  $P < 0.0001$  in all paired comparison; Figure 3A–D, and F, respectively). However, in the presence of BDNF, the number of the aforementioned sub-group T cells was significantly reduced in the mice of XT + BDNF group (CD3+ T cells:  $8.55 \pm 0.43 \times 10^8$  cells/mL, CD3+CD4+ T cells:  $4.36 \pm 0.33 \times 10^8$  cells/mL, CD3+CD4- T cells:  $2.16 \pm 0.18 \times 10^8$  cells/mL, IFN- $\gamma$ -producing CD3+CD4+ T cells:  $3.33 \pm 0.30 \times 10^7$  cells/mL, and IL-17-producing CD3+CD4+ T cells:  $1.21 \pm 0.15 \times 10^7$  cells/mL, respectively) compared to that in the group of XT only ( $P < 0.01$  in all paired comparison, Figure 3A–D, and F). In addition, the IL-4-producing CD3+CD4+ T cells and CD3+CD4+CD25+Foxp3+ T cells were significantly increased in the mice of XT + BDNF group ( $3.36 \pm 0.54 \times 10^7$  cells/mL and  $6.85 \pm 0.34 \times 10^6$  cells/mL, respectively) compared to those in the mice of XT only group ( $1.84 \pm 0.30 \times 10^7$  cells/mL and  $3.58 \pm 0.36 \times 10^6$  cells/mL, respectively,  $P < 0.05$ , Figure 3E and G).

### Effect of BDNF on the number of splenic B cells in the xenotransplanted mice

The number of CD3+CD19+ B cells was significantly decreased in the spleen of XT + BDNF group ( $6.01 \pm 0.52 \times 10^8$  cells/mL) compared to that in the sham control group ( $7.47 \pm 0.31 \times 10^8$  cells/mL,  $P < 0.0001$ ) or the XT only group ( $10.13 \pm 0.34 \times 10^8$  cells/mL,  $P < 0.0001$ , Figure 4A and B). There was no significant difference in the number of CD3+CD19+ B cells between the XT only group and the sham control mice ( $P=0.1128$ ).

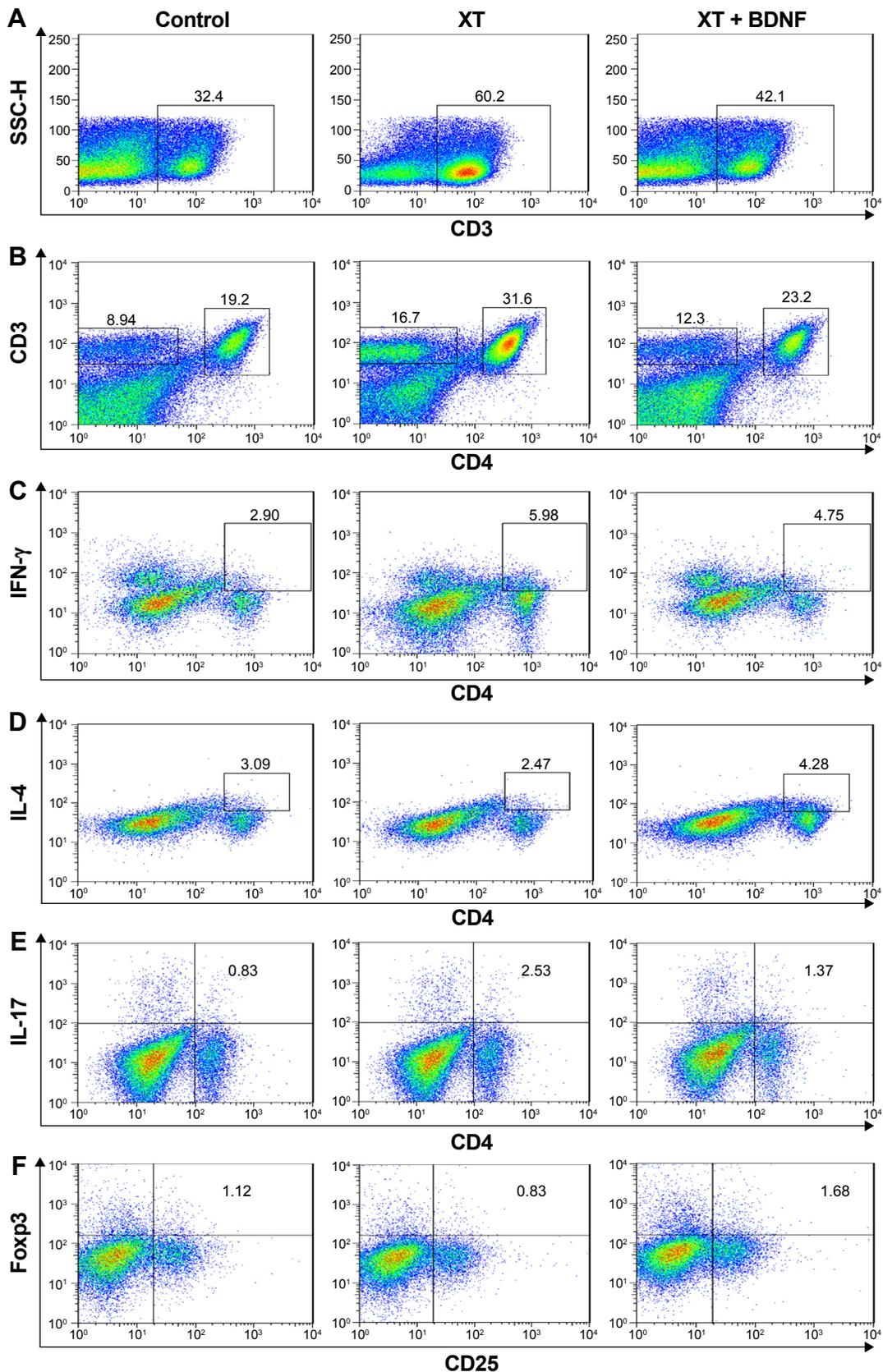
### Effect of BDNF on the levels of cytokines in the serum of xenotransplanted mice

Compared to the sham control group, the following cytokines were significantly increased in the serum of XT only mice: IL-6 ( $51.49 \pm 17.19$  pg/mL of control vs  $152.71 \pm 14.18$  pg/mL of XT only,  $P < 0.0001$ ), IL-17 ( $5.76 \pm 1.52$  pg/mL of control vs  $38.93 \pm 5.04$  pg/mL of XT only,  $P < 0.0001$ ), IFN- $\gamma$  ( $4.12 \pm 0.57$  pg/mL of control vs  $23.49 \pm 5.18$  pg/mL of XT only,  $P < 0.0001$ ), and TNF- $\alpha$  ( $7.15 \pm 1.85$  pg/mL of control vs  $34.74 \pm 3.05$  pg/mL of XT only,  $P < 0.0001$ ). In contrast, IL-4 and IL-10 were significantly decreased in the serum of XT only mice ( $1.89 \pm 0.66$  pg/mL and  $45.66 \pm 18.59$  pg/mL, respectively) compared to those in the sham control mice ( $3.39 \pm 0.68$  pg/mL,  $P=0.0076$ , and  $43.27 \pm 28.37$  pg/mL,  $P=0.0002$ , respectively).

Compared to XT only mice and XT + BDNF mice, the levels of IL-6 ( $152.71 \pm 14.18$  pg/mL of XT only vs  $84.78 \pm 7.84$  pg/mL of XT + BDNF,  $P < 0.0001$ ), IL-17 ( $38.93 \pm 5.04$  pg/mL of XT only vs  $24.16 \pm 1.44$  pg/mL of XT + BDNF,  $P=0.0002$ ), IFN- $\gamma$  ( $23.49 \pm 5.18$  pg/mL of XT only vs  $15.34 \pm 3.11$  pg/mL of XT + BDNF,  $P=0.0167$ ), and TNF- $\alpha$  ( $34.74 \pm 3.50$  pg/mL of XT only vs  $18.55 \pm 6.66$  pg/mL of XT + BDNF,  $P=0.0013$ ) were significantly decreased, while IL-4 ( $1.89 \pm 0.66$  pg/mL of XT only vs  $24.73 \pm 2.13$  pg/mL of XT + BDNF,  $P < 0.0001$ ) and IL-10 ( $45.66 \pm 18.59$  pg/mL of XT only vs  $375.06 \pm 57.11$  pg/mL of XT + BDNF,  $P < 0.0001$ ) were significantly increased (Figure 5).

## Discussion

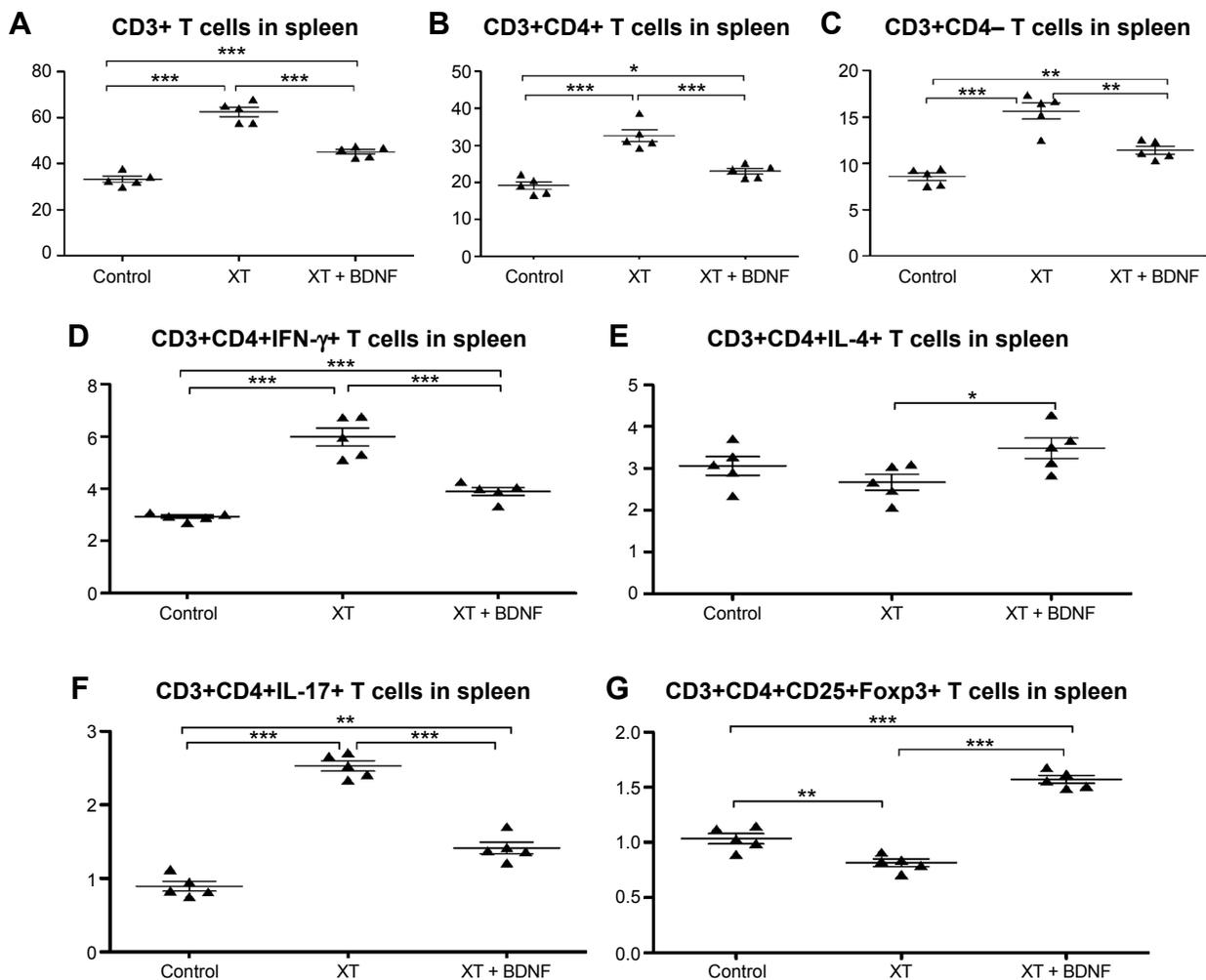
This study investigated the effect of BDNF on the immune reaction following peripheral nerve xenotransplantation from rats to mice. It was found that the number of NK cells, T cells, CD3+CD4+ T cells, CD3+CD4- T cells, Th1 cells, Th17 cells, and B cells was significantly increased in the mice receiving xenotransplantation and that BDNF could significantly suppress upregulation of the aforementioned T cells and B cells in the xenotransplanted mice. Paradoxically, the number of NKT cells, Th2 cells, and Tregs was significantly decreased at



**Figure 2** Flow cytometric analysis of splenic T cells.

**Notes:** Mice spleens were collected from the three groups on day 3 and flow cytometric analysis was performed as described in the method. (A) CD3+ T cells. (B) CD3+CD4+ T cells and CD3+CD4- T cells. (C) IFN- $\gamma$ -producing CD3+CD4+ T cells. (D) IL-4-producing CD3+CD4+ T cells. (E) IL-17-producing CD3+CD4+ T cells. (F) CD3+CD4+CD25+Foxp3+ T cells. Data in all panels are one representative result of two separate experiments with five animals in each group.

**Abbreviations:** IFN- $\gamma$ , interferon  $\gamma$ ; IL, interleukin; XT, xenotransplantation; BDNF, brain-derived neurotrophic factor.



**Figure 3** Comparison of T-cell subgroups between three treatment groups.

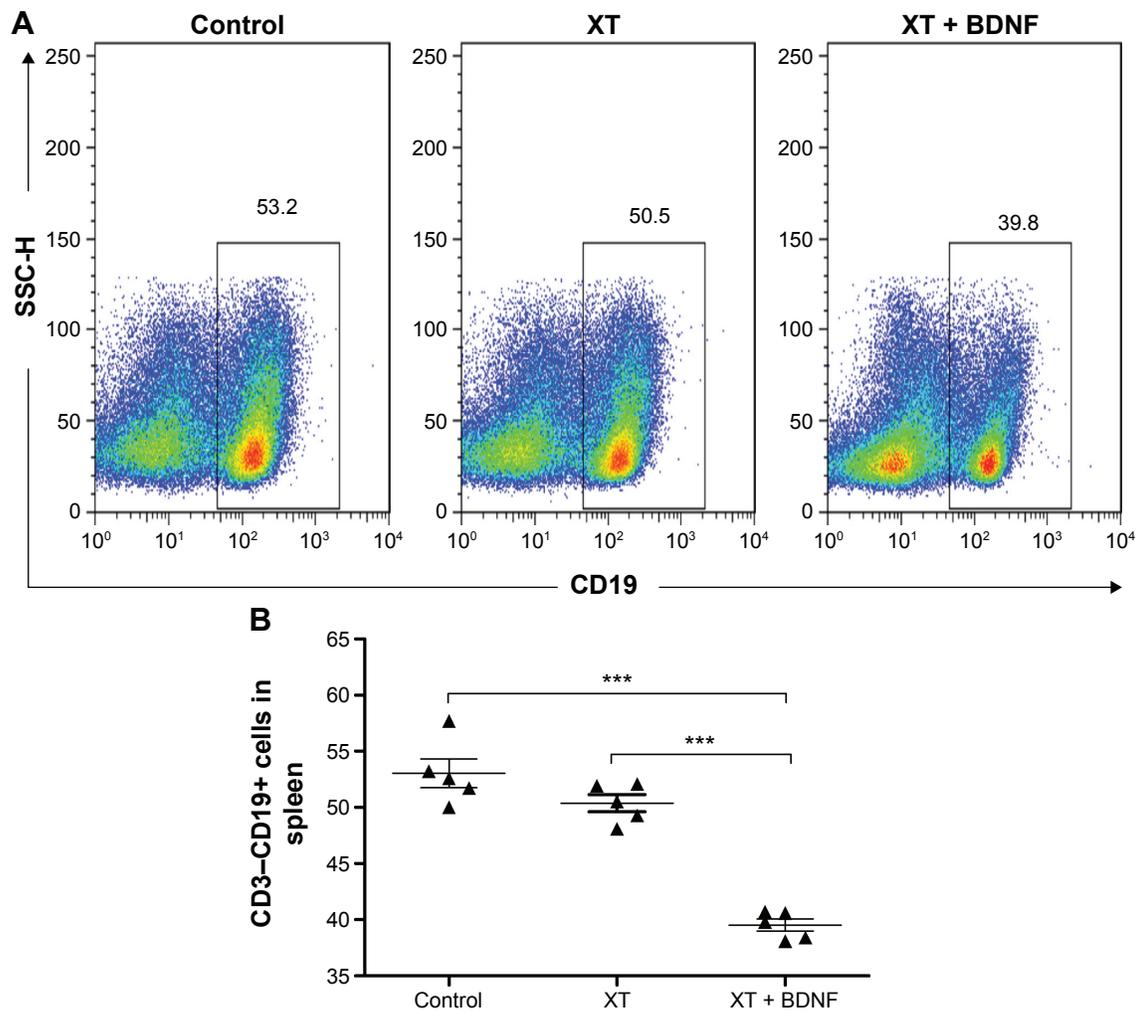
**Notes:** Percentage of splenic T-cell subsets was compared between the three treatment groups. (A) Comparison of CD3+ T cells. (B) Comparison of CD3+CD4+ T cells. (C) Comparison of CD3+CD4- T cells. (D) Comparison of IFN- $\gamma$ -producing CD3+CD4+ T cells. (E) Comparison of IL-4-producing CD3+CD4+ T cells. (F) Comparison of IL-17-producing CD3+CD4+ T cells. (G) Comparison of CD3+CD4+CD25+Foxp3+ T cells. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . Data presented are one representative result of two separate experiments with five mice in each group.

**Abbreviations:** IFN- $\gamma$ , interferon  $\gamma$ ; IL, interleukin; XT, xenotransplantation; BDNF, brain-derived neurotrophic factor.

the acute phase of peripheral nerve xenotransplantation in the mice, and BDNF could significantly upregulate NKT cells, Th2 cells, and Tregs in the mice receiving nerve xenotransplantation. Furthermore, serum levels of IL-6, IL-17, IFN- $\gamma$ , and TNF- $\alpha$  were significantly inhibited, whereas serum levels of IL-4 and IL-10 were significantly stimulated by BDNF in the xenotransplanted mice.

As a member of the neurotrophin family, BDNF was initially identified as a target-derived factor that promotes the survival of several populations of central neurons.<sup>11–14</sup> Both *in vitro*<sup>15</sup> and *in vivo*,<sup>16–18</sup> it has been reported that application of BDNF was effective for motoneuron survival. Induction of BDNF in the peripheral nerve and muscle would be important to stimulate Schwann cell differentiation and proliferation, a prerequisite for axonal regeneration. It has

been reported that BDNF mRNA and its TrkB receptor mRNA coexist in the same neuron,<sup>19,20</sup> and antisense BDNF oligonucleotides<sup>21,22</sup> or anti-BDNF antiserum<sup>23,24</sup> could reduce the survival of cultured neurons, even as isolated single cells. Thus, BDNF may act as an autocrine factor for maintaining neuronal survival during target-independent stages of development.<sup>25</sup> It has been reported that BDNF served as a self-amplifying autocrine factor in promoting axon formation in embryonic hippocampal neurons by triggering two-nested positive feedback mechanisms. First, BDNF elevates cytoplasmic cyclic adenosine monophosphate and protein kinase A activity, which triggers further secretion of BDNF and membrane insertion of its receptor TrkB. Second, BDNF/TrkB signaling activates PI3-kinase that promotes anterograde transport of TrkB in the putative



**Figure 4** Flow cytometric analysis of splenic B cells.

**Notes:** Mice spleens were collected from three groups on day 3 and used for flow cytometric analysis as described in the method. **(A)** One representative result of CD19 expression in splenic lymphocytes. All the graphs were gated from CD3<sup>-</sup> cells. **(B)** Comparison of CD3<sup>-</sup>CD19<sup>+</sup> B cells in the three groups. Vertical axis: percentage of CD3<sup>-</sup>CD19<sup>+</sup> cells; horizontal axis: three treatment groups. \*\*\* $P < 0.001$ . Data presented are one representative result of two separate experiments with five mice in each group.

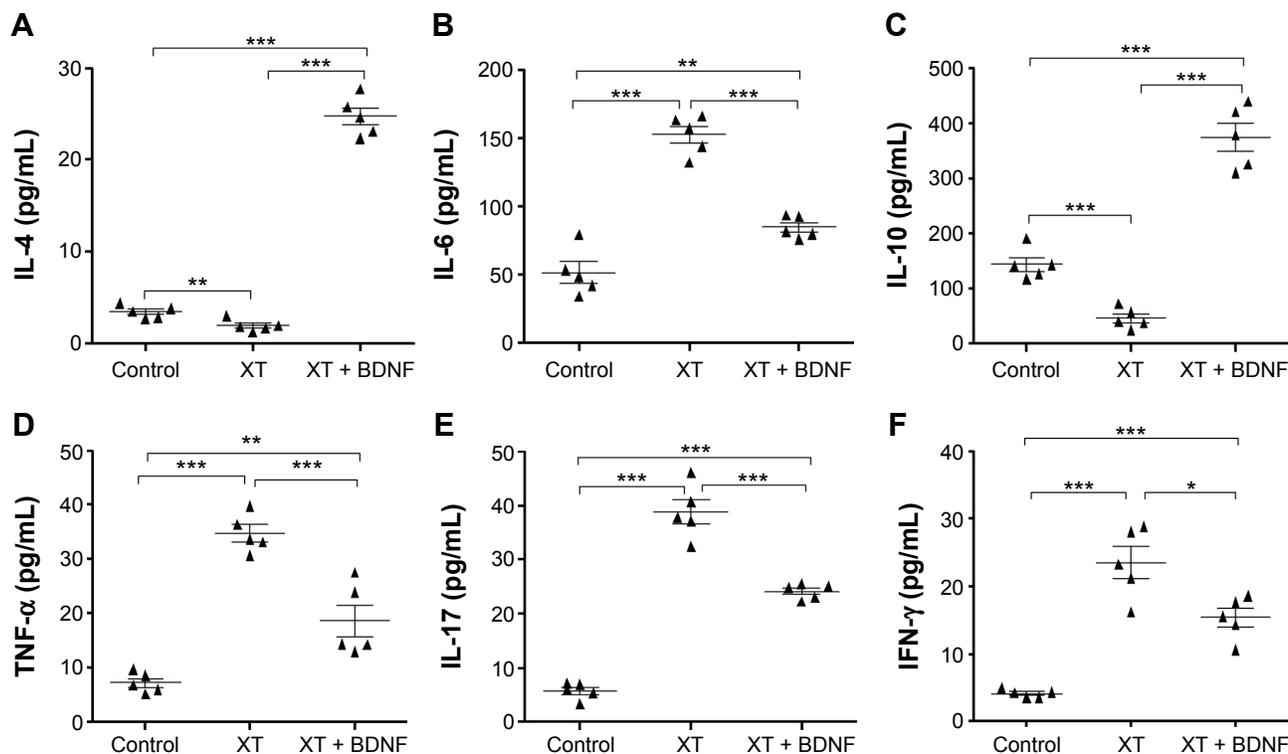
**Abbreviations:** XT, xenotransplantation; BDNF, brain-derived neurotrophic factor.

axon, further enhancing local BDNF/TrkB signaling. Together, these self-amplifying BDNF actions ensure stable elevation of local cyclic adenosine monophosphate/protein kinase A activity that is critical for axon differentiation and growth.

NK cells are large granular cytotoxic lymphocytes that represent a fundamental component of the innate immune system. In transplantation, NK cells are perhaps best known for their ability to directly reject major histocompatibility complex-mismatched bone marrow allografts, as illustrated by recent studies.<sup>26,27</sup> NK cells have the capacity to exacerbate multiple forms of allograft injury; in this regard, NK cells can induce cardiac, skin, and kidney allograft rejection.<sup>28–33</sup> In the current study, the number of NK cells after 3 days of peripheral nerve xenotransplantation was significantly

higher than that in the sham control mice, indicating that NK cells may induce the acute rejection of peripheral nerve xenotransplantation. However, in the presence of BDNF, the number of NK cells in the xenotransplanted mice was significantly low, suggesting that BDNF inhibits the rejection of peripheral nerve xenotransplantation by decreasing the level of NK cells.

NKT cells are innate-like T cells that recognize lipid antigens presented by the nonpolymorphic major histocompatibility complex class I-like molecule CD1d. It has been demonstrated that NKT cells are required for the induction of tolerance of islet xenografts and cardiac allografts.<sup>34,35</sup> In the current study, there was no significant difference in the number of NKT cells between the XT only mice and the sham control group, but it increased significantly in



**Figure 5** Cytometric bead array of IFN- $\gamma$ , TNF- $\alpha$ , IL-4, IL-6, IL-10, and IL-17.

**Notes:** Serum from each mouse was harvested and used for cytokine quantification by Cytometric Bead Array as described in the method. **(A)** Comparison of IL-4. **(B)** Comparison of IL-6. **(C)** Comparison of IL-10. **(D)** Comparison of TNF- $\alpha$ . **(E)** Comparison of IL-17. **(F)** Comparison of IFN- $\gamma$ . Vertical axis: level of cytokines (pg/mL); horizontal axis: three treatment groups. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . Data presented are one representative result of two separate experiments with five mice in each group.

**Abbreviations:** IFN- $\gamma$ , interferon  $\gamma$ ; IL, interleukin; XT, xenotransplantation; BDNF, brain-derived neurotrophic factor.

the mice of XT plus BDNF group, suggesting that BDNF enhances the effect of NKT cells in inducing peripheral nerve xenograft tolerance by increasing the number of NKT cells.

Following the activation of innate immune cells, such as NK and NKT cells, adaptive immune cells, including T cells and B cells, are activated during the acute rejection of peripheral nerve xenotransplantation. In this context, it has been demonstrated that cellular immunity, especially T cells, plays a vital role during organ xenotransplant rejection.<sup>36–39</sup> In addition to direct killing activity by cytotoxic T lymphocytes, xenograft rejection can occur through T cell-mediated mechanisms, including cytokine production, recruitment and activation of other cytotoxic cells, and by providing help for B cells that produce xenoreactive antibodies.<sup>40</sup> We demonstrated that both IFN- $\gamma$ - and IL-17-producing CD4+ and CD8+ T cells play a crucial role during the acute rejection of peripheral nerve xenotransplantation.<sup>41</sup> We further showed that the number of CD3+ T cells, Th1 cells, and Th17 cells was suppressed by BDNF, while the subsets of Th2 cells and Tregs were upregulated by BDNF in the xenotransplanted mice. In addition, serum

concentrations of IL-6, IL-17, TNF- $\alpha$ , and IFN- $\gamma$  were significantly inhibited by BDNF in the xenotransplanted mice. These results indicate that topical application of BDNF after peripheral nerve xenotransplantation results in the inhibition of Th1 and Th17 cells, but activation of Th2 cells and Tregs, and by using this mechanism, BDNF may inhibit the rejection of xenotransplanted peripheral nerve segment.

Besides T cell and its subgroups, B cells also play a crucial role in transplant rejection such as in kidney and liver transplantation by secreting target-specific antibodies. In the present study, B cells were also significantly suppressed by BDNF in the xenotransplanted mice, indicating that BDNF regulates target-specific antibodies by suppressing B cells.

In contrast to organ transplantation such as the kidney, heart, or liver transplantation, in which survival of the transplanted organs is crucial to have long-term organ function, the peripheral nerve xenograft functions for a limited time only. Thus, once the regenerative nerve can pass the nerve xenografts in a short period following the transplantation, the function of peripheral nerve xenografts is completed, regardless of the survival or death of the transplanted nerve.

The current study demonstrated that BDNF plays an essential role in inhibiting the acute rejection of peripheral nerve xenotransplantation by modulating the immune reaction in the xenotransplanted nerves.

## Conclusion

In summary, the current study demonstrated that the number of NK cells, Th1 cells, Th17 cells, and B cells in the xenotransplanted nerves was significantly reduced by BDNF, while the number of NKT, Th2 cells, and Tregs in the xenotransplanted spleen was significantly increased by BDNF. In addition, cytokines released by the aforementioned immune cells were also modulated by BDNF. These findings suggest that BDNF inhibits the peripheral nerve xenograft rejection by regulating the paradigm of NK/NKT and Th1/Th2/Th17/Tregs.

## Acknowledgments

This study was supported by the grants from the National Natural Science Foundation of China (Nos 81171145 and 81201382) and Post-doctor Science Foundation of China (No 2013M541315).

## Disclosure

The authors report no conflicts of interest in this work.

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