B cell-mediated immunomodulation by S-nitrosoglutathione (GSNO) in experimental autoimmune encephalomyelitis

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Research

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Abstract

Background Experimental autoimmune encephalomyelitis (EAE) is the most commonly used animal model for human multiple sclerosis (MS), a demyelinating autoimmune disease mediated by T and B lymphocytes. The aim of the present study was to investigate the role of S-nitrosoglutathione (GSNO), a physiological nitric oxide carrier molecule, in regulation of effector or regulatory B cell function as IL-6 and IL-10 expressions and thus the potential role of GSNO in targeting B cell-mediated immunopathogenesis in MS using EAE model.

Methods To this purpose, the in vivo EAE mouse model, generated by immunization with myelin oligodendrocyte glycoprotein (MOG) 35-55 peptide, or in vitro model of cultured B cells stimulated with lipopolysaccharide or anti-IgM antibody were treated with exogenous GSNO or N6022, an inhibitor of GSNO reductase (GSNOR; GSNO degrading enzyme) to increase endogenous GSNO, and then analyzed for B cell specific IL-6 and IL-10 expression.

Results In EAE model, administration of exogenous GSNO or inhibition of endogenous GSNO catabolism by N6022 treatment ameliorated the clinical disease with decreased CNS infiltration of B cells. In addition, GSNO/N6022 treatments increased the number of IL-10+ B cells but decreased the number of IL-6+ B cells in the CNS and spleen. Accordingly, GSNO/N6022 treatments increased the expression of IL-10 while reducing the IL-6 expression in the blood. Similar observations were also made in in vitro B cell culture model where GSNO treatment increased the IL-10+ B cells but decreased the IL-6+ B cells under BCR or TLR4 stimulatory conditions and under CD40 and BAFF co-stimulatory conditions. Accordingly, GSNO treatment increased the B cell production of IL-10 but reduced the IL-6 production under both stimulatory and co-stimulatory conditions. In vitro stimulation and co-stimulation of cultured naïve B cells increased two major distinct B cell populations; CD1dlow CD5high and CD1dhigh CD5high. In both populations, GSNO treatment increased the number of IL-10+ cells but decreased the IL-6+ cells.

Conclusion These data document, for the first time, that cellular GSNO homeostasis is a critical target for the regulation of IL-10+ B cells vs. IL-6+ B cells mediated immune balance under auto-immune disease conditions.

Full Text

This preprint is available for download as a PDF.

Figures

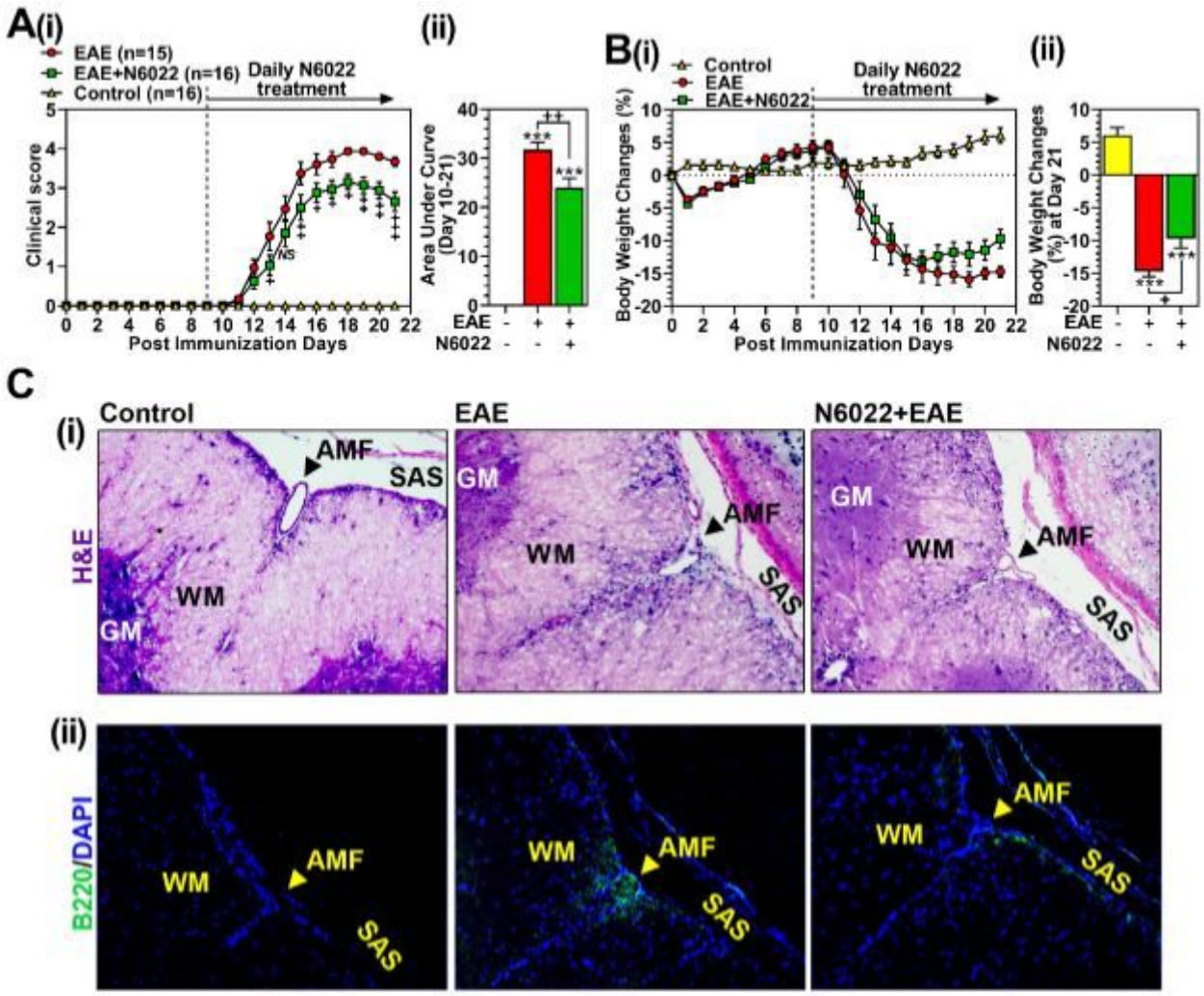

Figure 1

Effect of GSNOR inhibitor N6022 on EAE disease. C57BL/6 mice were immunized with MOG35-55 for the induction of EAE. EAE mice received daily doses of saline or N6022 (1 mg/kg/day/ip) starting before the onset of the disease (day 9 post-immunization). Daily clinical scores (A-i), area under each curve as a measure of quantitative clinical disease (A-ii), daily body weight changes (B-i), and statistic analysis of body weight changes at the day 21 postimmunization (B-ii) of control mice, EAE mice (treated with saline), and EAE mice treated with N6022 were analyzed. Data are expressed as mean ± standard deviation (SD). ** $p \leq 0.01$, *** $p \leq 0.001$ vs. control mice and + $p \leq 0.05$, ++ $p \leq 0.01$ vs. saline-treated EAE mice. At day 21 postimmunization, spinal lumbar cords were analyzed for mononuclear cell infiltration (H&E staining; C-i) and immunostaining for B220 (B cell marker; C-ii). AMF=anterior median fissure, GM=grey matter, SAS=subarachnoid space, WM=white matter. The n's represent the number of animals in each group.

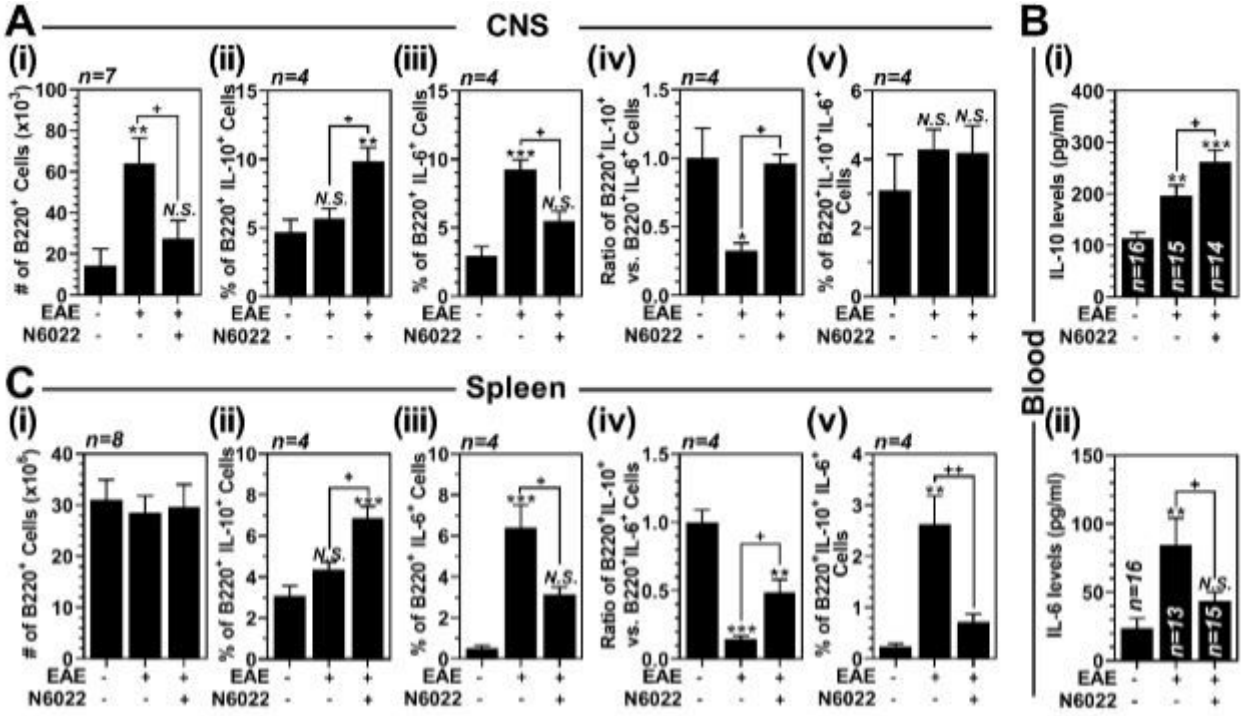

Figure 2

Effect of N6022 treatment on the B cell subsets expressing IL-10 and IL-6 in the CNS and spleen of EAE animals. C57BL/6 mice were immunized with MOG35-55 and receive daily doses of saline or N6022 (1 mg/kg/day/ip) starting on day 9 post-immunization. On the day 21 post-immunization, mononuclear and splenic cells were isolated from the CNS (A) and spleens (C) and the number of B220+ total B cells (A-i and C-i), IL-10+ B220+ B cells (A-ii and C-ii), IL-6+ B220+ B cells (A-iii and C-iii), the ratio of IL-10+ vs. IL-6+ B cells (A-iv and C-iv), and IL-10 and IL6 double-positive B220+ B cells (A-v and C-v) were analyzed by fluorescence flow cytometric analysis. The blood was collected on the same day and analyzed for blood levels of IL-10 (B-i) and IL-6 (B-ii). Data are expressed as mean ± standard deviation (SD); * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ vs. control mice; + $p \leq 0.05$, ++ $p \leq 0.01$ vs. saline-treated EAE mice. N.S.=not significant. The n's represent the number of samples in each group.

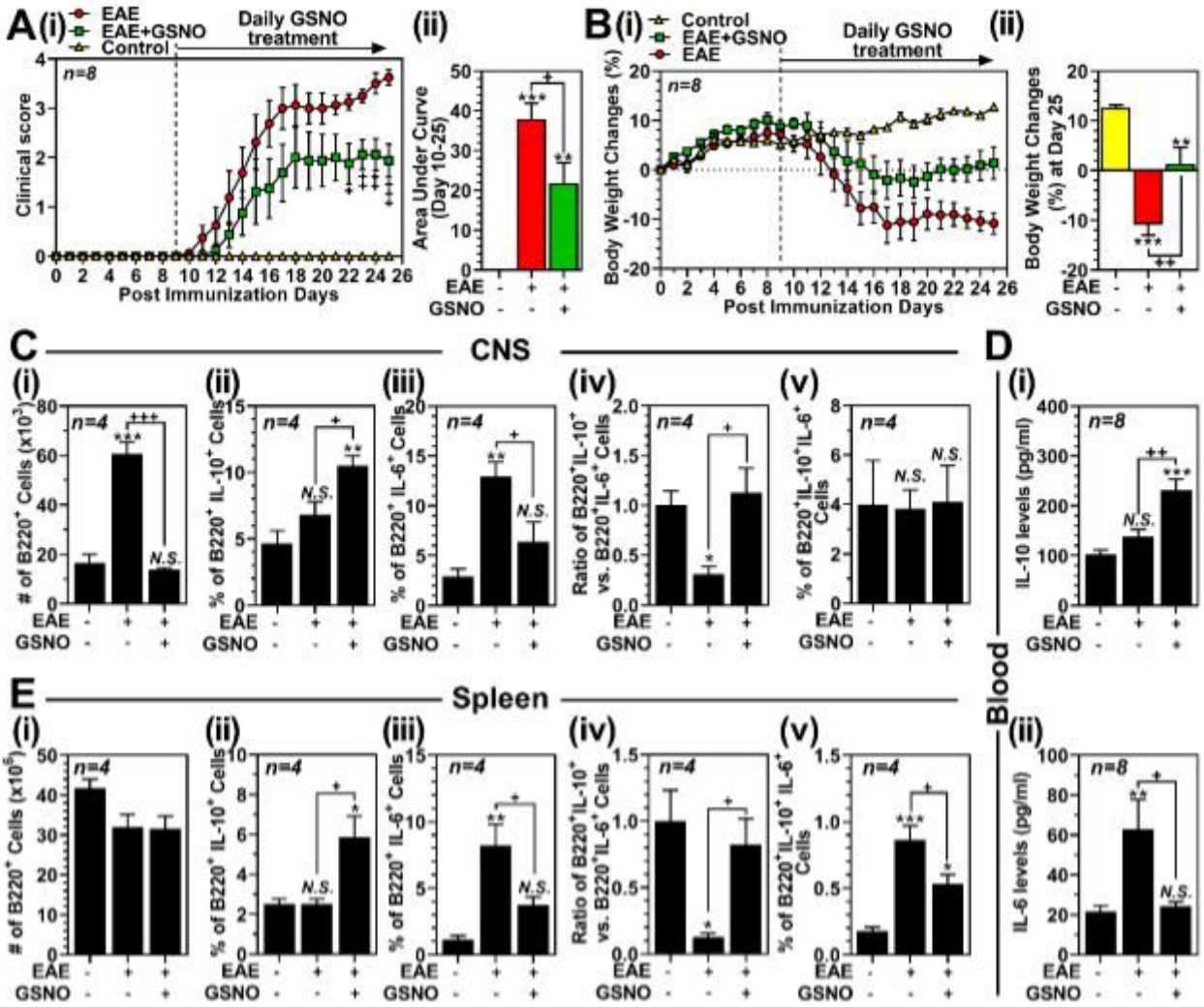

Figure 3

Effect of exogenous GSNO treatment on the B cell subsets expressing IL-10 and IL-6 in the CNS and spleen of EAE animals. C57BL/6 mice were immunized with MOG35-55 and receive daily doses of saline or GSNO (1 mg/kg/day/ip) starting on day 9 post-immunization. Daily clinical scores (A-i), area under each curve as a measure of quantitative clinical disease (A-ii), daily body weight changes (B-i), and statistic analysis of body weight changes at the day 25 postimmunization (B-ii) of control mice, EAE mice (treated with saline), and EAE mice treated with GSNO were analyzed. On the day 25 post-immunization, mononuclear and splenic cells were isolated from the CNS (C) and spleens (E) and the number of B220+ total B cells (A-i and C-i), IL10+ B220+ B cells (A-ii and C-ii), IL-6+ B220+ B cells (A-iii and C-iii), ratio of IL-10+ vs. IL-6+ B cells (A-iv and C-iv), and IL-10 and IL-6 double-positive B220+ B cells (A-v and C-v) were analyzed by fluorescence flow cytometric analysis. The blood was collected on the same day and analyzed for blood levels of IL-10 (D-i) and IL-6 (D-ii). Data are expressed as mean ± standard deviation (SD); * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ vs. control mice; + $p \leq 0.05$, ++ $p \leq 0.01$ vs. saline-treated EAE mice. N.S.=not significant. The n's represent the number of samples in each group.

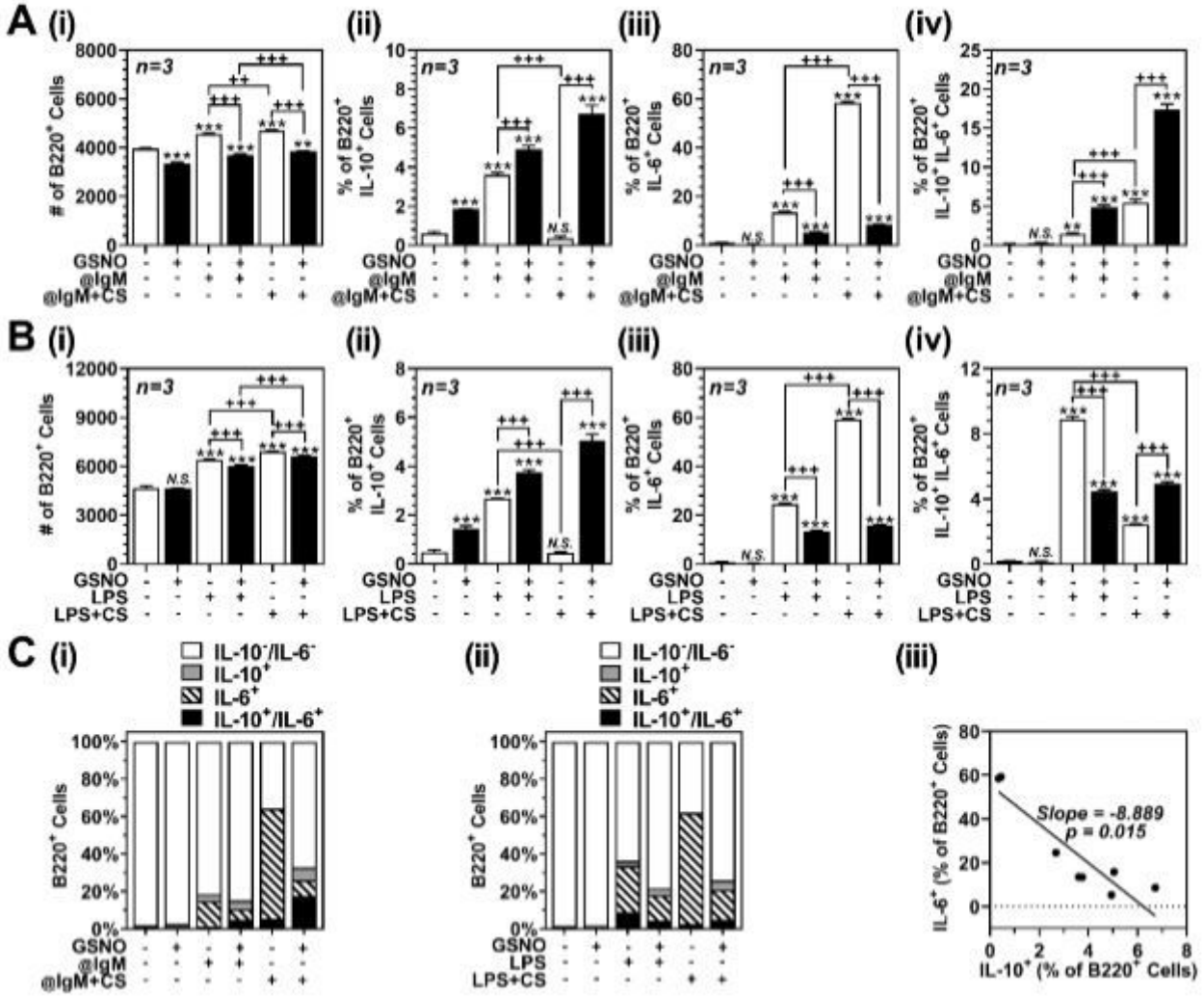

Figure 4

Effect of GSNO treatment on the B cell subsets expressing IL-10 and IL-6 under in vitro culture conditions. Naïve B cells were purified from C57BL/6 mice and stimulated with anti-IgM mAb (@IgM) or lipopolysaccharide (LPS) and co-stimulated (CS) with anti-CD40 mAb and BAFF as indicated and incubated for 48 hrs. One hour before the stimulation/co-stimulation, the cells were treated with vehicle or GSNO (50 μM). Following the incubation, numbers of B220+ total B cells (A-i and B-i), IL-10+ B220+ B cells (A-ii and B-ii), IL-6+ B220+ B cells (A-iii and B-iii), and IL-10 and IL-6 double-positive B220+ B cells (A-iv and B-iv) were analyzed by fluorescence flow cytometric analysis. Data are expressed as mean ± standard deviation (SD); ** $p \leq 0.01$, *** $p \leq 0.001$ vs. control; ++ $p \leq 0.01$, +++ $p \leq 0.001$ vs. as indicated. N.S.=not significant. The relative proportion of IL-10- IL-6-, IL-10+ IL-6-, IL-10- IL-6+, IL-10+ IL-6+ B cells were represented by stacked bar graph (C). Correlation between the numbers of IL-10+ vs. IL-6+ B cells was analyzed by nonlinear regression (curve fit) XY analysis (D). The n's represent the number of samples in each group. The cell culture experiments performed once using three different samples.

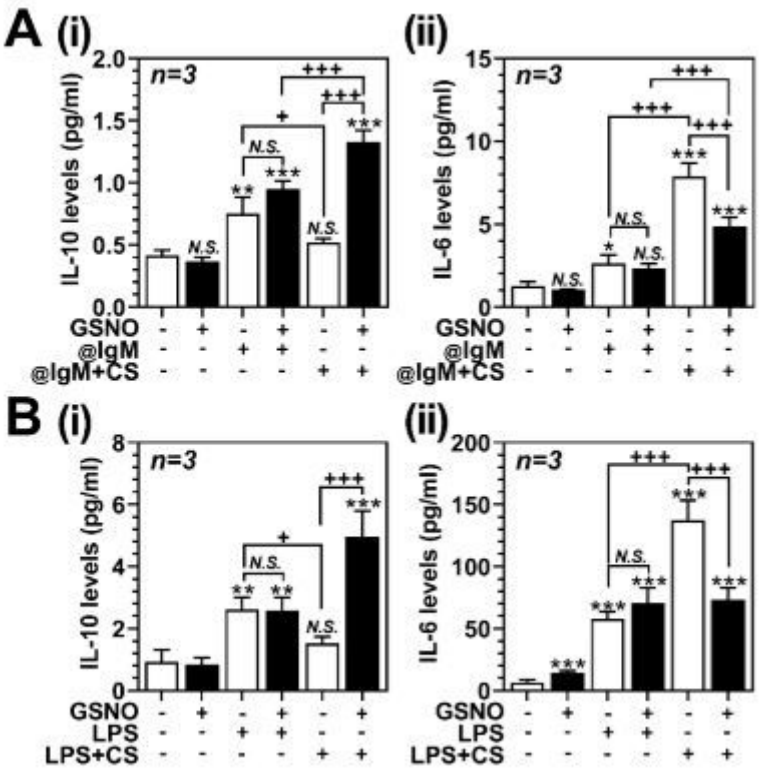

Figure 5

Effect of GSNO treatment on the B cell production of IL-10 and IL-6 under in vitro culture conditions. Naïve B cells were purified from C57BL/6 mice and stimulated with anti-IgM mAb (@IgM) or lipopolysaccharide (LPS) and co-stimulated (CS) with anti-CD40 mAb and BAFF as indicated and incubated for 48 hrs. One hour before the stimulation/co-stimulation, the cells were treated with vehicle or GSNO (50 μM). Following the incubation, B cell production of IL-10 (A-i and B-i) and IL-6 (A-ii and B-ii) was analyzed by ELISA using the culture media. Data are expressed as mean ± standard deviation (SD); ** $p \leq 0.01$, *** $p \leq 0.001$ vs. control; + $p \leq 0.05$, ++ $p \leq 0.01$, +++ $p \leq 0.001$ vs. as indicated. N.S.=not significant. The n's represent the number of samples in each group. The cell culture experiments performed once using three different samples.

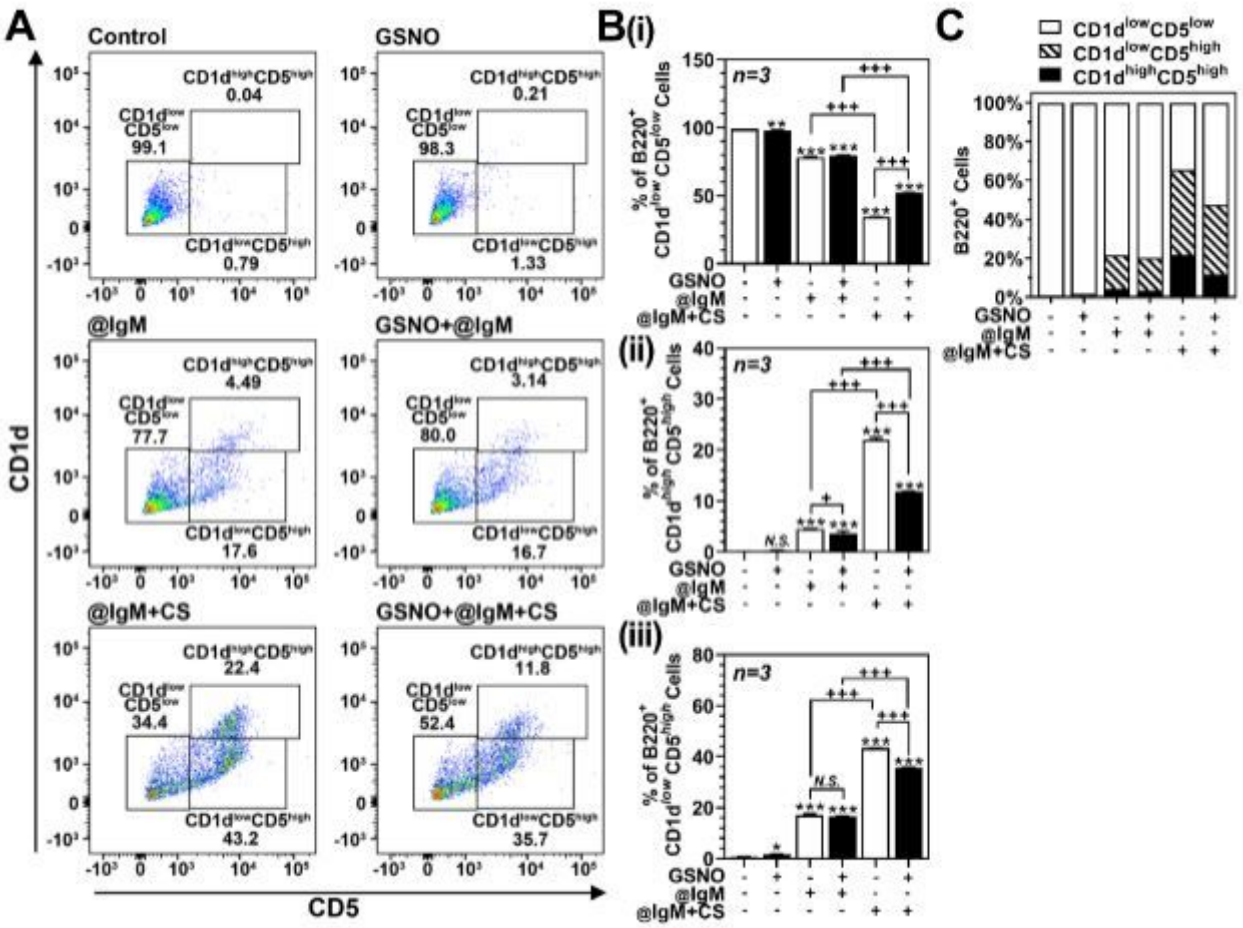

Figure 6

Effect of GSNO treatment on the expansion of CD1dhigh CD5high and CD1dlow CD5high B cell subsets under in vitro culture conditions stimulated with anti-IgM mAb. Naïve B cells were purified from C57BL/6 mice and stimulated with anti-IgM mAb (@IgM) and costimulated (CS) with anti-CD40 mAb and BAFF as indicated and incubated for 48 hrs. One hour before the stimulation/co-stimulation, the cells were treated with vehicle or GSNO (50 μM). Following the incubation, B cells were stained with antibodies specific to B220, CD1d, and CD5 and followed by fluorescence flow cytometric analysis. Following the gating of B220+ cells, distribution of CD1dlow CD5low, CD1dhigh CD5high, and CD1dlow CD5high B cells was represented by two-parametric scatter plots (A). In addition, percent of CD1dlow CD5low (B-i), CD1dhigh CD5high (Bii), and CD1dlow CD5high (B-iii) B cell numbers were represented by a bar graph. Data are expressed as mean ± standard deviation (SD); * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ vs. control; +++ $p \leq 0.001$ vs. as indicated. N.S.=not significant. The relative proportion of CD1dlow CD5low, CD1dhigh CD5high, and CD1dlow CD5high B cells was represented by a stacked bar graph (C). The n's represent the number of samples in each group. The cell culture experiments performed once using three different samples.

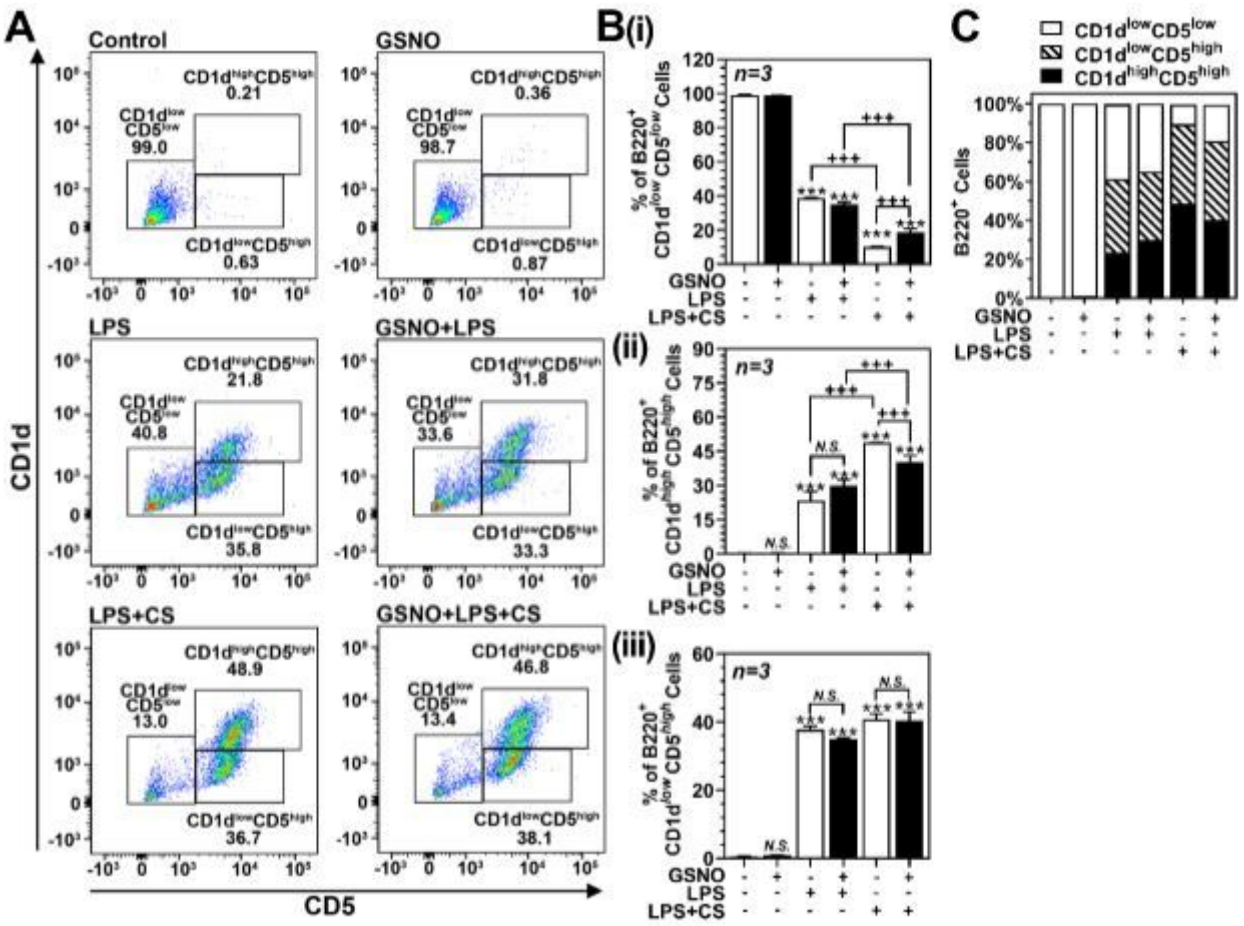

Figure 7

Effect of GSNO treatment on the expansion of CD1dhigh CD5high and CD1dlow CD5high B cell subsets under in vitro culture conditions stimulated with LPS. Naïve B cells were purified from C57BL/6 mice and stimulated with lipopolysaccharide (LPS) and co-stimulated (CS) with anti-CD40 mAb and BAFF as indicated and incubated for 48 hrs. One hour before the stimulation/co-stimulation, the cells were treated with vehicle or GSNO (50 μM). Following the incubation, B cells were stained with antibodies specific to B220, CD1d, and CD5 and followed by fluorescence flow cytometric analysis. Following the gating of B220+ cells, distribution of CD1dlow CD5low, CD1dhigh CD5high, and CD1dlow CD5high B cells was represented by two-parametric scatter plots (A). In addition, percent of CD1dlow CD5low (B-i), CD1dhigh CD5high (B-ii), and CD1dlow CD5high (B-iii) B cell numbers were represented by a bar graph. Data are expressed as mean ± standard deviation (SD); * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ vs. control; +++ $p \leq 0.001$ vs. as indicated. N.S.=not significant. The relative proportion of CD1dlow CD5low, CD1dhigh CD5high, and CD1dlow CD5high B cells was represented by a stacked bar graph (C). The n's represent the number of samples in each group. The cell culture experiments performed once using three different samples.

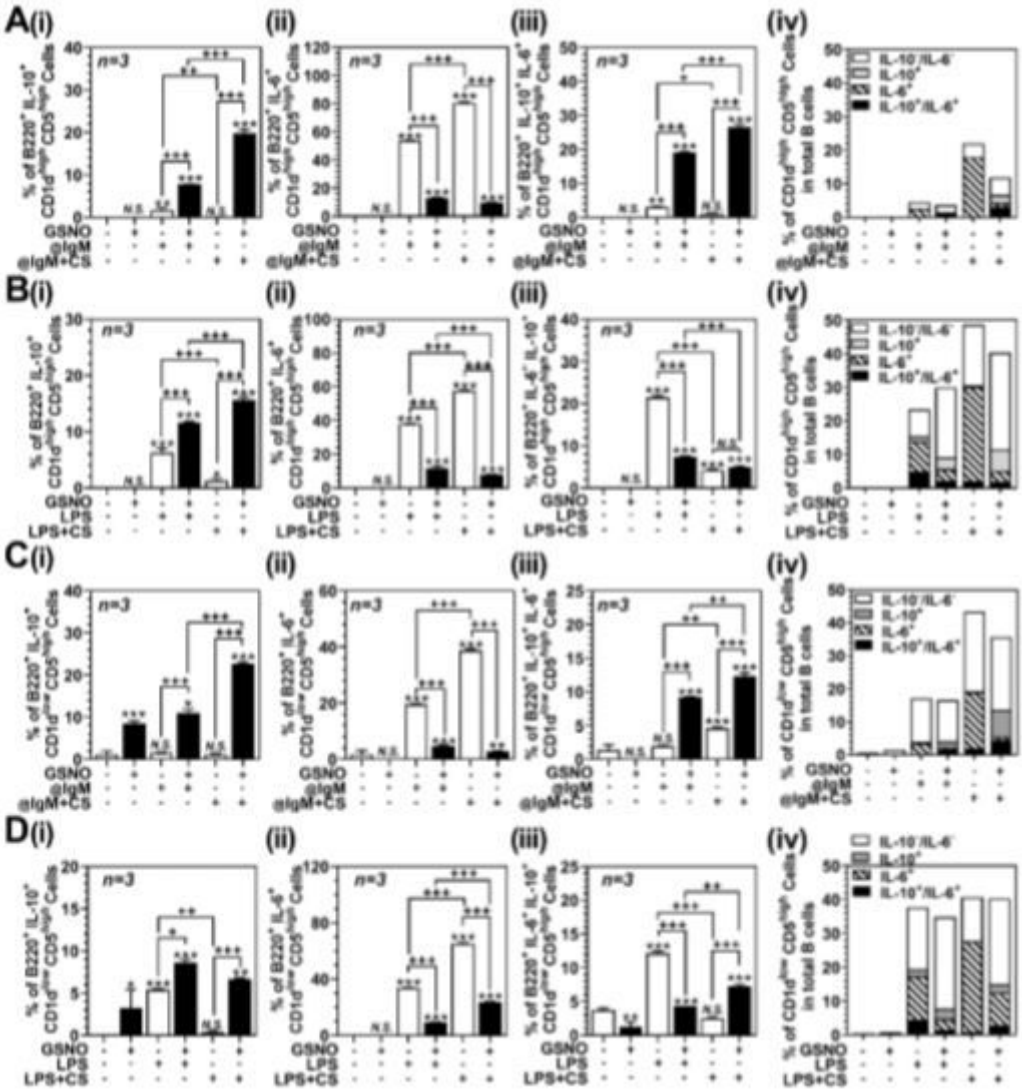

Figure 8

Effect of GSNO treatment on the expression of IL-10 and IL-6 by CD1dhigh CD5high and CD1dlow CD5high B cell subsets under in vitro culture conditions. Naïve B cells were purified from C57BL/6 mice and stimulated with anti-IgM mAb (@IgM) (A and C) or lipopolysaccharide (LPS) (B and D) and co-stimulated (CS) with anti-CD40 mAb and BAFF as indicated and incubated for 48 hrs. One hour before the stimulation/co-stimulation, the cells were treated with vehicle or GSNO (50 μM). Following the incubation, B cells were stained with antibodies specific to B220, CD1d, CD5, IL-10, and IL-6 and followed by fluorescence flow cytometric analysis. Following the serial gating of B220 and then CD1d/CD5, the proportion of IL10+ CD1dhigh CD5high (A-i and B-i), IL-6+ CD1dhigh CD5high (A-ii and B-ii), IL-10+ IL-6+ CD1dhigh CD5high (A-iii and B-iii), IL-10+ CD1dlow CD5high (C-i and D-i), IL-6+ CD1dlow CD5high (C-ii and D-ii), and IL-10+ IL-6+ CD1dlow CD5high (C-iii and D-iii) were represented bar graphs. In addition, their relative proportions were also represented by a stacked bar graph (A-iv, B-iv, C-iv, and D-iv). Data are expressed as mean ± standard deviation (SD); * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ vs. control; + $p \leq 0.05$, ++ $p \leq 0.01$, +++ $p \leq 0.001$ vs. as indicated. N.S.=not significant. The n's represent the number of samples in each group. The cell culture experiments performed once using three different samples.