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CD98 is a potential target for ablating B cell clonal expansion and autoantibody in multiple sclerosis

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Abstract

Current B cell-directed therapies for multiple sclerosis impact multiple B cell functions. CD98hc enables B cell clonal expansion and antibody production. I probed the relative importance of autoantibody secretion vs. other B cell functions in MS and targeted CD98hc as a possible therapeutic strategy. I report that loss of CD98hc function in B cells largely prevents autoantibody production while preserving antigen-presenting and T cell-directing capacities. Mice lacking CD98hc in B cells are protected from EAE; importantly this is overcome with autoantibody-containing plasma. Thus CD98hc blockade is a possible avenue to treat MS by inhibiting clonal expansion and autoantibody.

Keywords

CD98; EAE; B cell; Clonal Expansion; Autoantibody

Introduction

Multiple sclerosis (MS) patients that do not respond well to interferon and other first-line disease-modifying therapies (DMT) have an urgent need for new immune treatments. Recent studies using a pan-CD20+ B cell-depleting antibody (Rituxan) show that immune therapies directed against B cells can be effective for multiple sclerosis, although additional autoimmunity and PML risk are current concerns (Berger and Koralnik, 2005, Buttmann, 2010, Fontoura, 2010, Hauser et al., 2008). B cells are important in the pathogenesis of multiple sclerosis, but in their multiple roles may both contribute, and/or protect, from disease (Cross and Waubant, 2011, Mann et al., 2012). Initially they direct helper T cell responses by presenting antigen (APC function), controlling Treg numbers, and guiding effector T cell differentiation (Ray et al., 2012, Ray et al., 2011, von Budingen et al., 2011). Following clonal expansion, B cells differentiate into plasma cells, the major source of both protective and auto-antibodies. I previously described a protein, CD98 heavy chain

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(CD98hc) (Cantor and Ginsberg, 2012), which controls clonal expansion of B cells and their subsequent differentiation to plasma cells (Cantor et al., 2009). Genetic deletion of CD98hc blocks plasma cell formation, but maintains B cell numbers and preserves their ability to become activated and function as APC (Cantor et al., 2009). Thus I hypothesized that targeting CD98hc on B cells might selectively impair expansion-dependent B cell functions and delay or prevent experimental MS. Mice deficient for CD98hc on B cells showed significant protection from experimental autoimmune encephalomyelitis (EAE) induced with recombinant MOG (rMOG). Anti-MOG autoantibody levels were drastically reduced; however, B cell steady-state functions were preserved. Importantly, anti-MOG-containing mouse plasma could reconstitute disease in recipients lacking B cell CD98hc, supporting the importance of B cell autoantibody in EAE pathogenesis. Thus, I provide a new model to test the role of steady-state vs. clonally-expanded B cell functions in multiple sclerosis (MS), and reveal a new potential target for therapeutic blockade.

Methods

Mice and Antigens

Slc3a2^{f/f}, *CD19-Cre*⁺ mice were generated as previously described (Cantor et al, 2009, Feral et al., 2005). Littermate control *Slc3a2^{f/f}* mice were used whenever possible. OT-2 mice (Barnden et al., 1998) were purchased from Jackson Labs. All mice were housed and treated according to approved Institutional Animal Care and Use Committee (IACUC) guidelines. MOG₃₃₋₅₅ was purchased from Anaspec, and recombinant human MOG was a gift from Dr. Claude Bernard.

Standard EAE model

EAE was induced according to an established protocol (Stromnes and Goverman, 2006). Briefly, either MOG₃₃₋₅₅ (100 µg) or recombinant extracellular domain of human MOG protein (100 µg) emulsified in Complete Freund's Adjuvant (CFA) was injected s.c., with pertussis toxin i.v. injections (250ng) on days 0 and 3. Mice were clinically scored (0-5 scale) and weighed daily. According to IACUC regulations, any mice with clinical scores of 3 or more for >48h were euthanized. These scores were retained in calculating subsequent means.

Antigen Presentation Assay

Resting B cells were purified from spleens of *Slc3a2^{f/f}*, *CD19-Cre*⁺ or control *Slc3a2^{f/f}* littermate mice by negative depletion using magnetic beads (Dyna), with the addition of anti-CD98 antibody to deplete the few (~10-15%) CD98⁺ B cells in *Slc3a2^{f/f}*, *CD19-Cre*⁺ mouse samples (Cantor et al, 2009). B cells were cultured with 2.5 mg/ml ovalbumin and carboxyfluorescein succinimidyl ester (CFSE)-labelled resting OT-2 T cells purified from spleens of OT-2 mice by negative depletion (Dyna). After 3 days of co-culture, cells were harvested, stained for CD3⁺ T cells, and analyzed by flow cytometry.

Analysis of T helper subsets during EAE

EAE was induced as above and mice were sacrificed on day 12 or 14. Single-cell suspensions from spleens or collagenase-digested (Type IA, from Sigma) spinal cords were

cultured for 3h with PMA and Ionomycin in the presence of Brefeldin A (Biolegend). After surface staining using anti-CD4 antibody, cells were fixed and permeabilized using CytoFix/Perm kit (eBioscience), stained for intracellular FoxP3 (Treg), TNF- α , and IL-17A (Th17), and analyzed by flow cytometry.

Assessment and transfer of anti-MOG plasma

Mice were immunized with recombinant MOG according to the standard EAE induction protocol above. Plasma was obtained from blood collected before and 14 days after immunization. Plasma anti-MOG antibody levels in individual mice were tested by direct ELISA on plates coated with recombinant human MOG or control (ovalbumin) protein. O.D. values were compared to a standard curve generated from pooled *Slc3a2^{f/f}* plasma with undiluted pooled *Slc3a2^{f/f}* plasma set at a numerical value of 1 to yield relative antibody concentrations shown in figure 2A. Raw (blank-subtracted) O.D. values were used for figure S2. For plasma transfer experiments, EAE was induced using recombinant MOG in *Slc3a2^{f/f}*, *CD19-Cre+* and *Slc3a2^{f/f}* control mice according to the standard protocol, with injections of pooled *Slc3a2^{f/f}* plasma (125 μ l) i.p. on days 3, 6, 9, and 12.

Statistical Analysis

Mann-Whitney non-parametric statistical test was used for all comparisons. P-values are indicated in legends. $p < 0.05$ was considered significant.

Results

I previously demonstrated that CD98 heavy chain (CD98hc, encoded by the mouse *Slc3a2* gene), was required for clonal expansion of mouse B cells (Cantor et al, 2009). In order to investigate the role of B cell clonal expansion in development of MS, I experimentally induced autoimmune encephalomyelitis (EAE) in these mice with recombinant MOG (rMOG), a protocol that models the involvement of B cells (Stromnes and Goverman, 2006). Mice genetically lacking CD98 on B cells (*Slc3a2^{f/f}*, *CD19-Cre+*) developed significantly delayed and less severe rMOG-induced EAE than littermate *Slc3a2^{f/f}* controls (Fig 1A). However, MOG33-55 peptide-induced (B cell independent) disease occurred with similar kinetics and severity in *Slc3a2^{f/f}*, *CD19-Cre+* as *Slc3a2^{f/f}* controls (Fig 1B). Thus the protection from rMOG-induced disease in *Slc3a2^{f/f}*, *CD19-Cre+* mice shows that CD98hc is required on B cells for full development of EAE.

B cells have several functions in the immune response: acting as antigen-presentation cells (APC), immunomodulatory cells, and finally as antibody-secreting effector B cells. In order to narrow down the role of CD98hc on B cells in EAE, I tested the competence of CD98-null B cells in these tasks. CD98hc was recently reported necessary for macrophage phagocytosis and antigen-presentation (Tsumura et al., 2012). I thus tested whether CD98-null B cells could phagocytose, process, and present antigen to CD4 T cells using OT-2 T cell receptor transgenic T cells (Barnden et al, 1998), which respond to a class-II MHC immunodominant peptide from ovalbumin (OVA₃₂₃₋₃₃₉). CFSE-labeled OT-2 T cells divided vigorously in response to either CD98-null or CD98+ B cells and ovalbumin protein, whereas little or T cell proliferative response was observed in the absence of B cells

(Fig. 2A). These results show that CD98hc is not required on B cells for their ability to process and present antigen to T cells. Thus, protection from EAE in *Slc3a2^{ff}*, *CD19-Cre* mice cannot be attributed solely to a defect in antigen-presentation capacity.

B cells also regulate cell-mediated autoimmune responses by directing the differentiation of antigen-specific T cells (von Budingen et al, 2011, Weber and Hemmer, 2010). It is therefore possible that loss of B cell CD98hc protects from EAE by skewing the responding T cells to more regulatory (Treg) and less inflammatory types (Th17). I investigated this hypothesis by testing whether effector CD4⁺ T cell subsets exhibited a different distribution pattern in *Slc3a2^{ff}*, *CD19-Cre* mice compared with control in *Slc3a2^{ff}* littermates after induction of EAE with rMOG. At the peak of disease, I performed intracellular cytokine staining on CD4⁺ T cells to determine T helper subset distribution. Splenic T cells from *Slc3a2^{ff}*, *CD19-Cre* and control *Slc3a2^{ff}* littermates showed similar percentages of T cells that were positive for IL-17(Th17) or for Foxp3 (Treg) (Fig. 2B). The percentage of CD4⁺ T cells producing TNF- α , the only cytokine I could clearly detect in intrathecal T cells, was also similar between *Slc3a2^{ff}*, *CD19-Cre* mice and control *Slc3a2^{ff}* littermates (Fig. S1). Thus CD98hc-null B cells do not appear to skew T helper subset differentiation toward inflammatory subsets, which indicates that protection from rMOG EAE in *Slc3a2^{ff}*, *CD19-Cre* mice is not due to Th subset skewing.

In contrast to antigen-presenting and T cell-directing functions of B cells, secretion of high-affinity class-switched antibody is dependent on clonal expansion. I previously reported that CD98hc is required for B cell clonal expansion and production of antigen-specific antibody (Cantor et al, 2009), so a 3rd possible mechanism whereby loss of CD98 results in protection from EAE is loss of clonal expansion-dependent B cell effector functions. There is substantial data supporting the importance of autoantibody in the EAE model (Lyons et al., 2002, Raine et al., 1999), and evidence suggests roles for both anti-CNS autoantibody (Genain et al., 1999, Keegan et al., 2005, McLaughlin and Wucherpfennig, 2008, O'Connor et al., 2005, Pedotti et al., 2013, Zhou et al., 2006) and antibody-independent B cell functions (Cross et al., 2012, Cross et al., 2006, Hauser et al, 2008, Kappos et al., 2011, Naismith et al., 2010, Piccio et al., 2010) in the pathogenesis of human MS. To determine the importance of CD98hc in secretion of class-switched anti-CNS autoantibody, I measured the levels of anti-MOG IgG in *Slc3a2^{ff}*, *CD19-Cre* and in *Slc3a2^{ff}* control mice before and 2 weeks after disease induction with rMOG. *Slc3a2^{ff}*, *CD19-Cre* mice had dramatically reduced concentrations of anti-MOG IgG in blood plasma relative to *Slc3a2^{ff}* controls (Fig. 3A, S2). This data suggests that loss of CD98 on B cells ablates production of anti-MOG autoantibody, which could explain the protection of *Slc3a2^{ff}*, *CD19-Cre* mice from EAE. I next tested directly whether *Slc3a2^{ff}*, *CD19-Cre* mice are protected from EAE by a lack of pathogenic autoantibody. I induced EAE in *Slc3a2^{ff}*, *CD19-Cre* and *Slc3a2^{ff}* controls using rMOG as before. On days 3, 6, 9, and 12 after induction, I transferred pooled plasma from *Slc3a2^{ff}* mice i.v into *Slc3a2^{ff}*, *CD19-Cre* EAE-induced mice. Serum from *Slc3a2^{ff}* mice was able to overcome the protection from EAE present in *Slc3a2^{ff}*, *CD19-Cre* mice (Fig. 3B). Taken together, these data demonstrate that CD98hc is required for full development of EAE due to its ability to enable B cell clonal expansion and allow production of anti-CNS autoantibody.

Discussion

Targeting specific B cell functions that contribute to disease pathogenesis is critical to improving B cell-directed therapies for multiple sclerosis. Using specific genetic deletion in mice, I found that CD98hc-dependent B cell clonal expansion and autoantibody production are crucial steps in the MOG-induced EAE model of MS. Loss of B cell CD98hc resulted in significant protection from rMOG-induced EAE, identifying this protein as a potential therapeutic target. This potential is enhanced by the maintenance of other B cell functions such as antigen presentation and modulation of T cell responses. These B cell functions could be beneficial in maintaining protective immunity and anti-inflammatory immune regulation. Approx. 10-15% of B cells in *Slc3a2^{ff}*, *CD19-Cre* mice are CD98+ and have a strong selective advantage (Cantor et al, 2009) that could explain the presence of some pathology in *Slc3a2^{ff}*, *CD19-Cre* mice. Another possibility might be that the other B cell functions contribute significantly to disease, as recent analysis of promising anti-CD20 monoclonal antibody studies in MS patients indicates (Cross et al, 2012, Cross et al, 2006, Hauser et al, 2008, Kappos et al, 2011, Piccio et al, 2010) In either case, inhibition of clonal expansion without perturbing B steady-state functions represents a highly focused and novel therapeutic approach for inflammatory disease that may benefit a subset of MS patients with antibody-dependent disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights for “CD98 is a potential target for ablating B cell clonal expansion and autoantibody in MS”

- CD98 is required on B cells for efficient induction of MOG-induced EAE.
- Loss of B cell CD98 blocks autoantibody production after immunization with MOG.
- Targeting CD98 does not appear to affect steady-state B cell functions.
- CD98 is a potential therapeutic target in the antibody-dependent subset of MS.

Figure 1.

EAE incidence and severity in the *Slc3a2^{ff}*, *CD19-Cre* mouse. (A) *rMOG-induced EAE*.

Experimental Autoimmune Encephalomyelitis (EAE) was induced in *Slc3a2^{ff}*, *CD19-Cre* and control *Slc3a2^{ff}* mice by subcutaneous immunization with recombinant MOG protein (100μg) and i.v. injection of pertussis toxin on days 0 and 3. Mice were monitored daily for weight loss and progressive paralysis/loss of motor control, graded on a 0-5 scale. Error bars represent S.E.M. for the indicated group sizes. Experiment was repeated 3 times. (B) *MOG₃₃₋₅₅ peptide-induced EAE*. EAE was induced as in (A), except with MOG₃₃₋₅₅ peptide. No significant differences in disease were observed between *Slc3a2^{ff}*, *CD19-Cre* and control *Slc3a2^{ff}* mice. * $p < 0.05$, ** $p < 0.025$

Figure 2.

Antigen presentation and regulation of T cell responses by CD98-null B cells. **(A)** Antigen presentation by CD98-null B cells. B cells were purified from spleens of *Slc3a2^{ff}*, *CD19-Cre* or control littermate *Slc3a2^{ff}* mice and cultured with ovalbumin and CFSE-labeled OT-2 responder T cells. After 3 days, cultures were harvested and stained using fluorochrome-conjugated anti-CD3 antibody and analyzed for proliferation (CFSE dye dilution) by flow cytometry. Unfilled overlaid peaks are cultures lacking B cells. **(B)** B cell regulation of anti-MOG T cell response. EAE was induced with recombinant MOG and pertussis toxin. Mice were sacrificed at the peak of disease (day 14); single-cell suspensions from excised spleens were cultured in the presence of PMA/Ionomycin for 3-4 hours and stained for surface CD4 and intracellular IL-17 and Foxp3. Representative dot plots are shown above and data are summarized by bar graphs underneath. Error bars depict S.E.M. of *n*=4 mice per group. No statistically significant differences were observed. Experiment was performed twice.

Figure 3.

B cell effector function in *Slc3a2^{ff}*, *CD19-Cre* mice. **(A)** Anti-MOG antibody levels in *Slc3a2^{ff}*, *CD19-Cre* mice. *Slc3a2^{ff}*, *CD19-Cre* and control *Slc3a2^{ff}* mice were immunized with recombinant MOG protein as for EAE induction. Blood was collected before immunization/induction and on day 14. Relative anti-MOG antibody levels in plasma were determined by direct ELISA. Experiment was repeated twice. **(B)** Disease complementation by plasma transfer. Pooled sera from *Slc3a2^{ff}* samples in **(A)** were administered i.p. (125 μ l) on days 3, 6, 9, and 12. Mice were monitored daily for clinical signs of disease. Bar graph summarizes mean clinical scores for each group at the peak of disease (day 17). Error bars represent S.E.M. for the indicated group sizes. * $p < 0.015$, ** $p < 0.05$, *** $p < 0.02$