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Siglec-G Deficiency Leads to Autoimmunity in Aging C57BL/6 Mice

Jennifer Müller,* Benjamin Lunz,* Inessa Schwab,* Andreas Acs,* Falk Nimmerjahn,* Christoph Daniel,† and Lars Nitschke*

Siglec-G, a member of the sialic acid–binding Ig-like lectin (Siglec) family, is expressed on B cell and dendritic cell surfaces. It acts as an inhibitory coreceptor and modulates B cell activation, especially on B1 cells, as Siglec-G–deficient mice show mainly a B1 cell–restricted phenotype resulting in increased B1 cell numbers. Although higher B1 cell numbers are discussed to be associated with autoimmunity, loss of Siglec-G does not result in autoimmune disease in BALB/c mice. However, there is evidence from Siglec-G × CD22 double-deficient mice and Siglec-G<sup>−/−</sup> mice on an autoimmune-prone MRL/lpr background that Siglec-G is important to maintain tolerance in B cells. In this study, we analyzed the role of Siglec-G in induction and maintenance of B cell tolerance on C57BL/6 background and in the FcγRIIb-deficient background. We find that aging Siglec-G–deficient and Siglec-G × FcγRIIb double-deficient mice develop an autoimmune phenotype with elevated autoantibody levels and mild glomerulonephritis. Aging Siglec-G–deficient mice have elevated numbers of plasma cells and germinal center B cells, as well as a higher number of activated CD4<sup>+</sup> T cells, which likely all contribute to autoantibody production. Additional loss of the inhibitory receptor FcγRIIb in Siglec-G<sup>−/−</sup> mice does not result in exacerbation of disease. These results indicate that Siglec-G is important to maintain tolerance in B cells and prevent autoimmunity.

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A ctivation of B cells and Ab production need to be tightly regulated to prevent the generation of autoantibodies during immune responses. There are several tolerance mechanisms that sense autoreactive B cells, deplete them from bone marrow or the periphery, and protect from autoimmune diseases. The most important mechanisms of central B cell tolerance, that is, receptor editing, B cell depletion, and anergy induction, are dependent on BCR signaling strength after self-antigen encounter (1).

ITIM-containing inhibitory receptors on B cells are essential for down-modulation of the BCR signal and are therefore key regulators for the maintenance of B cell tolerance (2). Ag binding by the BCR induces phosphorylation of ITIMs on inhibitory receptors by the tyrosine kinase Lyn and recruitment of the negative regulator of BCR calcium (Ca<sup>2+</sup>) signaling, the Src homology region 2 domain–containing phosphatase (SHP)-1 (3). Deficiencies in inhibitory receptors, such as CD72, PIR-B, or FcγRIIb, can lead to autoimmunity and to systemic lupus erythematosus (SLE)–like disease in mice (4–6). Additionally, the loss of the downstream signaling molecules Lyn or SHP-1 results in strongly elevated BCR signaling and development of autoreactive B cells (7, 8). In contrast, deficiency in the sialic acid–binding Ig-like lectin (Siglec) family members CD22 (on C57BL/6 [B6] background) or Siglec-G (on BALB/c background), which are two other important inhibitory receptors on B cells, does not lead to autoimmunity in mice (9–12). There appears to be a functional redundancy of both molecules for B cell tolerance induction, as Siglec-G × CD22 double-deficient mice do show an autoimmune phenotype at old age with high levels of IgG autoantibodies (with specificities including ssDNA, dsDNA, and RNA) and development of glomerulonephritis (13). However, also the single loss of one of these SiglecS can contribute to development of autoimmunity, as Siglec-G<sup>−/−</sup> mice crossed to the autoimmune prone MRL/lpr background or CD22<sup>−/−</sup> mice crossed to the Y chromosome–linked autoimmune accelerator (yaa) background show an autoimmune phenotype (14, 15). Furthermore, murine B cells lacking the Siglec ligand–modifying enzyme sialic acid acetyleralase, which affects CD22 and Siglec-G sialic acid binding, develop a lupus-like autoimmunity disease (16). Defective sialic acid acetyleralase function is also genetically linked to a variety of human autoimmune diseases (17).

Siglec-G (or the human ortholog Siglec-10) is constitutively expressed on B cell surfaces and acts as an inhibitory coreceptor of the BCR by negatively modulating its Ca<sup>2+</sup> response (9). The extracellular ligand-binding domain of the transmembrane protein Siglec-G recognizes exclusively α2,3- and α2,6-linked sialic acids, terminal carbohydrates abundantly expressed in higher vertebrates and found on many soluble and cell surface–bound glycoproteins (18). The cytoplasmic signaling domain of Siglec-G carries an ITIM- and an ITIM-like motif. For Siglec-G, it was shown that it binds and activates the phosphatase SHP-1, whereas Siglec-10 binds both SHP-1 and SHP-2 phosphatases. These tyrosine phosphatases can dephosphorylate positive regulators of the BCR signaling cascade and cause a dampening of the BCR signal (19, 20). Although Siglec-G is expressed on all B cell subsets (19, 21),
deficiency of Siglec-G in mice causes a strong enlargement of the B1 cell population, but not of B2 cells, indicating that Siglec-G has mainly a B1 cell–restricted inhibitory function. Additionally, Siglec-G–deficient B cells are hyperreactive and exhibit a highly elevated Ca\(^{2+}\) flux as well as a prolonged live span only in B1 but not in B2 cells (9). Recently it was demonstrated that the binding of Siglec-G to its ligand is crucial for its inhibitory function, as the Siglec-G–IgM association was ablated on B1 cells expressing Siglec-G with a mutated ligand-binding domain. B1 cell surfaces show a higher expression of α2,3-linked sialic acids, which is the preferential ligand for Siglec-G. It is therefore likely that the differential abundance of Siglec-G ligands on B1 and B2 cells leads to this specific phenotype in Siglec-G–deficient mice (21). FcγRIIB, an inhibitory IgG-binding Fc receptor, which is constitutively expressed on B cells, has a variety of functions in humoral immunity. In contrast to BCR stimulation alone, coligation of FcγRIIB and the BCR by IgG immune complexes, consisting of IgG and Ag, reduces Ca\(^{2+}\) signaling and proliferation in B cells (22). Crosslinking of FcγRIIB with the BCR induces phosphorylation of the intracellular ITIM and recruitment of SHIP-1 or SHIP-2, which then inhibit positive regulators of BCR signaling (23). On plasma cells, which do not express BCR molecules anymore, crosslinking of FcγRIIB by immune complexes induces apoptosis through an proapoptotic pathway, which does not require ITIMs or SHIP-1 (24-26). Because IgG is the major Ab isotype responsible for tissue inflammation during autoimmune diseases, FcγRIIB function is highly important for the inhibition of IgG-mediated activation of B cells and prevention of autoimmune production by B cells (27). FcγRIIB-deficient mice on a mixed 129Sv/B6 background (FcγRIIB\(^{129/2}\)) develop severe, lethal lupus with high titers of IgG autoantibodies, whereas FcγRIIB-deficient mice on a pure B6 background (FcγRIIB\(^{b6/2}\)) exhibit a hyperactive phenotype, but no lupus-like autoimmune disease (4, 28). The FcγRIIB gene, located on chromosome 1 of the mouse genome, is next to the Sle1b locus, which is associated with a strong autoimmune phenotype in mixed 129/B6 backgrounds (29). Within this locus the SLAM cluster, consisting of genes for stimulatory and inhibitory signaling molecules, was identified to cause autoimmunity in mixed strains. The fact that the 129Sv-derived Sle1b haplotype causes loss of tolerance in combination with the B6 genome explains the much stronger autoimmune phenotype of FcγRIIB\(^{129/2}\) mice compared with FcγRIIB\(^{b6/2}\) mice. Although FcγRIIB deficiency does not result in autoimmunity, it could be demonstrated that loss of FcγRIIB in mice amplifies spontaneous autoimmunity in the context of autoimmune susceptibility loci, such as yaa or Sle1b (28). In humans, both a FcγRIIB promoter polymorphism and an allelic FcγRIIB I1232V variant are associated with a higher risk of SLE development (30-32).

Siglec-G deficiency on the BALB/c background does not result in autoimmune disease (9). In this study we addressed the question of whether Siglec-G deficiency in a B6 genome can lead to a break of tolerance and to development of spontaneous autoimmunity. We therefore backcrossed Siglec-G–deficient BALB/c mice to the B6 genome (Siglec-G\(^{b6/2}\)) and tested for the development of autoimmune disease both in the Siglec-G–deficient as well as in the FcγRIIB\(^{b6/2}\) × Siglec-G\(^{b6/2}\) double-deficient background. Our results show that the loss of Siglec-G in the B6 genome causes a break in tolerance with or without deficiency of FcγRIIB when compared with wild-type (WT) littermate controls. Siglec-G–deficient mice develop high autoantibody titers and mild glomerular kidney damage. However, the combined loss of both inhibitory receptors does not lead to a stronger autoimmune phenotype compared with Siglec-G deficiency alone.

**Materials and Methods**

**Mice**

Siglec-G–deficient BALB/c mice (9) were backcrossed to the B6 background (Siglec-G\(^{b6/2}\)) by marker-assisted speed congenics (tested in the N\(_2\) and N\(_3\) generations), followed by two random backcrosses to the B6 background. The resulting Siglec-G–deficient generation (N\(_5\)) was crossed to FcγRIIB–deficient mice, which were derived from B6 embryonic stem cells (33) (all mice were then an N\(_6\) backcross to B6). These mice were then intercrossed to obtain Siglec-G\(^{−/−}\), FcγRIIB\(^{−/−}\), Siglec-G\(^{−/−}\) × FcγRIIB\(^{−/−}\) double-deficient, or control mice. To determine the backcrossing efficiency, eight mice of all genotypes from the intercrossed N\(_6\) generation were tested with 69 genomic single nucleotide polymorphism (SNP) markers (LGC Genomics, KASP genotyping) covering all mouse chromosomes. This analysis showed that the intercrossed N\(_6\) animals had on average 94.6% B6 homozygosity. WT controls were littermates of heterozygous breedings or age-matched from the same (N\(_6\)) background. Animal experiments were approved by a local ethics committee (Regierung Mittelfranken).

**ELISA**

We measured Ig serum levels from naive mice by standard ELISA methods (34). Sera and standard were applied to the plates in serial 1:3 dilutions in 1% (w/v) BSA, 0.05% (w/v) sodium azide in PBS and incubated overnight at \(^\circ\)C. Isotype-specific Abs coupled to alkaline phosphatase (AP; SouthernBiotech, Birmingham, AL) were used for detection. Plates were measured at 405-nm wavelength in the ELISA reader (VersaMaxPLUS) and analyzed using Softmax Pro software. The OD values of the serial dilutions were fit to a curve using the four-parameter fit.

**Serum Ig level.** To determine the total Ig titer in sera of naive mice, 96-well PolySorp ELISA plates (Nunc) were coated with 1 µg/ml goat anti-mouse isotype-specific Abs (SouthernBiotech). Mouse Ig isotype mAb (1 µg/ml) served as standard (SouthernBiotech). Goat anti-mouse secondary AP-conjugated Ab (1 µg/ml) was used for detection.

**Anti-DNA Ab titer.** For detection of dsDNA and ssDNA total IgG titers, MaxiSorp plates were precoated with 0.01% poly-L-lysine diluted in H\(_2\)O. Precoated plates were coated with 20 µg/ml calf thymus dsDNA or ssDNA. ssDNA was obtained by incubation of dsDNA at 98 °C for 25 min. A sera pool of aged MRL/lpr mice served as standard. Goat-anti-mouse IgG (1 µg/ml) coupled to AP was used for detection.

**Rheumatoid factor IgM.** For detection of rheumatoid factor IgM titers, MaxiSorp plates were coated with 10 µg/ml purified rabbit IgG (Sigma-Aldrich). A sera pool of aged MRL/lpr mice served as standard. Goat-animal IgG (1 µg/ml) coupled to AP was used for detection.

**Anti-phosphorylcholine Abs.** For detection of anti-phosphorylcholine (PC) total IgG and IgM titers, MaxiSorp plates were coated with 10 µg/ml PC-BSA-FITC (Biosearch Technologies) in PBS. A sera pool of aged MRL/lpr mice served as standard. Goat-anti-mouse IgG or IgM (1 µg/ml) coupled to AP was used for detection.

**ELISPOT**

Total IgG- or IgM-secreting cells were determined by ELISPOT assay. Ninety-six–well MaxiSorp plates were coated with goat anti-mouse IgG or IgG and saturated with 1% gelatin in PBS. Bone marrow or spleen cells (1 × 10\(^6\)) were 1:3 serially diluted and cultured overnight at 37 °C/5% CO\(_2\). Anti-nuclear Abs (ANAs) were detected by the use of ImmuGlo ANA HEp-2 substrate (Immco Diagnostics) according to the manufacturer’s instructions. Slides were incubated with 1:300 diluted sera for 30 min at room temperature. Anti-nuclear total IgG was detected with Alexa Fluor 488–conjugated rabbit anti-mouse IgG (Invitrogen). The slides were analyzed with a ×10 magnification on a fluorescence microscope. Fluorescence intensity of cells compared with a standard of pooled MRL/lpr serum was determined with ImageJ64.

**Blood urea nitrogen**

An enzymatic blood urea nitrogen (BUN) kit (Stanbio Laboratory) was used according to the manufacturer’s instructions to determine the urea nitrogen content in blood. Sera were diluted 1:100 in enzymatic reagent on 96-well MaxiSorp plates. After incubation in color reagent, the samples were measured at 600-nm wavelength in the ELISA reader (VersaMaxPLUS).
and analyzed using SoftMax Pro software. Values of <35 mg/dl were considered normal.

**Immunohistochemistry**

Spleens and kidneys were harvested and snap-frozen in TissueTek OCT Cryomold (Sakura). Three consecutive 8-μm cryostat sections were fixed on poly-L-lysine slides with ice-cold acetone for 5 min and rehydrated with 1× PBS for 5 min. Spleen sections were saturated with 20% horse serum, 20% FCS, 1% anti-CD16/32 (2.4G2, own hybridoma) in 1× PBS and kidney sections were saturated with 20% horse serum in 1× PBS. Spleen cryostat sections were stained with anti–IgD-bio (eBioscience), anti–peanut agglutinin (PNA–FITC (Vector Laboratories), anti–IgM–FITC (own hybridoma, clone 29-11), anti–IgG-Alexa Fluor 488 (Invitrogen), and streptavidin–Cy3 (GE Healthcare). Spleen cryostat sections were stained for immunocomplex depositions with anti–IgG-Alexa Fluor 488 (Invitrogen), anti–IgG1–FITC (BD Biosciences), anti–IgG2b–FITC (BD Biosciences), anti–IgG2c–FITC (SouthernBiotech), and anti–IgG3–FITC (SouthernBiotech). All stained sections were fixed with mounting medium and analyzed on a fluorescence microscope with the same exposure time the next day. Fluorescence intensity was determined with ImageJ64.

**Histology**

Kidneys were fixed in 4% formalin overnight and stored in 70% ethanol. Sections, stainings, and scoring were performed as previously described (14).

**Flow cytometry**

Single-cell suspensions of bone marrow, spleen, kidney, and peritoneal cavity were treated with Gey’s solution to deplete erythrocytes. Kidneys were predigested with collagenase D (1 mg/ml; from Clostridium histolyticum, Roche) and DNase I (100 ng/ml; DNase I from bovine pancreas; Roche) in RPMI 1640 medium for 30 min at 37°C. Staining was done with the following Abs (conjugated with allopbyocyanin, biotin, FITC, PerCP, Cy5, or PE): anti–B220 (RA3-6B2; eBioscience), anti–GL7 (GL7; eBioscience), anti–IgM (11/41; eBioscience), anti–IgD (11-26C; our hybridoma), anti–CD11d (213; our hybridoma), anti–CD4 (4D.K; BD Biosciences), anti–CD5 (53-7-3; BD Biosciences), anti–CD21 (7G8; our hybridoma), anti–CD23 (B3B4; eBioscience), anti–CD95 (Jo2; BD Pharmingen), anti–CD138 (281-2; BD Biosciences), anti–CD5 (53-7-3; eBioscience), anti–IgM (11/41; eBioscience), anti–IgD (11-26C; our hybridoma), anti–CD11d (213; our hybridoma), anti–CD4 (4D.K; BD Biosciences), anti–CD5 (53-7-3; BD Biosciences), anti–CD8 (53-6.7; eBioscience), anti–IgM (11/41; eBioscience), and anti–IgD (11-26C; our hybridoma) at a 1:150 dilution as described (14). Staining was done with steptavidin PE-Cy5 or PerCP-Cy5.5 (eBioscience). For intracellular staining, cells were fixed with 1× Cytofix (BD) and stained with anti–IgG2b–FITC (BD Biosciences), anti–IgG2a–FITC (BD Biosciences), anti–IgG1–FITC (BD Biosciences), and anti–IgG3–FITC (BD Biosciences). Intracellular staining for IgG was determined with the BD Cytofix/Cytoperm solution kit (BD Biosciences) according to the manufacturer’s instructions.

**Results**

**Higher plasma cell numbers and spontaneous germinal center formation in aged Siglec-G<sup>−/−</sup> and Siglec-G<sup>−/−</sup> × FcγRIIb<sup>−/−</sup> double-deficient mice**

Siglec-G-deficient mice on a BALB/c background have higher levels of IgM and more IgM-secreting plasma cells in spleen and bone marrow (9). Correspondingly, autoreactive Abs of the IgM isotype are elevated in these mice, although they do not develop autoimmune disease. To study the contribution of Siglec-G on a B6 background to autoimmunity, Siglec-G<sup>−/−</sup> (BALB/c) mice were backcrossed to the B6 background by marker-assisted and random backcrossing. The resulting Siglec-G<sup>−/−</sup> mice were further crossed to the autoimmune-prone FcγRIIb<sup>−/−</sup> strain, which is on a pure B6 background. These B6-backcrossed mice (N<sub>s</sub> generation) were tested with 69 SNP markers to be ~95% homozygous for the B6 background. In particular, chromosomes 1, 3, and 13, which contain loci that can contribute to autoimmunity in mixed mouse background strains (29, 35), were determined to be 100% B6 in all tested B6-backcrossed mice (not shown). In these B6-backcrossed mice, parameters for B cell hyperactivity, such as spontaneous plasma cell and germinal center formation or Ig serum levels, were analyzed. First, we examined the natural serum Ig level in sera of 12- and 48-week-old mice, as altered Ig level in blood can be a sign for a dysregulated Ig production by plasma cells. Young Siglec-G<sup>−/−</sup> and Siglec-G<sup>−/−</sup> × FcγRIIb<sup>−/−</sup> double-deficient mice exhibit diminished levels of IgG1, IgG2b, and IgG2c subclasses, which, except for IgG2c in Siglec-G<sup>−/−</sup> × FcγRIIb<sup>−/−</sup> double-deficient mice, are adjusted to normal levels in older mice or even increased compared with WT controls. Aged Siglec-G<sup>−/−</sup> and Siglec-G<sup>−/−</sup> × FcγRIIb<sup>−/−</sup> double-deficient mice exhibit increased titers of IgM and IgG3 compared with WT littermates (Fig. 1A). According to elevated Ig serum titer, higher numbers of IgM and IgG plasma cells and plasmablasts can be detected in spleen sections (Fig. 1B) as well as in FACS analysis and ELISPOT of spleen and bone marrow (Fig. 1C, Table I) in aged Siglec-G<sup>−/−</sup> and Siglec-G<sup>−/−</sup> × FcγRIIb<sup>−/−</sup> double-deficient mice. To confirm that the IgG-stained spleen sections are indeed plasma cells, we showed intracellular IgG production of cells in these sections by use of an additional DAPI staining (Supplemental Fig. 1). Furthermore, formation of spontaneous germinal centers occurs in old Siglec-G<sup>−/−</sup>, FcγRIIb<sup>−/−</sup>, and Siglec-G<sup>−/−</sup> × FcγRIIb<sup>−/−</sup> double-deficient mice (Fig. 1B), whereas a mild splenomegaly occurs only in double-deficient male mice (Fig. 1D).

**Altered B cell numbers and enhanced T cell activation in Siglec-G<sup>−/−</sup> and Siglec-G<sup>−/−</sup> × FcγRIIb<sup>−/−</sup> double-deficient mice**

Because both Siglec-G and FcγRIIb are expressed on B lymphocytes, we analyzed whether loss of one or both inhibitory receptors results in altered B cell numbers. Flow cytometric analysis in bone marrow and spleen of 12 wk-old Siglec-G<sup>−/−</sup>, FcγRIIb<sup>−/−</sup>, and Siglec-G<sup>−/−</sup> × FcγRIIb<sup>−/−</sup> double-deficient mice revealed no gross changes in total B cell numbers for typical developmental stages compared with WT littermate controls, apart from elevated B1 cell numbers in Siglec-G<sup>−/−</sup> and Siglec-G<sup>−/−</sup> × FcγRIIb<sup>−/−</sup> double-deficient mice (Supplemental Table I). In older mice (48 wk) peritoneal B1a cells of all null-mutant mice were strongly elevated (Table I). This result is consistent with the phenotype being observed in Siglec-G–deficient mice on a BALB/c background and in FcγRIIb–deficient mice (9, 36). Additionally, the peritoneal B1b/B2 cell population was significantly increased compared with WT controls in aged Siglec-G<sup>−/−</sup>, FcγRIIb<sup>−/−</sup>, and Siglec-G<sup>−/−</sup> × FcγRIIb<sup>−/−</sup> double-deficient mice. The other changed B lineage populations were increased numbers of plasmablasts and plasma cells in Siglec-G<sup>−/−</sup> and Siglec-G<sup>−/−</sup> × FcγRIIb<sup>−/−</sup> double-deficient mice (Table I). B cells of aged Siglec-G<sup>−/−</sup> and Siglec-G<sup>−/−</sup> × FcγRIIb<sup>−/−</sup> double-deficient mice did show slightly increased expression of the activation marker CD86 (Fig. 2A).

Because Siglec-G is also expressed on dendritic cells (DCs) (19, 21), we analyzed DC numbers in aging mice and also determined activation markers such as MHC class II and CD86 on DCs. We did not detect any significant changes in the three knockout mouse strains (not shown). However, examination of T cell populations revealed a higher CD4<sup>+</sup>/CD8<sup>+</sup> T cell ratio in these mice, resulting from reduced total CD8<sup>+</sup> T cell numbers and a proportional increase of the CD4<sup>+</sup> T cell population (Fig. 2B). Further analysis of T cell populations revealed a higher activation status of CD4 T cells in Siglec-G<sup>−/−</sup> mice, whereas no changes were found in the CD8 T cell population. Specifically, CD4 T cells of Siglec-G<sup>−/−</sup> mice expressed higher CD69 levels (Fig. 2C). There was a tendency of higher T follicular helper cell numbers (identified as CD4<sup>+</sup>CXCR5<sup>+</sup>PD-1<sup>+</sup>) in Siglec-G<sup>−/−</sup> mice (Fig. 2D). In about half of the Siglec-G<sup>−/−</sup> mice we also noted an increased CXCR5<sup>+</sup>PD-1<sup>+</sup> population (Fig. 2D), which may be T follicular helper precursors (37). Furthermore, a higher number of effector memory CD4 T cells (CD62L<sup>−</sup>CD44<sup>+</sup>) was detected in Siglec-
GB6/2 mice (Fig. 2E). This higher activation status of CD4 T cells in Siglec-G B6/2 mice may depend on a higher Ag presentation of DCs, which lack the inhibitory protein Siglec-G.

Aged Siglec-G–deficient and Siglec-G/B6/2 × FcγRIIb/B6/2 double-deficient mice show increased autoantibody titers

FcγRIIb deficiency enhances spontaneous autoimmunity in mice, which are susceptible to lupus-like diseases (28). To address the question whether Siglec-G deficiency can lead to a break of tolerance and acts to enhance autoimmunity in FcγRIIb/B6/2 mice, respectively, we analyzed Abs titers against various autoantigens in sera of mutant mice over time. IgG autoantibodies against ssDNA and dsDNA are significantly increased in sera of 24-wk-old Siglec-G/B6/2 and Siglec-G/B6/2 × FcγRIIb/B6/2 double-deficient mice compared with WT sera and steadily rise in aging mice. Rheumatoid factor IgM is elevated in 48- and 70-wk-old...

FIGURE 1. Dysregulated Ig production and higher plasma and germinal center cell numbers in aged Siglec-G/B6/2, FcγRIIb/B6/2, or Siglec-G/B6/2 × FcγRIIb/B6/2 double-deficient mice. (A) Serum Ig levels of 12- and 48-wk-old WT littermates, Siglec-G/B6/2, FcγRIIb/B6/2, and Siglec-G/B6/2 × FcγRIIb/B6/2 double-deficient mice were measured by ELISA. Each symbol represents the serum of a single mouse. (B) IgM (upper row) and IgG (middle row) plasma cells (green) in spleens of 70-wk-old WT, Siglec-G/B6/2, FcγRIIb/B6/2, and Siglec-G/B6/2 × FcγRIIb/B6/2 double-deficient mice were analyzed on cryosections with fluorescent-conjugated Abs against IgM and total IgG. Germinal center B cells (green) were detected with anti-PNA Ab (lower row). B cell follicles were detected with anti-IgD Abs (red). Numbers of germinal centers per area were quantified by counting PNA+ follicles per section. Original magnification ×10. Each symbol represents analysis of one spleen section. Data represent typical results of one of six to eight independent experiments. (C) IgM- and IgG-producing plasma cells in bone marrow and spleens of naive 48-wk-old WT, Siglec-G/B6/2, FcγRIIb/B6/2, and Siglec-G/B6/2 × FcγRIIb/B6/2 double-deficient mice were detected by ELISPOT. Each symbol represents the number of plasma cells per 1 × 10⁷ total cells isolated from bone marrow or spleen of a single mouse in four independent experiments. (D) Spleen weight of 48- and 70-wk-old animals (n = 5–10) was determined with fine scales. Mice with spleens >200 mg were defined as mice with splenomegaly. *p < 0.05, **p < 0.005, ***p < 0.001 by Mann–Whitney U test and unpaired t test.
Siglec-G<sup>−/−</sup> mice but not in Siglec-G<sub>B6</sub>−/− × FcγRIIb<sup>−/−</sup> double-deficient mice (Fig. 3A). ANA levels were increased in sera of 24-wk-old Siglec-G<sub>B6</sub>−/− and Siglec-G<sub>B6</sub>−/− × FcγRIIb<sub>B6</sub>−/− double-deficient mice. At 48 and 70 wk of age, all mutant mouse strains (Siglec-G<sub>B6</sub>−/−, FcγRIIb<sub>B6</sub>−/−, and Siglec-G<sub>B6</sub>−/− × FcγRIIb<sub>B6</sub>−/− double-deficient mice) had elevated ANA levels compared with WT controls (Fig. 3B). No gender-specific differences were detected in any autoantibody levels that were analyzed. Because of the elevated B1 cell population, we also examined PC-specific Abs, which are typically produced by B1 cells. We found elevated titers of PC-specific Abs in sera of 24-wk-old Siglec-G<sub>B6</sub>−/− and Siglec-G<sub>B6</sub>−/− × FcγRIIb<sub>B6</sub>−/− double-deficient mice (Supplemental Fig. 2).

Elevated immune complex depositions and mild, nonlethal glomerular damage in kidneys of Siglec-G<sub>B6</sub>−/− and FcγRIIb<sub>B6</sub>−/− mice

Because high titers of autoantibodies against DNA and nuclear Ags were detected in sera of Siglec-G<sub>B6</sub>−/− and Siglec-G<sub>B6</sub>−/− × FcγRIIb<sub>B6</sub>−/− double-deficient mice, we addressed the question of whether immune complexes consisting of autoantibiotic Abs and autoantigen accumulate in kidneys of aged mice with defective inhibitory receptors. Glomerular nephritis results from an inflammatory response, which is caused by IgG immune complex depositions in glomeruli. Elevated depositions of total IgG, IgG1, IgG2b, IgG2c, and IgG3 immune complexes can be detected in kidney sections of 48- to 70-wk-old Siglec-G<sub>B6</sub>−/− and Siglec-G<sub>B6</sub>−/− × FcγRIIb<sub>B6</sub>−/− double-deficient mice (Fig. 4A).

The severity of glomerular nephritis can be determined by the BUN concentration and level of proteinuria. Sera of aged MRL/lpr mice served as positive control, as these mice exhibit severe kidney damage by immune complex depositions. BUN levels of all mutant mice were in a range of 25–35 mg/dl and therefore normal, when compared with WT controls (Fig. 4B). A further method to measure kidney damage is to determine the amount of protein in the urine. All tested mice did not show elevated protein contents with >300 mg/dl in urine (not shown). Histopathological evaluation of aging healthy WT mice showed mild glomerular alterations, including mesangial matrix expansion as a sign of senescence (Fig. 5, WT). The strongest pathological alterations were found in kidneys of Siglec-G<sub>B6</sub>−/− mice showing renal injury typical for experimental lupus nephritis, including intracapillary thrombus formation, abnormal glomerular basement membrane, mesangial cell proliferation, and matrix expansion (Fig. 5, Siglec-G<sup>−/−</sup>). FcγRIIb-deficient mice showed similar alterations as identified in Siglec-G<sub>B6</sub>−/− mice, but intracapillary thrombus formation was rarely found in these animals (Fig. 5, FcγRIIb<sup>−/−</sup>). Surprisingly, renal alterations in Siglec-G<sub>B6</sub>−/− × FcγRIIb<sub>B6</sub>−/− double-deficient mice were less pronounced compared with kidney biopsies from Siglec-G<sub>B6</sub>−/− or FcγRIIb<sub>B6</sub>−/− mice (Fig. 5, Siglec-G<sup>−/−</sup> × FcγRIIb<sup>−/−</sup>). Female Siglec-G<sub>B6</sub>−/−, FcγRIIb<sub>B6</sub>−/−, and Siglec-G<sub>B6</sub>−/− × FcγRIIb<sub>B6</sub>−/− double-deficient mice animals showed significantly higher glomerular alterations compared with WT controls than did male animals (Fig. 5, lower panels). Tubulointerstitial infiltration with inflammatory cells, tubular atrophy, and fibrosis were rarely found in all investigated groups and not dependent on the genotype. To determine whether kidney alterations were also accompanied by lymphocyte infiltration, lymphocyte populations in kidneys of 48-wk-old mice were examined. Elevated B cell and plasmablast numbers were found in Siglec-G<sub>B6</sub>−/− and Siglec-G<sub>B6</sub>−/− × FcγRIIb<sub>B6</sub>−/− double-deficient mice compared with littermate controls, whereas plasma cell and T cell numbers were normal in these mice (Supplemental Fig. 3).

Discussion

Siglec-G is an inhibitory coreceptor of the BCR and modulates the B cell activation strength after Ag encounter, especially on B1 cells. In this study we reveal that loss of Siglec-G in mice on the B6 background results in autoimmunity characterized by autoantibody and enhanced plasma cell production as well as by development of mild, nonlethal glomerular damage in the kidney. Combined deficiency of Siglec-G and the inhibitory Fc receptor FcγRIIb does not lead in general to increased severity of autoimmunity compared with Siglec-G deficiency alone. The numbers of B1 and B2 cells in the peritoneal cavity, germinal center B cells in the spleen, as well as IgM and IgG plasma cells in spleen and bone marrow are increased in aged Siglec-G<sub>B6</sub>−/−-deficient and Siglec-G<sub>B6</sub>−/− × FcγRIIb<sub>B6</sub>−/− double-deficient mice. The elevation of plasma cell numbers in these mice is reflected by higher titers of serum IgM and some IgG subclasses. Also, Siglec-G<sub>B6</sub>−/− mice show an enhanced CD4 T cell activation. Additionally, higher levels of autoantibodies against ssDNA, dsDNA, and rheumatoid factor can be detected in both aged Siglec-G<sub>B6</sub>−/− and Siglec-G<sub>B6</sub>−/− × FcγRIIb<sub>B6</sub>−/− double-deficient mice. A mild form of glomerulonephritis with a significantly elevated glomerular alteration score

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**Table I. Absolute cell numbers and surface marker expression of lymphocyte populations in 48-wk-old WT (C57BL/6), Siglec-G<sup>−/−</sup>, FcγRIIb<sup>−/−</sup>, and Siglec-G<sub>B6</sub>−/− × FcγRIIb<sub>B6</sub>−/− double-deficient mice**

<table>
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<tr>
<th></th>
<th>WT</th>
<th>Siglec-G&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>FcγRIIb&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>Siglec-G&lt;sub&gt;B6&lt;/sub&gt;−/− × FcγRIIb&lt;sub&gt;B6&lt;/sub&gt;−/−</th>
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<tr>
<td>Bone marrow (&lt;10&lt;sup&gt;5&lt;/sup&gt;)</td>
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<td>Immature cells (B220&lt;sup&gt;+&lt;/sup&gt;IgM&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>3.0 ± 1.2</td>
<td>3.1 ± 1.3</td>
<td>2.8 ± 2.1</td>
<td>3.6 ± 1.9</td>
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<tr>
<td>Transitional B cells (B220&lt;sup&gt;+&lt;/sup&gt;IgM&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>1.1 ± 0.3</td>
<td>1.0 ± 0.5</td>
<td>1.1 ± 0.5</td>
<td>1.1 ± 0.6</td>
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<tr>
<td>Mature B cells (B220&lt;sup&gt;+&lt;/sup&gt;IgD&lt;sup&gt;-&lt;/sup&gt;)</td>
<td>11.4 ± 4.4</td>
<td>9.1 ± 3.8</td>
<td>8.2 ± 5.1</td>
<td>11.1 ± 4.3</td>
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<tr>
<td>Plasma cells (CD138&lt;sup&gt;+&lt;/sup&gt;Ig κ + λ L chain&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>0.14 ± 0.05</td>
<td>0.25 ± 0.06**</td>
<td>0.14 ± 0.08</td>
<td>0.23 ± 0.08*</td>
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<td>Spleen (&lt;10&lt;sup&gt;6&lt;/sup&gt;)</td>
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<td>Follicular B cells (CD21&lt;sup&gt;+&lt;/sup&gt;CD23&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>22.9 ± 10.3</td>
<td>17.6 ± 5.7</td>
<td>24.2 ± 13.5</td>
<td>21.1 ± 12.0</td>
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<td>B1a cells (CD5&lt;sup&gt;+&lt;/sup&gt;CD220&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>2.0 ± 1.4</td>
<td>8.4 ± 5.0**</td>
<td>3.4 ± 1.7*</td>
<td>4.2 ± 0.9*</td>
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<td>Plasmablasts (B220&lt;sup&gt;+&lt;/sup&gt;CD138&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>2.5 ± 0.9</td>
<td>4.2 ± 1.8*</td>
<td>2.9 ± 1.1</td>
<td>3.7 ± 1.8*</td>
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<tr>
<td>Plasma cells (B220&lt;sup&gt;+&lt;/sup&gt;CD138&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>0.9 ± 0.6</td>
<td>1.9 ± 1.0*</td>
<td>0.7 ± 0.3</td>
<td>2.8 ± 0.4**</td>
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<td>CD4&lt;sup&gt;+&lt;/sup&gt; T cells (CD4&lt;sup&gt;+&lt;/sup&gt;CD8&lt;sup&gt;−&lt;/sup&gt;)</td>
<td>15.1 ± 4.1</td>
<td>20.1 ± 13.1</td>
<td>13.4 ± 3.4</td>
<td>17.2 ± 7.2</td>
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<td>CD8&lt;sup&gt;+&lt;/sup&gt; T cells (CD4&lt;sup&gt;+&lt;/sup&gt;CD8&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>6.5 ± 2.0</td>
<td>5.6 ± 3.0</td>
<td>4.6 ± 1.2</td>
<td>3.9 ± 1.2**</td>
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<td>CD4/CD8 ratio</td>
<td>2.5 ± 0.8</td>
<td>4.0 ± 1.7*</td>
<td>3.1 ± 1.0</td>
<td>4.4 ± 1.4**</td>
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<td>Peritoneal cavity (&lt;10&lt;sup&gt;9&lt;/sup&gt;)</td>
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<td>B1a cells (CD5&lt;sup&gt;+&lt;/sup&gt;CD220&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>2.3 ± 1.5</td>
<td>7.1 ± 4.7**</td>
<td>10.0 ± 7.0*</td>
<td>18.1 ± 16.7**</td>
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<td>B1b/B2 cells (CD5&lt;sup&gt;+&lt;/sup&gt;CD220&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>6.5 ± 3.8</td>
<td>11.9 ± 9.2*</td>
<td>13.0 ± 9.8*</td>
<td>15.6 ± 7.4**</td>
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Cell numbers and surface marker expression of indicated populations are given as means ± SD. Eight to thirteen mice were included in quantification of total cell numbers. Significant changes are shown in bold with *p < 0.05, **p < 0.005, and ***p < 0.001.
occurs in aged female Siglec-G−/− and FcγRIIb−/− deficient mice and in Siglec-G−/− × FcγRIIb−/− double-deficient (DKO) mice. The histogram (right panel) shows a representative result for each genotype. (B) Ratio of CD4+ to CD8+ total T cell numbers. (C) CD69 gMFI of CD4+ T cells. The histogram (right panel) shows a representative result for each genotype. (D) CD4+CXCR5 hiPD-1 hi T follicular helper cell numbers of 48-wk-old WT littermates, Siglec-G−/−, FcγRIIb−/−, and Siglec-G−/− × FcγRIIb−/− double-deficient mice. One representative result is shown in FACS blots for each genotype. (E) Total naive (CD44 loCD62L hi), effector memory (CD44 hiCD62L hi), and central memory (CD44 hiCD62L lo) CD4+ T cell numbers of 48-wk-old WT littermates, Siglec-G−/−, FcγRIIb−/−, and Siglec-G−/− × FcγRIIb−/− double-deficient mice are shown. One representative result is shown in FACS blots for each genotype. All graphs (A–E) show the results of 12–14 mice per group. *p < 0.05, ***p < 0.001 by Mann–Whitney U test and unpaired t test.
A hallmark of many autoimmune diseases is the occurrence of pathogenic autoantibodies produced by self-reactive plasma cells (39, 40). In Siglec-G<sup>B6<sup>−/−</sup></sup> mice we found increased numbers of IgM- and IgG-producing plasmablasts and plasma cells in spleen and bone marrow. Siglec-G is a modulator of BCR signaling mainly in B1 cells, and Siglec-G deficiency results in strongly elevated B1 cell numbers in the peritoneal cavity (9). In this study we found not only higher numbers of B1a cells in peritoneal cavities of all three double-deficient mice detected on HEp2- slides. Pooled sera of MRL/lpr mice (39 wk old) served as positive control. Original magnification $\times 10$. Left panel, The mean pixel intensity determined from 10 randomly analyzed cells in a sample is shown. Each symbol represents the IgG serum ANA level of a single mouse. The graph shows the summary of six to eight independent experiments. **$p < 0.005$, ***$p < 0.001$ by Mann–Whitney $U$ test.

B cells normally differentiate into plasmablasts and plasma cells after Ag encounter and T cell help in lymphatic organs in a tightly regulated manner. Recently, Siglec-G expression was confirmed on DCs, which are responsible for T cell priming during an immune response (19, 21). Therefore, loss of Siglec-G on DCs could potentially result in an enhanced activation of T cells, which in turn leads to an uncontrolled activation of Siglec-G<sup>B6</sup>−/− deficient B cells by T cells. Because CD4 T cells of Siglec-G−/− mice were increased and had upregulated activation markers and increased effector memory phenotype, we think that it is likely that higher activation of CD4 T cells contributes to the development of autoimmune. Although Siglec-G−/− mice show mainly enhanced signaling and functional consequences in B1 cells, Siglec-G can also inhibit conventional B2 cell responses, as has been demonstrated by transfections of B cell lines or by use of synthetic Siglec-G ligands (9, 19). It is therefore likely that dysregulation of T cell activation combined with overstimulation of conventional B cells contributes to the higher generation of autoantibody-producing plasma cells of IgM and IgG isotypes in Siglec-G<sup>B6</sup>−/− mice. A skewed CD4<sup>+</sup>/CD8<sup>+</sup> ratio of T cell numbers and elevation of activated T cells are reported to be typical signs of an autoimmune phenotype (28, 45). We do not know why single Siglec-G<sup>B6</sup>−/− mice showed a stronger T cell activation phenotype than did double-deficient (Siglec-G<sup>B6</sup>−/− × FcγRIIB<sup>B6</sup>−/−) mice.

FcγRIIB limits the accumulation of IgG<sup>+</sup> plasma cells and modifies autoimmune susceptibility in autoimmune-prone strains of mice.
FIGURE 4. Normal urea nitrogen levels in sera, but increased immune complex deposition, in aged Siglec-G<sup>−/−</sup>, FcyRllb<sup>−/−</sup>, and Siglec-G<sub>B6</sub>−/− × FcyRllb<sub>B6</sub>−/− double-deficient mice. (A) Immune complex depositions in kidneys of 70-wk-old mice were analyzed on cryosections with fluorescent-conjugated Abs against total IgG and IgG subclasses. Upper panels, Example of stainings of 70-wk-old WT (littermate controls), Siglec-G<sup>−/−</sup>, FcyRllb<sup>−/−</sup>, and Siglec-G<sub>B6</sub>−/− × FcyRllb<sub>B6</sub>−/− double-deficient kidneys. Original magnification ×20. Data represent typical results of one of three independent experiments. Lower panels, Fluorescence intensity of immune complex depositions, which was quantified with ImageJ64. Each symbol represents the mean fluorescence intensity of Ig depositions of a single kidney. The graphs show the summary of three independent experiments. (B) Urea nitrogen contents in blood of WT, Siglec-G<sup>−/−</sup>, FcyRllb<sup>−/−</sup>, and Siglec-G<sub>B6</sub>−/− × FcyRllb<sub>B6</sub>−/− double-deficient mice were determined with an enzymatic BUN kit. A serum pool of MRL/lpr mice served as positive control for elevated urea nitrogen levels. Each symbol represents the urea nitrogen level of a single mouse (mg/dl). The graphs show the summary of 10–14 independent experiments. *p < 0.05, **p < 0.005, ***p < 0.001 by Mann–Whitney U test.

(28, 46). Although Siglec-G<sub>B6</sub>−/− mice show an autoimmune phenotype, additional FcyRllb deficiency does not exacerbate autoimmune disease. A possible explanation may be that FcyRllb only modifies development of spontaneous autoimmune in strains with more severe and earlier onset of autoimmune disease, such as in mice with yaa or MRL/lpr background. Because FcyRllb is also expressed on follicular DCs, which present Ags in germinal center responses and are involved in selection of high-affinity Abs (47), this Fc receptor may also control B cell tolerance by mechanisms that are distinct to Siglec-G.

Self-reactive Abs are crucial for onset of lupus-like diseases, and autoreactive B2 cells need to go through germinal center response to produce high-affinity IgG autoantibodies (48). In contrast to WT mice, spontaneous formation of germinal centers was observed in all three aged genetically modified mouse strains to the same degree. This indicates that loss of Siglec-G or FcyRllb does not only influence the activation of B1 cells, but it also induces dysregulation in B2 cells, which leads to spontaneous germinal center reactions. Consistent with an elevation of plasma cell numbers and spontaneous germinal center formation, we can detect significantly increased titers of anti-ssDNA and -dsDNA as well as rheumatoid factor autoantibodies in aged Siglec-G<sub>B6</sub>−/− and Siglec-G<sub>B6</sub>−/− × FcyRllb<sub>B6</sub>−/− double-deficient mice. However, ANA titers are increased in all three analyzed mouse strains compared with WT controls. Anti-DNA and anti-nuclear autoantibody titers are more elevated in aged Siglec-G<sup>−/−</sup> × FcyRllb<sup>−/−</sup> mice than in Siglec-G<sub>B6</sub>−/− mice, indicating that combined loss of the two inhibitory receptors further promotes the development of autoantibodies. In ongoing germinal center responses, B cells require high SHP-1 and SHIP-1 phosphatase activity (49). Loss of Siglec-G and FcyRllb may result in reduced phosphatase recruitment to the BCR, thereby disturbing normal germinal center responses and promoting generation of self-reactive Abs.

Aggregates of anti-DNA Abs or ANAs and apoptotic material can form immune complexes, which are deposited in kidneys. The resulting inflammation can cause kidney damage (40). Although BUN and proteinuria are not elevated over controls in aged Siglec-G<sub>B6</sub>−/−, FcyRllb<sub>B6</sub>−/−, or Siglec-G<sub>B6</sub>−/− × FcyRllb<sub>B6</sub>−/− double-deficient mice, IgG immune complex depositions can be found in kidneys of these mice. Surprisingly, the combined loss of both inhibitory receptors does not increase glomerular damage compared with loss of Siglec-G alone, but is rather reduced. IgG2c is strongly diminished or even not detectable in sera of aged Siglec-G<sub>B6</sub>−/− × FcyRllb<sub>B6</sub>−/− mice, which indicates that this IgG subclass may play a stronger role in the development of glomerular damage than do the other IgG subclasses. Reduced titers of IgG2a, which is the BALB/c allele corresponding to the B6 IgG2c allele (50), could also be found in Siglec-G-deficient mice on BALB/c background (9), indicating that the diminished IgG2c level in young Siglec-G<sub>B6</sub>−/− and young and aged Siglec-G<sub>B6</sub>−/− × FcyRllb<sub>B6</sub>−/− double-deficient mice could be caused by the loss of Siglec-G. Isotype switching to IgG2c subclass is mainly induced by Th1-type cytokines such as IFN-α or IFN-γ (51, 52), indicating that this response may be diminished in Siglec-G<sub>B6</sub>−/− and Siglec-G<sub>B6</sub>−/− × FcyRllb<sub>B6</sub>−/− mice. Because Siglec-G is also expressed on DCs (19, 21), an altered cytokine production by DCs induced by the loss of Siglec-G may affect Th1 cytokines.

Of note, although lupus disease is reported to progress in a gender-specific way (53), there where no overall differences in autoantibody titers and disease score between male and female
animals. An exception can be found in kidneys of female Siglec-G<sup>B6</sup>−/− mice, which have stronger elevated glomerular alteration scores than do male littermates. This is consistent with the observations of more affected female Siglec-G-deficient mice on MRL/lpr background (14), indicating that Siglec-G may be a factor regulating gender-specific progression of autoimmunity. The alterations in female Siglec-G<sup>B6</sup>−/− mice included thrombus formation, which is one of the histopathological changes that occur during lupus nephritis and is a consequence of endothelial damage and platelet aggregation. Thrombi in the setting of SLE are also found in humans and may be the consequence of anti-phospholipid syndrome, thrombotic microangiopathy, or SLE vasculopathy (54). We think it is unlikely that the enhanced anti-phospholipid syndrome, thrombotic microangiopathy, or SLE are also found in humans and may be the consequence of anti-phospholipid formation, which is one of the histopathological changes that increased injury, especially on endothelial cells in these mice.

B cells or plasmablasts may contribute to autoantibody production directly in the kidneys of aged Siglec-G<sup>B6</sup>−/− and Siglec-G<sup>B6</sup>−/− × FcγRIIB<sup>B6</sup>−/− double-deficient mice, as they were found in elevated numbers by flow cytometry. There is evidence that plasma cells can be found in inflamed kidneys and contribute to lupus nephritis (55). However, there is no significant decrease in survival in these animals (not shown). Siglec-G<sup>−/−</sup>, FcγRIIB<sup>−/−</sup>, and Siglec-G<sup>B6</sup>−/− × FcγRIIB<sup>B6</sup>−/− double-deficient animals were also analyzed on the BALB/c background. In this genetic background no autoantibodies and no glomerular damages were detected up to the age of 6 mo (not shown). This difference of autoimmunity between B6 and BALB/c mice has also been described for FcγRIIB<sup>129</sup>−/− mice that were backcrossed to these backgrounds and showed different susceptibility (4). It can only be speculated why both FcγRIIB<sup>−/−</sup> mice and Siglec-G<sup>B6</sup>−/− mice show this differential susceptibility to autoimmunity on these two mouse strain backgrounds. It has been observed that in the B6 background preferentially a Th1-type cytokine response is induced in infections, whereas in BALB/c a Th2 cytokine response is typical (56). This differential cytokine expression of the two strains may be important for driving inflammation and autoimmune disease. Taken together, loss of Siglec-G on the B6 background regulates not only B1 cells, but also conventional B2 cells and leads to activated CD4 T cells, which together result in a loss of tolerance and development of a mild autoimmune disease. It is not clear which B cell subpopulation contributes to disease progression. Elevated B1 cells are sometimes associated with autoimmunity, which was shown by analysis of SHP-1-deficient and NZB/W mice (8, 43, 57). However, Siglec-G-deficient mice on BALB/c background, which have hyperresponsive B1 cells and an enlarged B1a cell population, do not develop autoimmune disease (9). Supporting evidence for B2 cell involvement of the Siglec-G deficiency in autoimmunity comes from Siglec-G-deficient mice on the autoimmune-prone MRL/lpr background. These mice have normal numbers of B1a cells, but more activated conventional B cells and an earlier onset and more severe autoimmune disease compared with MRL/lpr controls, indicating a role for B2 cells, rather than B1 cells, in this lupus model (14). Additionally, it could be shown that Siglec-G ligands can induce tolerance in both B1 and B2 cells after co-presentation of Ag and high-affinity Siglec-G ligands on liposomes (19).

We conclude that Siglec-G regulates the immune system by controlling both B1 and B2 cell activation and contributes to maintenance of tolerance toward self-antigens. Aging Siglec-G<sup>B6</sup>−/− deficient mice develop spontaneous autoimmunity, which was not observed in the BALB/c background. Siglec-G/Siglec-10, as a BCR signaling inhibitor and keeper of tolerance, could be used as potential targets for treatment of autoimmune diseases in both Ab- and ligand-based strategies (2).

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**Disclosures**

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