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The major histocompatibility complex haplotype represents the most prevalent genetic risk factor for the development of autoimmune diseases. However, the mechanisms by which major histocompatibility complex–associated genetic susceptibility translates into autoimmune disease are not fully understood. Epidermolysis bullosa acquisita is an autoimmune skin-blistering disease driven by autoantibodies to type VII collagen. Here, we investigated autoantigen-specific plasma cells, $CD4^+$ T cells, and IgG fraction crystallizable glycosylation in murine epidermolysis bullosa acquisita in congenic mouse strains with the disease-permitting H2s or disease-nonpermitting H2b major histocompatibility complex II haplotypes. Mice with an H2s haplotype showed increased numbers of autoreactive $CD4^+$ T cells and elevated IL-21 and IFN-γ production, associated with a higher frequency of IgG autoantibodies with an agalactosylated, proinflammatory *N*-glycan moiety. Mechanistically, we show that the altered antibody glycosylation leads to increased ROS release from neutrophils, the main drivers of autoimmune inflammation in this model. These results indicate that major histocompatibility complex II–associated susceptibility to autoimmune diseases acuminates in a proinflammatory IgG fraction crystallizable *N*-glycosylation pattern and provide a mechanistic link to increased ROS release by neutrophils.

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INTRODUCTION

Genetic predisposition considerably contributes to the development of most autoimmune diseases. They are frequently associated with the expression of certain major histocompatibility complex (MHC) haplotypes (Cho and Feldman, 2015), which represent the most prevalent genetic risk factors for the development of rheumatoid arthritis, type 1 diabetes, and multiple sclerosis, among others (Tsai and Santamaria, 2013). Autoimmune disorders with characteristic autoantibodies are typically associated with specific *MHCII* alleles (Sollid et al., 2014). MHCII molecules are of crucial importance for the positive selection of nonself-reactive $CD4^+$ T-cell clones and the deletion of autoreactive cells from this population during central tolerance development (Hengartner et al., 1988; Kisielow et al., 1988; Lu et al., 2019).

Epidermolysis bullosa acquisita (EBA) is an autoimmune disease of the skin and/or mucous membranes characterized by subepidermal skin blistering and autoantibodies to type VII collagen (Kasperkiewicz et al., 2016). Susceptibility to EBA development is associated with the MHCII haplotype HLA-DR in patients (Gammon et al., 1988; Zumelzu et al., 2011) and the MHCII haplotype H2s in mice (Ludwig et al., 2011). Immunization of susceptible mice such as congenic B6.s mice expressing the MHCII haplotype H2s with the von Willebrand factor A–like domain 2 (vWFA2) of type VII collagen together with the adjuvant Titermax results in the development of experimental EBA (Iwata et al., 2013). T cells can amplify autoantibody-induced tissue injury (Bieber et al., 2016), whereas regulatory T cells (Tregs) can dampen IgG autoantibody production in autoimmune-blistering skin diseases (Bieber et al., 2017b; Haeberle et al., 2018).

IgG autoantibodies induce a cascade of events that leads to neutrophil recruitment into the skin, their subsequent Fcγ receptor (FcγR)-dependent activation, and ROS and protease release that mediates EBA skin disease (Ludwig et al., 2017; Recke et al., 2015).

The pathogenicity of the fragment crystallizable (Fc) part of IgG autoantibodies varies depending on their subclass and the type of Fc *N*-glycosylation (Nimmerjahn and Ravetch, 2008, 2005). Whereas low-galactosylated IgG antibodies correlate with proinflammatory effector functions, galactosylated and terminally sialylated IgG antibodies associate

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Abbreviations: dLN, draining lymph node; EBA, epidermolysis bullosa acquisita; Fc, fragment crystallizable; FcγR, Fc-gamma receptor; MHC, major histocompatibility complex; Tfh, T follicular helper; Treg, regulatory T cell; vWFA2, von Willebrand factor A–like domain 2

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with less inflammatory functions or even show immunosuppressive properties (Bartsch et al., 2018; Collin and Ehlers, 2013; Hess et al., 2013; Karsten et al., 2012). The Fc *N*-glycosylation pattern of IgG antibodies is determined by T-cell cytokines such as IFN-γ and IL-21 (Collin and Ehlers, 2013; Hess et al., 2013; Pfeifle et al., 2017).

Here, we compared EBA development in susceptible B6.s mice and nonsusceptible C57BL/6J mice and investigated the corresponding autoimmune response.

RESULTS

Nonsusceptible C57BL/6J mice develop lower IgG autoantibody serum levels than congenic susceptible B6.s mice

Susceptibility to immunization-induced EBA in mice is associated with MHCII H2s haplotype (Hammers et al., 2011; Ludwig et al., 2011). To study the role of the genetic susceptibility for the development of this autoimmune disorder, nonsusceptible C57BL/6J mice expressing the MHCII H2b and congenic susceptible B6.s mice (B6.SJL-H2^s C3^c/1CyJ) carrying the H2s haplotype were immunized with vWFA2, which encompasses an immunogenic domain within the noncollagenous NC1 domain of murine type VII collagen. Within 4–5 weeks after immunization, all the B6.s mice developed skin inflammation (Figure 1a). In line with previous observations, C57BL/6J mice did not show any signs of clinical disease (Ludwig et al., 2011). Compared with B6.s mice, C57BL/6J mice exhibited a delayed and approximately 10-fold lower anti-vWFA2 autoantibody titer (Figure 1b). Of note, despite these quantitative differences, we found considerable amounts of circulating vWFA2-specific IgG in C57BL/6J mice. Moreover, some individual diseased B6.s mice developed only moderate autoantibody levels, which were comparable with those of nondiseased C57BL/6J mice, indicating that the disease phenotype of B6.s mice is not merely the consequence of increased autoantibody production. Whereas sections from C57BL/6J mice showed no C3 depositions, the presence of IgG deposits at the dermal–epidermal junction could be not excluded (Supplementary Figure S1). In contrast, the sera from B6.s mice showed increased IgG binding to the skin in an indirect immunofluorescence staining assay (Supplementary Figure S2). The differences in the average levels of vWFA2-specific serum antibodies between B6.s and C57BL/6J mice were apparent for IgG1, IgG2b, IgG2c, and IgM (Figure 1c).

In conclusion, the considerable production of IgG and IgM autoantibodies observed in the serum of C57BL/6J mice suggests that despite mediating nonsusceptibility to EBA development, expression of the H2b haplotype is not sufficient to guarantee tight immunological tolerance that efficiently depletes or anergizes vWFA2 autoantigen-reactive B and T cells.

IgG autoantibodies from C57BL/6J and B6.s mice differ with respect to their Fc *N*-glycosylation pattern

Because some individual diseased B6.s mice developed only moderate autoantibody levels comparable with nondiseased C57BL/6J mice, we speculated that in addition to the quantitative differences of autoantibodies, other factors may also contribute to the differential disease development in the two mouse strains. This idea was further strengthened by the observation that the disease score of individual mice did not correlate with the respective autoantibody titer (Supplementary Figure S3). To test whether anti-vWFA2 autoantibodies from C57BL/6J and B6.s mice exhibited different pathogenic potential, equal amounts of purified anti-vWFA2 IgG from the two mouse strains were tested for their capacity to induce an oxidative burst in innate effector cells isolated from a naive C57BL/6J mouse. This assay was selected because immune complex–induced neutrophil activation is a key pathogenic driver of skin inflammation in experimental EBA (Ludwig et al., 2017, 2013). IgG autoantibodies collected from B6.s mice sera induced an oxidative burst approximately 30% higher than that induced by the IgG autoantibodies from nonsusceptible C57BL/6J mice (Figure 2).

The capacity of IgG antibodies to activate innate immune cells strongly depends on their IgG Fc *N*-glycosylation pattern (Epp et al., 2018; Ludwig et al., 2017; Nimmerjahn and Ravetch, 2008, 2005). Previously, we found that highly galactosylated IgG1 immune complexes can even exert anti-inflammatory properties in experimental EBA (Karsten et al., 2012) and that highly sialylated IgG1 immune complexes containing autoantigen have reduced disease symptoms in experimental rheumatoid arthritis (Bartsch et al., 2018). To assess whether B6.s mice generated autoantibodies with a more proinflammatory IgG glycosylation pattern than C57BL/6J mice, Fc glycan structures of purified vWFA2-specific IgG1 autoantibodies from both strains were analyzed by liquid chromatography–mass spectrometry/mass spectrometry (Figure 3a and Supplementary Figure S4). Anti-vWFA2–specific IgG1 was the most frequently induced IgG subclass, and the corresponding sufficient liquid chromatography–mass spectrometry/mass spectrometry signals could be detected in all samples, whereas autoantigen-specific IgG2 signals were in several probes under the detection limit. However, all murine IgG subclasses have shown comparable shifts in previous experiments after immunization with, for example, different adjuvants (Epp et al., 2018).

First, we determined the *N*-glycan pattern of total serum IgG1. We found a similar frequency of the agalactosylated IgG-G0 type in C57BL/6J and B6.s mice. In contrast, the IgG1-G1 and IgG-G2 types were higher in B6.s than in C57BL/6J mice, whereas the frequency of sialylated total IgG1 was lower in B6.s than in C57BL/6J mice. Next, we analyzed the *N*-glycan pattern in vWFA2-specific IgG1 autoantibodies. Here, we observed a markedly higher frequency of IgG1 with an agalactosylated G0 type in B6.s than with C57BL/6 J mice. This increase in IgG1-GO and to a lesser extent IgG1-G1 glycan type was associated with a massively reduced frequency of double galactosylated (IgG1-G2) and sialylated (IgG1-G1S1/G2S1/G2S2) *N*-glycans in serum anti-vWFA2–specific IgG1 from vWFA2-immunized B6.s mice compared with IgG1 antibodies from C57BL/6J mice (Figure 3b–d). In conclusion, the total IgG1 and purified anti-vWFA2–specific IgG1 from vWFA2-immunized B6.s mice showed a more proinflammatory Fc *N*-glycosylation pattern than the IgG1 antibodies from C57BL/6J mice.

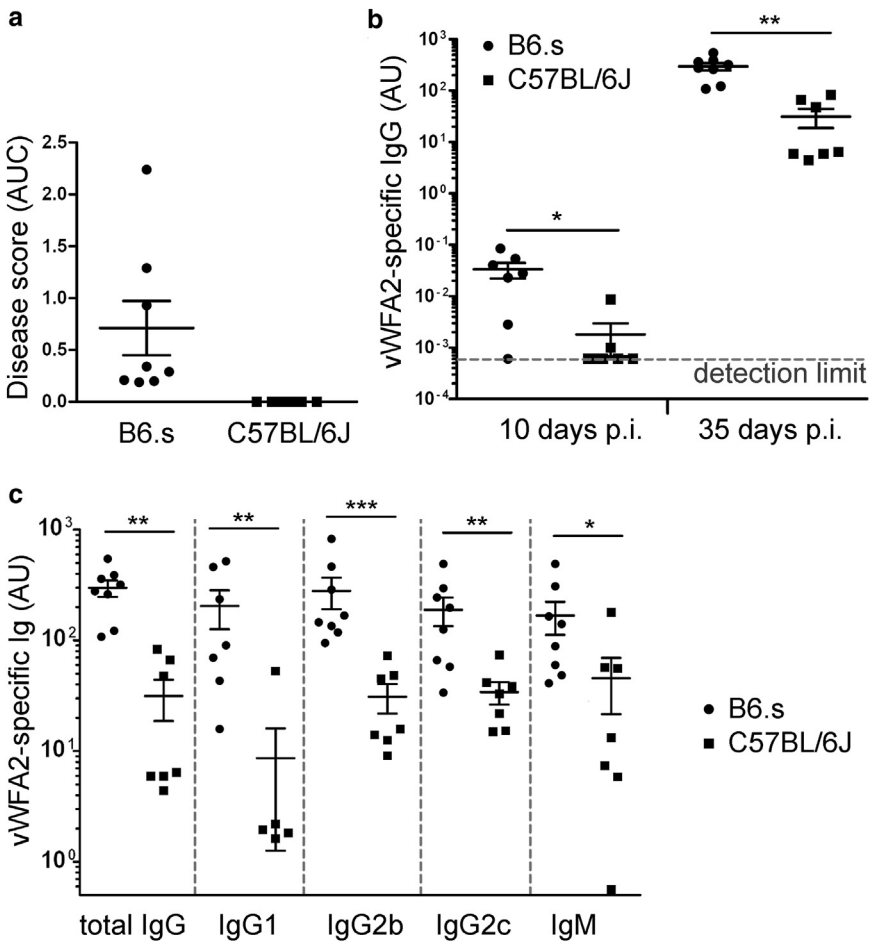

Figure 1. Disease score and autoantibody titer of vWFA2-immunized mice. Mice were immunized with vWFA2 andTitermax. (**a**) EBA disease score (affected body surface area) was measured weekly for 5 weeks after immunization and is shown as AUC. Pooled data are shown from two independent experiments; n = 7–8. (**b**) vWFA2-specific serum IgG at 10 days and 5 weeks after immunization, as measured by ELISA (pooled data are shown from two independent experiments are shown; n = 7–8; statistic: 2-tailed Mann–Whitney test). (**c**) vWFA2-specific autoantibodies of different subclasses at week 5 after immunization (pooled data from two independent experiments are shown; n = 7–8; statistic: two-tailed Mann-Whitney test). Data are expressed as mean ± SEM. $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$. AU, arbitrary unit; EBA, epidermolysis bullosa acquisita; AUC, area under the curve; p.i., postimmunization; vWFA2, von Willebrand factor A–like domain 2.

The IgG1 Fc *N*-glycosylation pattern determines the strength of ROS release from neutrophils

Agalactosylated IgGs of the G0 type promote inflammation through the modulation of macrophages and dendritic cell functions (Anthony et al., 2011; Bartsch et al., 2018; Bayry et al., 2003; Oefner et al., 2012). However, the impact of differential IgG Fc *N*-glycosylation on neutrophils is not well-established, although a recent paper suggests that this mechanism may play a role in IgG-mediated allergic reactions (Epp et al., 2018). Because the release of ROS from activated neutrophils is supposed to be the major effector mechanism mediating EBA skin inflammation, we next investigated the impact of IgG1 Fc *N*-glycosylation on ROS release by neutrophils (Koga et al., 2018; Sadeghi et al., 2015). For this purpose, neutrophils were isolated from naive C57BL/6J mice bone marrow and stimulated with differentially glycosylated monoclonal murine anti-trinitrophenyl IgG1 antibodies. Neutrophils incubated with degalactosylated IgG1 exhibited a much stronger ROS release than the neutrophils stimulated with the same monoclonal IgG1 but with a highly sialylated *N*-glycan moiety (Figure 4). Stimulation with immune complexes did not increase the frequencies of dead or apoptotic neutrophils in culture (Supplementary Figure S5).

The differences in ROS release mediated by the differentially glycosylated monoclonal IgG1 antibodies were in the same range as the differences in ROS release induced by the differentially glycosylated IgG autoantibodies from C57BL/6J and B6.s mice. These findings suggest that the different IgG1 Fc *N*-glycosylation patterns have a major impact on the pathogenic potential of the IgG autoantibodies from these two mouse strains.

Collectively, these data demonstrate that genetic susceptibility to EBA development is associated with an increased autoantibody response and with the production of IgG autoantibodies that exhibit an Fc *N*-glycosylation pattern that drives strong neutrophil activation.

B6.s mice yield higher numbers of vWFA2-specific plasma cells and vWFA2-specific T follicular helper cells than C57BL/6J mice

T-cell–derived cytokines have a strong impact on IgG Fc *N*-glycosylation, which is mediated by glycosyltransferases in antibody-secreting plasma cells (Hess et al., 2013; Pfeifle et al., 2017). Next, we determined the cellular B- and T-cell responses to vWFA2 in the immunization-induced EBA model. Autoreactive plasma cells are preferentially found in the draining lymph nodes (dLNs) (Tiburzy et al., 2013). Plasma cells specific for vWFA2 were identified by intracellular staining with antigen as described (Kulkarni et al., 2016; Tiburzy et al., 2013). On day 10 after immunization, dLN of B6.s mice contained approximately 1 million vWFA2-specific plasma cells, whereas <1,000 cells were found in these tissues in C57BL/6J mice (Supplementary Figure S6).

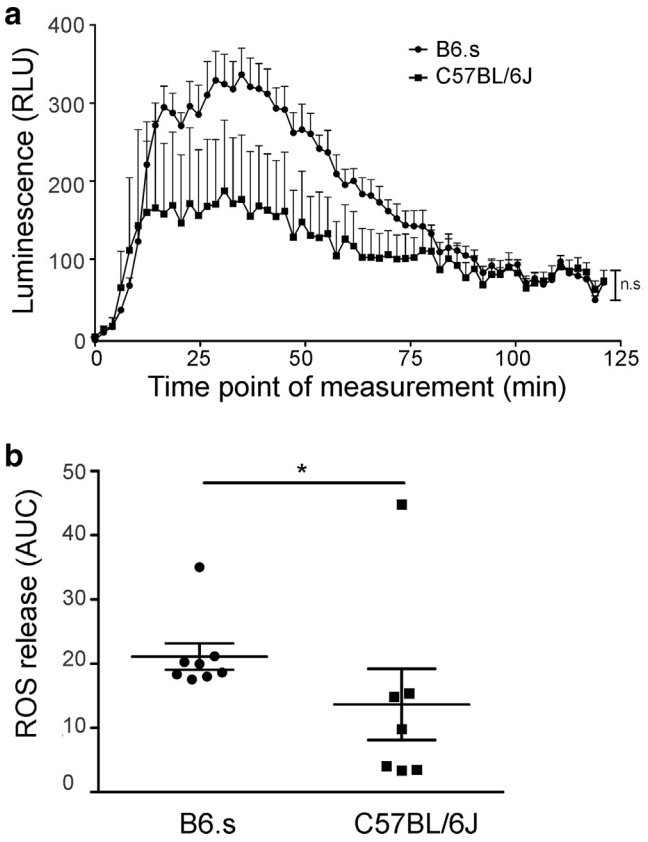

Figure 2. Oxidative burst induction by autoantibodies. Mice were immunized with vWFA2 andTitermax. Sera were collected 5 weeks after immunization and normalized to their individual IgG autoantibody content. Sera were collected from two independent experiments. (**a**) The immune complex–stimulated release of ROS was measured with a luminol-amplified luminescence assay. Increase of luminescence due to the release of ROS by immune complex–activated bone marrow cells from a naive C57BL/6J mouse over time (n = 7–8; statistic: two-way ANOVA). (**b**) Total ROS release defined as the AUC. Each dot represents data from the serum of a single mouse (n = 7–8; statistic: two-tailed Mann–Whitney test). Data are expressed as mean ± SEM. *$P \leq 0.05$. AUC, area under the curve; min, minute; n.s., not significant; RLU, relative luminescence unit; vWFA2, von Willebrand factor A–like domain 2.

After 5 weeks of immunization, vWFA2-specific plasma cell frequencies and numbers in C57BL/6J mice reached about 10% of those in B6.s mice.

The MHC has no direct impact on B-cell development or activation but modulates the T-cell response, which is crucial for mounting an autoreactive B-cell response (Iwata et al., 2013). Therefore, we studied vWFA2-specific $CD4^+$ T cells by the rapid antigen-induced upregulation of CD154 (Frentsch et al., 2005; Meier et al., 2008; Tiburzy et al., 2013). In the dLNs and spleens of B6.s mice, we found some 4-fold higher frequencies and numbers of vWFA2-specific $CD4^+$ T cells than in those of C57BL/6J mice (Figure 5 and Supplementary Figure S7). T follicular helper (Tfh) cells represent the subpopulation of $CD4^+$ T cells relevant for supporting the corresponding B-cell response (Liu et al., 2013; Wan et al., 2019). Although B6.s mice showed only approximately 1.5-fold higher frequencies of total Tfh cells, the percentage of vWFA2-specific cells among the Tfh population was 3-fold higher in B6.s mice than in C57BL/6J mice (Supplementary Figure S8). The absolute numbers of total Tfh cells and vWFA2-specific Tfh population cells showed similar differences between the two mouse strains (Supplementary Figure S9).

Together, our observations suggest that genetic susceptibility to EBA development is associated with an increased formation of autoreactive Tfh cells, which promote the autoreactive plasma cell response and the secretion of vWFA2-specific autoantibodies.

B6.s and C57BL/6J mice exhibit altered Treg function and different cytokine profiles of Tfh cells

To test whether the discrepancies in the Treg frequencies could provide the basis for the quantitatively different populations of Tfh cells and autoantibody levels in C57BL/6J and B6.s mice, Tregs were quantified in dLNs. These tissues are the major sites of antibody production in this model (Tiburzy et al., 2013). Neither the frequencies of total $CD4^+$/$FoxP3^+$ Tregs nor the proportion of vWFA2-reactive Treg populations was different between EBA-susceptible B6.s mice and non-susceptible C57BL/6J mice on day 10 after immunization (Supplementary Figure S10). At that time, the two mouse strains showed already substantial differences in their autoreactive plasma cell responses and autoantibody levels. These results indicate that the different MHCII haplotypes had no impact on the frequencies of total or autoantigen-specific Tregs that could be relevant for the massive differences observed in the levels of vWFA2-specific autoantibodies. However, Tregs from susceptible B6.s mice showed a lower capacity to suppress the proliferation of T-effector cells in vitro than the Tregs from C57BL/6J mice (Supplementary Figure S11).

IL-10 is important for the immunosuppressive function of Tregs (Jin et al., 2013; Kemper et al., 2003; Roncarolo et al., 2018; Sojka and Fowell, 2011). As observed by intracellular staining after short-term restimulation with phorbol 12-myristate 13-acetate/ionomycin/lipopolysaccharide, $CD4^+$ T cells from C57BL/6J and B6.s mice contained similar frequencies and numbers of IL-10–producing cells (Supplementary Figures S12 and S13a). In contrast, $CD4^+$ T cells from susceptible B6.s mice produced more IFN-γ and IL-21 than those from nonsusceptible C57BL/6J mice. Moreover, Tfh cells from B6.s mice produced more proinflammatory IFN-γ as well as more IL-10 and IL-21 (Figure 6 and Supplementary Figure S13b). Both IL-10 and IL-21 are known to promote plasma cell formation and antibody production (Guthmiller et al., 2017; Heine et al., 2014; Linterman et al., 2010; Odegard et al., 2008; Xin et al., 2018), whereas T-cell–derived IFN-γ promotes the generation of IgG with a proinflammatory Fc *N*-glycosylation pattern (Hess et al., 2013). Furthermore, IL-21 can suppress the expression of β-galactoside α2,6-sialyltransferase in antibody-secreting plasma cells, which controls the post-translational transfer of sialic acid to IgG-linked glycans (Pfeifle et al., 2017).

The majority of the antigen-presenting cell populations did not show significant differences in the expression of the costimulatory molecules CD80 and CD86 or the checkpoint molecules PD-L1 and indoleamine 2,3-dioxygenase between vWFA2-immunized B6.s and C57BL/6J mice. However, red

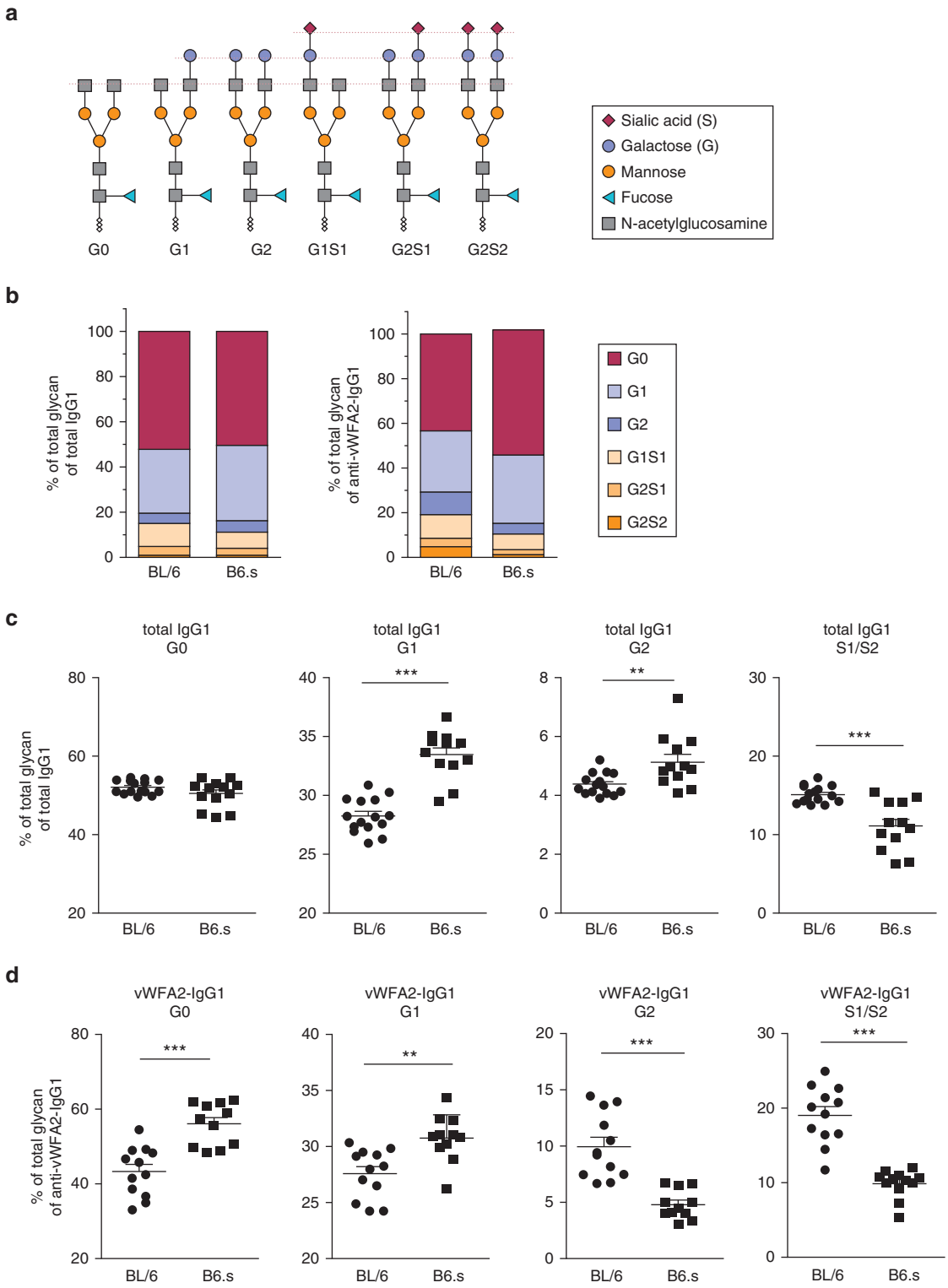

Figure 3. Antibody glycosylation in vWFA2-immunized mice. (**a**) Schematic Fc glycan pattern of murine IgG Fc glycan-composition. Fc *N*-glycosylation pattern of the (**b, c**) total and (**d**) vWFA2-specific IgG1 autoantibodies in B6.s and C57BL/6J (BL/6) mice at 12 weeks after induction of experimental EBA by immunization with vWFA2. IgG from sera was purified using protein G-sepharose beads (total IgG) or beads coated with vWFA2 and subsequently digested with trypsin. Purification of *N*-linked glycans and glycan analysis were performed using LC-MS/MS as described in the Materials and Methods (n = 11–15; statistic: two-tailed unpaired *t*-test). $^{**}p \leq 0.01$; $^{***}p \leq 0.001$. Data are expressed as mean ± SEM. EBA, epidermolysis bullosa acquisita; Fc, fragment crystallizable; G, galactose; LC-MC/MC, liquid chromatography–mass spectrometry/mass spectrometry; S, sialic acid; vWFA2, von Willebrand factor A–like domain 2.

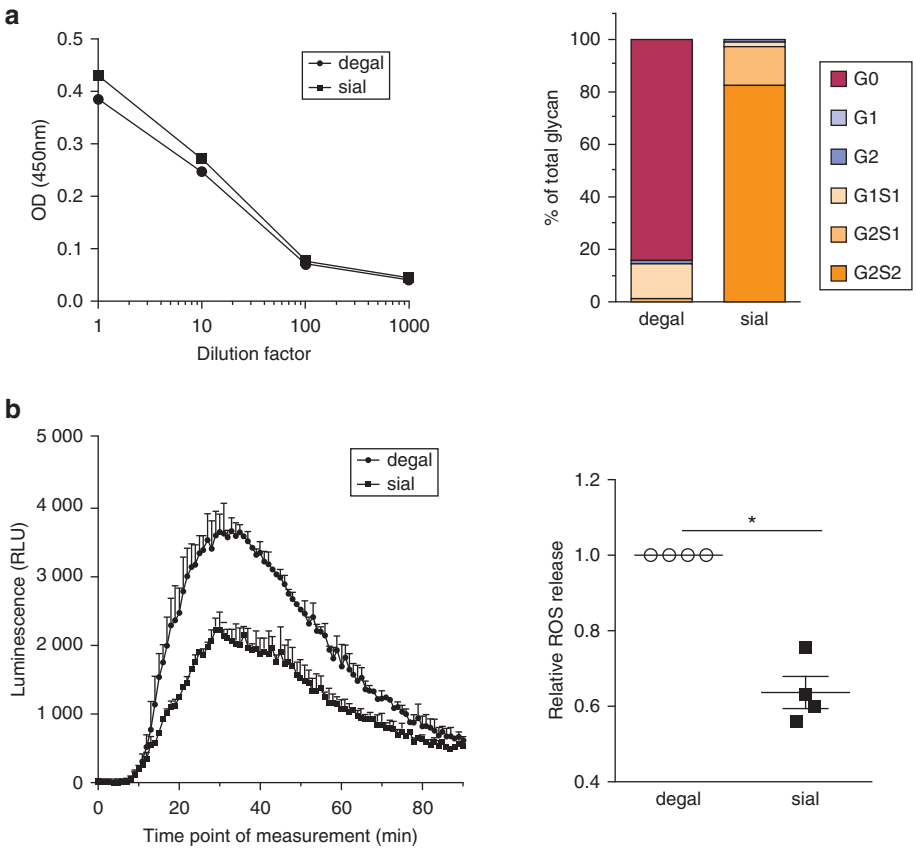

Figure 4. Sialylation of murine IgG1 attenuates neutrophilic activation and release of ROS in vitro. (**a**) In vitro differentially glycosylated murine IgG1 (clone H5) showed similar antigen binding as tested by ELISA (left) but different glycosylation pattern (right). (**b**) Representative analysis of ROS release by isolated neutrophils from naive C57BL/6J mice with a luminol-amplified luminescence assay. Neutrophils were stimulated with either 7.5 μg/ml immobilized degal (>85% G0) or sial (<5% nonsial) mouse IgG1 antibodies. Total ROS release was calculated as AUC and AUC values of independent experiments normalized to degal IgG1. Data from two individual experiments are shown (n = 4; statistic: two-tailed Mann–Whitney test). Data are expressed as mean ± SEM. *$P \leq 0.05$. AUC, area under the curve; degal, degalactosylated; G, galactose; min, minute; OD, optical density; RLU, relative luminescence unit; S, sialic acid; sial, sialylated.

pulp macrophages and monocyte-derived macrophages from B6.s mice expressed higher levels of $CD80^+/CD86^+$, and indoleamine 2,3-dioxygenase was also upregulated in the subcapsular sinus macrophages in the dLNs of B6.s mice (Supplementary Figures S14 and S15).

Collectively, these results suggest that the H2s MHCII haplotype balances proinflammatory autoantibody IgG Fc *N*-glycosylation through reduced Treg suppression capacities, altered antigen-presenting cell functions, and the regulation of Tfh cells that produce IFN-γ and IL-21, resulting in IgG autoantibodies with a high potential to activate neutrophils.

DISCUSSION

Like for most other autoimmune disorders, genetic predisposition has a major impact on the susceptibility to murine immunization-induced EBA (Ludwig et al., 2012, 2011), and clinical studies indicate that this is also true for human EBA (Gammon et al., 1988; Zumelzu et al., 2011).

Our data suggest that in addition to the increased formation of autoreactive lymphocytes, genetic susceptibility to EBA is also associated with qualitative changes in the response of $CD4^+$ T-effector cells, Tfh cells, and antibody glycosylation. An earlier study showed that skin depositions of susceptible B6.s mice predominantly contain antibodies of the complement-fixing IgG2b/c subclasses, whereas the skin of C57BL/6J mice showed more IgG1 that has less inflammatory potential. On the basis of this finding and the observation that B6.s mice produce more IFN-γ, it was concluded that a T helper type 1 polarization in B6.s mice results in the predominant production of complement-fixing, pathogenic IgG2b/c antibodies (Hammers et al., 2011). Our data do not support the idea that a shift of the IgG2b/c to IgG1 ratio is involved in the differential disease susceptibility of B6.s mice compared with that of C57BL/6J mice. This observation does not exclude the possibility that altered ratios of inflammatory-to-noninflammatory autoantibody subclasses contribute to disease development but that they are not the consequence of a difference in the MHCII haplotype.

We found that autoantibodies from B6.s mice exhibited an increased potential to induce an oxidative burst in innate effector cells, which is important to drive autoimmune-blistering skin diseases (Caielli et al., 2012; Ludwig et al., 2017; Sadeghi et al., 2015; Sezin et al., 2017). The differential inflammatory potential of autoantibodies from susceptible and nonsusceptible mouse strains was associated with different IgG Fc *N*-glycosylation pattern. By using differentially glycosylated monoclonal IgG1, we could clearly demonstrate that ROS release by neutrophils, the critical effector mechanism of EBA skin disease, strongly depends on the antibody glycosylation pattern. These observations are in line with the notion that IgG Fc *N*-glycosylation tips the balance of the proinflammatory versus anti-inflammatory potential of IgG antibodies (Bartsch et al., 2018; Oefner et al., 2012; Seeling et al., 2017) and identifies neutrophils as the prominent targets of IgG autoantibodies exhibiting a proinflammatory Fc *N*-glycosylation pattern.

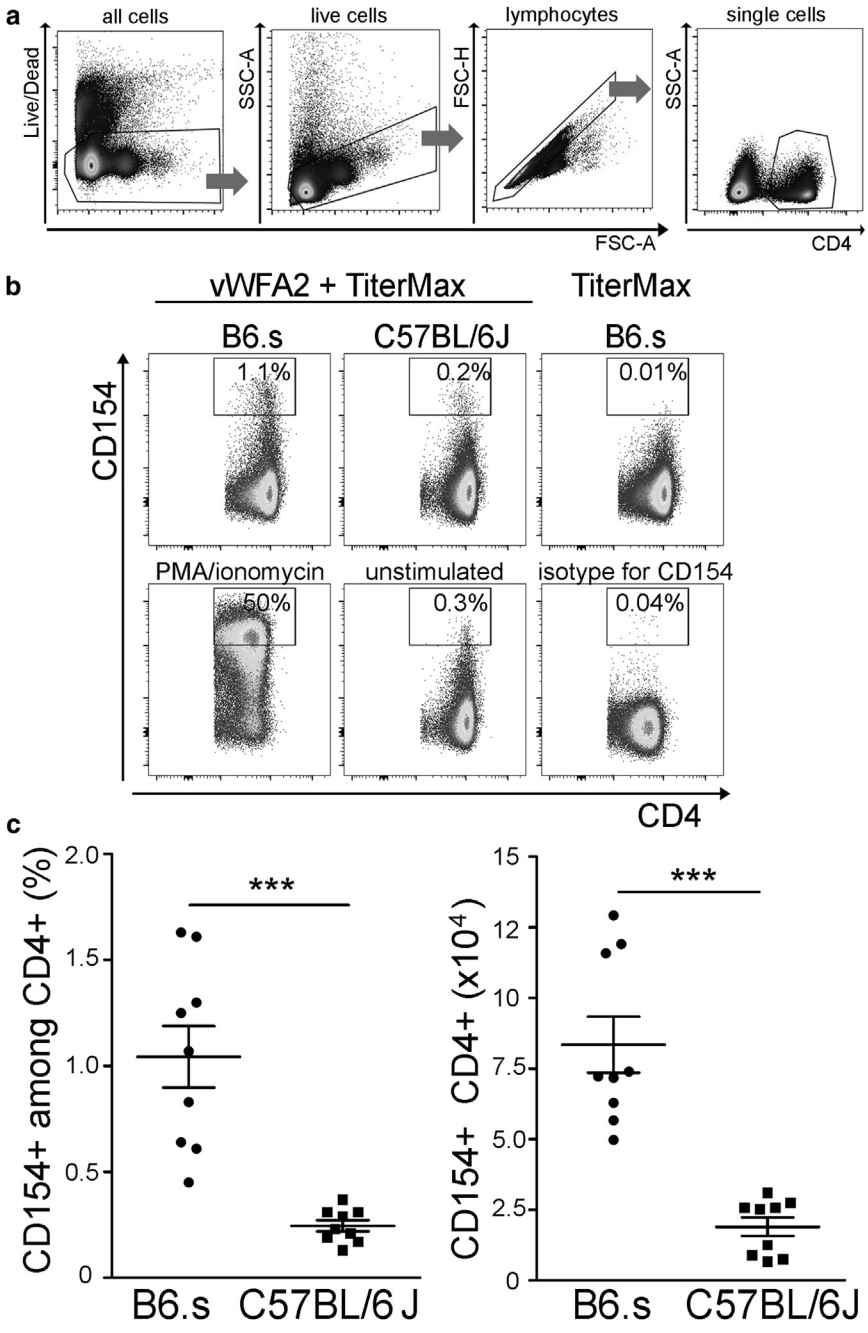

Figure 5. Autoreactive CD4$^+$ T cells in vWFA2-immunized mice. Mice were immunized with vWFA2 andTitermax, and cells from dLN were analyzed by flow cytometry 10 days later. (**a**) Single-cell suspensions were stimulated by autoantigen or PMA/ionomycin as described in the Supplementary Materials and Methods. Gating: dead cells, debris, and doublets were excluded by live and/or dead stain and FSC and/or SSC. Antigen-specific cells were identified among the CD4-positive population as indicated. (**b**) vWFA2-specific CD4$^+$ T cells were identified by CD154 expression after restimulation. Positive control: PMA/ionomycin/LPS-stimulated cells (lower panel, left). Negative controls: cells from a mouse immunized with Titermax only (upper panel, right), unstimulated cells (lower panel, middle), and cells stained with an isotype control (lower panel, right). (**c**) Frequency of vWFA2-specific CD154$^+$ cells among CD4$^+$ T cells (left) and cell numbers of vWFA2-specific CD154$^+$ and/or CD4$^+$ T cells (right). Each dot represents data from a single mouse (n = 9; statistic: two-tailed unpaired *t*-test). Data are expressed as mean ± SEM. ***$p \leq 0.001$. dLN, draining lymph node; FSC, forward scatter; LPS, lipopolysaccharide; PMA, phorbol 12-myristate 13-acetate; SSC, sideward scatter; vWFA2, von Willebrand factor A–like domain 2.

The type of Fc *N*-glycosylation correlates with different pathogenic or immunosuppressive effector functions of IgG by changing the interaction with complement and FcγRs (Epp et al., 2018; Karsten et al., 2012; Nimmerjahn and Ravetch, 2008). This interaction between IgG and FcγRs on innate effector cells such as neutrophils is of crucial importance for the activation of these cells. These findings imply that differential IgG Fc *N*-glycosylation contributes to EBA susceptibility of B6.s mice. Our findings indicate that measurement of the IgG Fc *N*-glycosylation pattern and IgG subclass distribution could help to improve the predictive value of autoantibody diagnostics in autoimmune-blistering diseases.

Although IgG1 exhibits a higher affinity for the inhibitory FcγRIIB than for the activating FcγRs (Nimmerjahn and Ravetch, 2008), we found that degalactosylated IgG1 was able to induce robust ROS release by neutrophils. Sialylated IgG1 antibodies show reduced binding to both the activating FcγRs and the inhibitory FcγRIIB (Kaneko et al., 2006). Therefore, it is unlikely that the reduced ROS release induction by sialylated IgG1 is mediated through direct stimulation of FcγRIIB. Because sialylated IgG1-antigen immune complexes are known to inhibit proinflammatory immune responses independent of FcγRIIB (Collin and Ehlers, 2013; Oefner et al., 2012), differential binding of IgG1 to C-type lectin–like receptor Dectin-1 could modulate its association with FcγRIIB and eventually affect neutrophil activation, a mechanism that was shown to have a strong impact on chemoattractant receptor–driven extracellular

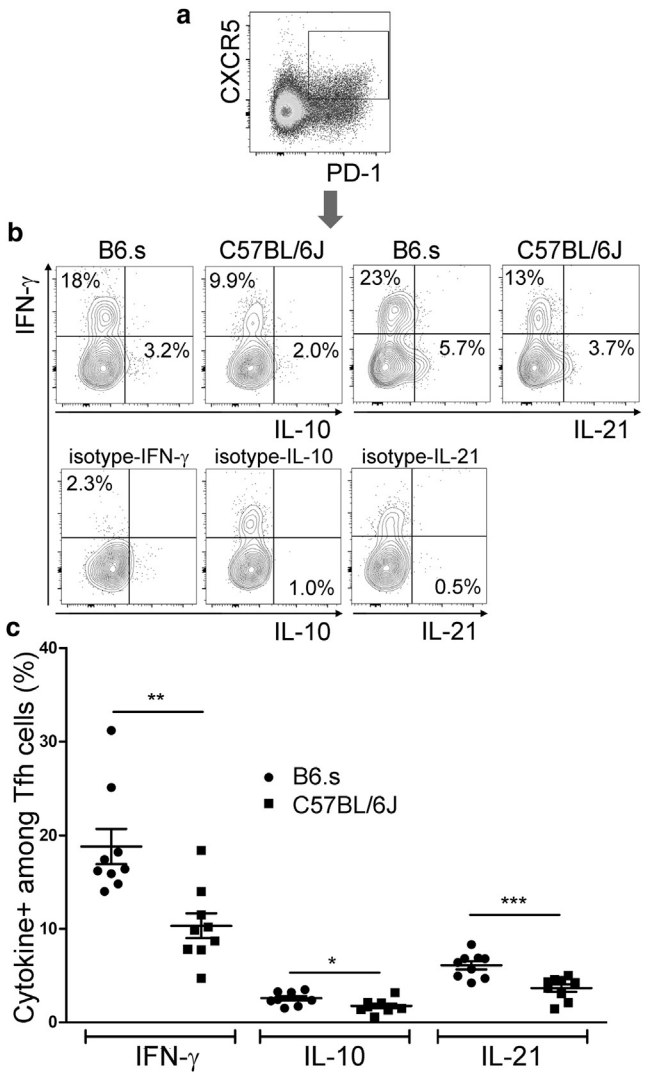

Figure 6. Cytokine expression by Tfh cells from vWFA2-immunized mice. B6.S and C57BL/6J mice were immunized with vWFA2 andTitermax, and cells from dLN were collected 10 days later. Cells were restimulated by PMA/Ionomycin/LPS and stained for intracellular cytokine expression. (**a**) $CD4^+$ cells were gated, and Tfh cells were identified by the expression of CXCR5 and PD-1. (**b**) Representative flow cytometry data of cytokine stainings and isotype controls are shown as indicated. (**c**) Frequencies of IFN-γ^+, IL-10^+, and IL-21^+ cells among Tfh cells. Each dot represents data from a single mouse (n = 9; statistic: two-tailed unpaired *t*-test). Data are expressed as mean ± SEM. $^*P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$. dLN, draining lymph node; LPS, lipopolysaccharide; PMA, phorbol 12-myristate 13-acetate; Tfh, T follicular helper; vWFA2, von Willebrand factor A–like domain 2.

signal–regulated kinase 1/2 phosphorylation and neutrophil migration (Karsten et al., 2012). Alternatively, the weakened binding to activating FcγRs could be an explanation for the reduced neutrophil activation.

In this study, we confirm that a nonsusceptible genotype is not necessarily associated with the absence of an autoimmune reaction, as evidenced by a considerable autoreactive B- and T-cell response in C57BL/6J mice. Moreover, some individual nondiseased C57BL/6J mice developed autoantibody levels comparable with those of diseased B6.s mice, and the autoantibody levels in the serum of individual B6.s mice correlated poorly with their disease score. Hence, the control of immunological tolerance, which deletes or anergizes autoreactive lymphocytes, is not the only mechanism by which genetic susceptibility modulates the development of EBA. This seems to be similar in humans, where antibodies reactive to skin autoantigens were also found in some individuals, which did not show any skin pathology (Prüßmann et al., 2015).

In patients, susceptibility to EBA development is associated with the MHCII haplotype HLA-DR (Gammon et al., 1988; Zumelzu et al., 2011), whereas murine EBA is associated with the H2s haplotype (Ludwig et al., 2011). Our data indicate that this MHCII haplotype is associated with different T-cell responses and differentially glycosylated IgG antibodies with distinct potencies to induce ROS release by neutrophils, a major effector cell in blistering skin diseases.

Tregs can limit autoantibody-mediated skin inflammation in patients and mouse models (Bieber et al., 2017b; Haeberle et al., 2018; Muramatsu et al., 2018). Interestingly, we found that B6.s and C57BL/6J mice showed similar populations of Tregs, which however exhibit distinct suppressive capacities. We suspect that the reduced Treg function in B6.s mice allows for the observed increased production of proinflammatory IFN-γ and IL-21 by Tfh cells.

Collectively, we propose that the MHCII haplotype determines the suppressive capacity of Tregs and the cytokine profile of T-effector and Tfh cells, eventually leading to a change in the ratio between inflammatory and less inflammatory antibody subclasses and IgG Fc *N*-glycosylation patterns. Mechanistically, our data link proinflammatory IgG Fc *N*-glycosylation with an increased potency of IgG autoantibody-mediated ROS release by neutrophils, which is the main driver of EBA skin inflammation but also important in many other diseases. Furthermore, they indicate that merely the determination of the autoantibody levels alone is not sufficient for optimal diagnosis but that the analysis of the IgG Fc *N*-glycosylation pattern and/or the measurement of the actual pathogenic potency of serum autoantibodies are needed to improve autoantibody diagnostics.

MATERIALS AND METHODS

EBA induction and scoring

Production and purification of recombinant murine vWFA2, immunization, and scoring were performed as described (Bieber et al., 2017a; Hirose et al., 2017; Iwata et al., 2013). Details are found in Supplementary Materials and Methods.

Flow cytometry

Antigen-specific plasma cells were stained with antigen, as previously described (Kulkarni et al., 2016; Tiburzy et al., 2013). Antigen-specific T cells were analyzed by the rapid antigen-induced upregulation of CD154 (Frentsch et al., 2005; Tiburzy et al., 2013). Details are found in Supplementary Materials and Methods.

Determination of the IgG Fc glycan composition by liquid chromatography–mass spectrometry/mass spectrometry analysis

Purified IgG was analyzed, and data were processed as described (de Haan et al., 2017; Falck et al., 2017). Details are found in Supplementary Materials and Methods.

Study approval

The animal experiments conducted in this study were done in strict accordance with the German regulations of the Society for Laboratory Animal Science and the European Health Law of the Federation of Laboratory Animal Science Associations. All animal experiments were approved by the respective local committees on the ethics of animal experiments of the state Schleswig-Holstein (Ministerium für Landwirtschaft, Umwelt und ländliche Räume des Landes Schleswig-Holstein). Approval numbers are V242-65339/2018 (12-2/18), V242-22797/2016 (43-4/16), V242-43397/2016 (84-7/16).

Data availability statement

Datasets related to this article can be found at https://doi.org/10.17632/9pgfdgjfc7.2, an open-source online data repository hosted at Mendeley Data.

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CONFLICT OF INTEREST

The authors state no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: AKC, YCB, AK, JK, ME, RAM; Investigation: AKC, YCB, AK, GK, GML, LNA, TL, JP, CNR, JT, SK, AGS, CE; Project Administration: AKC, RAM; Resources: RJL, KB, MW, ME, LD, JK; Supervision: AKC, JK, MW, KB, ME, RAM; Writing - Original Draft Preparation: RAM; Writing - Review and Editing: AKC, YCB, AK, JK, RJL, SMI, KB, ME, RAM

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2020.06.022.

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