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Cell Depletion in Mice That Express Diphtheria Toxin Receptor under the Control of SiglecH Encompasses More Than Plasmacytoid Dendritic Cells

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Susan Gilfillan,* William Vermi,*§ Christiane Ruedl,§ Giorgio Trinchieri,† and Marco Colonna* Plasmacytoid dendritic cells (pDC) produce IFN-I in response to viruses and are routinely identified in mice by SiglecH expression. SiglecH is a sialic acid–binding Ig-like lectin that has an immunomodulatory role during viral infections. In this study, we evaluated the impact of SiglecH deficiency on cytokine responses in the presence and absence of pDC. We found that lack of SiglecH enhanced IFN-I responses to viral infection, regardless of whether pDC were depleted. We also examined the expression pattern of SiglecH and observed that it was expressed by specialized macrophages and progenitors of classical dendritic cells and pDC. Accordingly, marginal zone macrophages and pDC precursors were eliminated in newly generated SiglecH–diphtheria toxin receptor (DTR)–transgenic (Tg) mice but not in CLEC4C-DTR–Tg mice after diphtheria toxin (DT) treatment. Using two bacterial models, we found that SiglecH-DTR–Tg mice injected with DT had altered bacterial uptake and were more susceptible to lethal Listeria monocytogenes infection than were DT-treated CLEC4C-DTR–Tg mice. Taken together, our findings suggest that lack of SiglecH may affect cytokine responses by cell types other than pDC during viral infections, perhaps by altering viral distribution or burden, and that cell depletion in SiglecH-DTR–Tg mice encompasses more than pDC. The Journal of Immunology, 2014, 192: 4409–4416.

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Abbreviations used in this article: BAC, bacterial artificial chromosome; BM, bone marrow; cDC, classical dendritic cell; DC, dendritic cell; DT, diphtheria toxin; DTR, DT receptor; LM-OVA, Listeria monocytogenes expressing OVA; LN, lymph node; MCMV, murine CMV; MM, metallophilic macrophage; MZM, marginal zone macrophage; pDC, plasmacytoid DC; p.i., postinfection; prepDC, precursor of pDC; qPCR, quantitative PCR; RT, room temperature; Tg, transgenic; WT, wild-type.
Given that SiglecH deficiency may affect cytokine responses by cells other than pDC, we decided to evaluate the expression pattern of SiglecH in vivo using anti-SiglecH mAb and heterozygous SiglecH<sup>ΔH/++</sup> mice. We found that SiglecH was expressed by pDC and specialized macrophage subsets, such as marginal zone macrophages (M2M), lymph node (LN) medullary macrophages, and microglia. SiglecH also was expressed by immediate precursors of pDC (pre-pDC) in the bone marrow (BM), which have the plasticity to differentiate into pDC and classical DC (cDC) (12, 13). Analysis of Siglec-H-DTR–Tg and CLEC4-DTR–Tg mice indicated that M2M and pre-pDC were eliminated in DT-treated Siglec-H-DTR–Tg mice but not in CLEC4-DTR–Tg mice. Therefore, administration of DT to mice that express DTR under the control of the SiglecH promoter might affect a large number of APCs. Indeed, using two bacterial infection models, we found that SiglecH-DTR–Tg mice injected with DT had altered bacterial uptake and were more susceptible to lethal Listeria monocytogenes infection than were DT-treated CLEC4-DTR–Tg mice. Thus, we envision that the broad expression pattern of SiglecH potentially explains why data derived from inducible pDC-ablation models may be different.

Materials and Methods

Mice, treatments, and infections

Animal studies were approved by the Washington University Animal Studies Committee. Siglec-H-eGFP knockout mice and CLEC4-C4-DTR–Tg mice, both on a C57BL/6 background, were bred in-house (3). Siglec-H-DTR–Tg mice were generated and bred at the National Institutes of Health (C57BL/6) or at Nanyang Technological University (BMALc). CLEC4-C4-DTR–Tg mouse and SiglecH-DTR–Tg mice were injected i.p. with 100–200 ng or 200–500 ng DT (Sigma–Aldrich), respectively. Non-Tg control mice also were injected with DT in some experiments. CpgA 2216 (Oriosp; 6 μg/mouse) was complexed with DOTAP and injected i.v. HSV-1 KOS strain was injected i.v. at 1 × 10<sup>7</sup> PFU. MCMV Smith strain was injected i.p. at 5 × 10<sup>5</sup> PFU. L. monocytogenes expressing OVA (LM-OVA) (14) was injected i.p. at 2.5 × 10<sup>7</sup> CFU. Alexa Fluor 647–labeled, heat-killed Streplococcus pneumoniae R36A was a generous gift from J.F. Kearney (University of Alabama at Birmingham, Birmingham, AL) and was injected i.v. at ~1 × 10<sup>8</sup> CFU/mouse.

Generation of Siglec-H-DTR–Tg mice

C57BL/6–Tg(Siglec-H/ΔH-EGFP)NCR–Tg mice were generated by bacterial artificial chromosome (BAC) recombination. The BAC clone encoding the complete SiglecH gene locus (RP24-265E12) was obtained from the BACPAC Resources Center at Children’s Hospital Oakland Research Institute (Oakland, CA). The BAC clone was modified by recombination using a shuttle vector containing a bicistronic cassette consisting of the cDNA sequences encoding for human DTR and eGFP. The cassette was flanked by two homologous regions targeting the transgenes to the desired site of insertion (SiglecH exon I, after the second triplet of the open reading frame). The modified BAC clone was linearized and injected into the pronuclei of fertilized C57BL/6/NCr oocytes at the Laboratory Animal Science Program facility (National Cancer Institute). Single-cell embryos were implanted in pseudoglandular females, and litters were screened to select Tg mouse founders. Two Tg mouse lines with high transgene expression were established. The plasmid containing the human DTR sequence used in the shuttle vector preparation was a generous gift of Dr. T. Walzer (Université de Lyon, Marseille, France). Siglec-H-DTR–Tg mice on a BALB/c background were generated via BALB/c embryonic stem cells transfected with recombined BAC clones (Siglec-H: RP24-265E12) carrying insertions of human DTR sequences encoding for human DTR and eGFP. The cassette was flanked by two homologous regions targeting the transgenes to the desired site of insertion (SiglecH exon I, after the second triplet of the open reading frame). The modified BAC clone was linearized and injected into the pronuclei of fertilized C57BL/6/NCr oocytes at the Laboratory Animal Science Program facility (National Cancer Institute). Single-cell embryos were implanted in pseudoglandular females, and litters were screened to select Tg mouse founders. Two Tg mouse lines with high transgene expression were established. The plasmid containing the human DTR sequence used in the shuttle vector preparation was a generous gift of Dr. T. Walzer (Université de Lyon, Marseille, France). Siglec-H-DTR–Tg mice on a BALB/c background were generated via BALB/c embryonic stem cells transfected with recombined BAC clones (Siglec-H: RP24-265E12) carrying insertions of human DTR sequences encoding for human DTR and eGFP. The cassette was flanked by two homologous regions targeting the transgenes to the desired site of insertion (SiglecH exon I, after the second triplet of the open reading frame).

Generation of Siglec-H-DTR–Tg BM chimeras

BM from C57BL/6 Siglec-H-DTR–Tg mice was prepared from tibias and femurs. Microglia were isolated as described (16). pDC were enriched from BM by negative selection using the Plasmacytoid Dendritic Cell Isolation Kit II (Miltenyi Biotec) and stimulated with CpGA (3 or 6 μg/ml). Purity was ~50% after enrichment. FACS-sorted pDC (purity > 98%) were stimulated with CpGA (6 μg/ml) or MCMV tissue culture stock (multiplicity of infection: 10:1). Cells were cultured in 96-well flat bottom plates at 0.25–1 × 10<sup>5</sup> cells/well with CpGA or MCMV. Sorted prepDC (1 × 10<sup>3</sup> cells/well) were cultured in 96-well flat-bottom plates in complete medium with GM-CSF (1 ng/ml) or Fli3L (10 ng/ml) (PeproTech) for 3 d.

Ab, flow cytometry, and cell sorting

Ab were purchased from BioLegend, eBioscience, or BD Biosciences. The following clones were used: SiglecH (551 or 440c), CD11c (HL3), Ly6C (AL-21), CCR9 (eBioCW-1.2), B220 (RA3-6B2), CD11b (M1/70), CD45 (30-F11), CD4 (GK1.5), CD8α (53-67), Gr-1 (RB6-8C5), F4/80 (BM8), and IA/IE (MS/114.15.2). Flow cytometry was conducted on a FACS Calibur or FACS Canto (BD Biosciences) and analyzed with FlowJo software (TreeStar). Cell sorting was performed on a FACSAria II (BD Biosciences). Mature pDC and prepDC were defined as sorted as SiglecH<sup>+/B220+</sup>CCR9<sup>+</sup> and SiglecH<sup>+/B220+</sup>CCR9<sup>+</sup> cells, respectively. pDC were sorted from spleens into CD11<sup>ci</sup>B220<sup>+</sup>MHCII<sup>+</sup>CDC8<sup>+</sup> and CD11<sup>ci</sup>B220<sup>+</sup>MHCII<sup>+</sup>CDC8<sup>+</sup> subsets.

Cytokine analysis and quantitative PCR

IFN-α levels were determined by ELISA (PBL Interferon Source). Cytokines were measured by Cytometric Bead Array (BD Biosciences). Expression levels of SiglecH and E2-2 were measured by quantitative PCR (qPCR) and normalized to GAPDH. Primers used for qPCR included SiglecH forward primers: 5’-ATT TTG TCT GTG AGG AAA GGA TGC 3’ and 5’-AAT TCA CAG AAT ACC AAA AGT TGA G-3’. Cells were cultured in 96-well flat-bottom plates in complete media for 24 h. Analysis of WT and SiglecH-eGFP reporter mice. (A) SiglecH–GFP<sup>+/+</sup> mice were infected or not with HSV-1, and spleens were analyzed 8 and 24 h later for pDC (Ly6C<sup>+</sup>SiglecH<sup>+</sup>eGFP<sup>+</sup>). (B) Body weights and levels of blood urea nitrogen, creatinine, and total protein in blood from WT and SiglecH<sup>+/GFP</sup> mice. Data are from two independent experiments. (C) Representative light microscopy images of H&E-stained kidney sections revealed no pathologic abnormalities in SiglecH-deficient mice (original magnification ×40).
Blood work and kidney histology

Whole blood was collected by cardiac puncture into EDTA tubes for complete blood counts or in serum-collection tubes for measurement of blood urea nitrogen, creatinine, and total protein. Tests were performed by the Division of Comparative Medicine at Washington University School of Medicine. For H&E staining, kidneys were fixed with 10% buffered formalin solution and embedded in paraffin; 5-μm sections were prepared and stained with H&E.

Immunohistochemistry and immunofluorescence

For immunohistochemistry, 5-μm frozen tissue sections were used for immunohistochemical staining to visualize SiglecH+ cells in spleens and LN. Digital images were taken using an Olympus BX60 microscope, captured using a DP-70 Olympus digital camera, and processed using Analysis Image Processing software (Olympus). For immunofluorescence, 8-μm frozen spleen sections were fixed in acetone for 5 min at room temperature (RT) and stored at −80°C. Frozen sections were blocked with 10% horse serum for 20 min at RT. Primary Ab to MARCO, Gr-1, SIGN-R1, and Sialoadhesin were applied to tissue sections for 30 min at RT. After washing with PBS, fluorescent-conjugated anti-rat secondary Ab were added to slides for 30 min at RT. Slides were mounted with Fluoromount-G and imaged on a Zeiss LSM 510 META confocal laser scanning microscope. Immunofluorescence images were adjusted globally for brightness and contrast using Adobe Photoshop CS6.

Statistical analysis

Statistical significance was analyzed with an unpaired, two-tailed Student t test or Mann–Whitney U test. The p values < 0.05 were considered statistically significant. For susceptibility studies, p values were determined by the log-rank test.

Results

SiglecH effectively identifies pDC in steady-state and during infection

SiglecH is a DAP12-associated receptor used to discriminate pDC from other cell types in mice (9, 10). Our previous work (17) showed that pDC numbers are reduced in spleens of infected mice, which appeared to be a consequence of cell death. A recent study (11) indicated that, following stimulation with CpG or infection with MCMV, SiglecH is downregulated in a TLR9/MyD88-dependent manner. To determine whether reduced pDC numbers during...
infection are due to reduced detection of SiglecH expression, we compared SiglecH and eGFP expression in pDC from spleens of SiglecH<sup>GFP/+</sup> mice that were infected or not with HSV-1. Using markers to identify pDC, such as Ly6C<sup>+</sup> and CD11c<sup>+</sup>, we found that frequencies of Ly6C<sup>+</sup>eGFP<sup>+</sup> and Ly6C<sup>+</sup>SiglecH<sup>+</sup> cells were comparable and reduced in spleen to a similar extent 8 and 24 h postinfection (p.i.) (Fig. 1A, data not shown), indicating that anti-SiglecH Ab is effective at identifying pDC in the steady-state and during viral infection.

**SiglecH<sup>GFP/+</sup> mice are healthy in steady-state**

A recent report (18) suggested that an independently derived SiglecH-deficient mouse strain had abnormal kidney pathology and function. Therefore, we evaluated body weight and performed blood work analyses on WT and SiglecH<sup>GFP/+</sup> mice (Fig. 1). WT and age/gender-matched SiglecH<sup>GFP/+</sup> mice had comparable body weights, percentages of segmented neutrophils and lymphocytes in circulation, and levels of blood urea nitrogen, creatinine, and total protein (Fig. 1B, data not shown). Consistent with the absence of biochemical abnormalities in renal function, no pathologic changes were observed by light microscopy in the glomeruli or tubules of SiglecH<sup>GFP/+</sup> mice (Fig. 1C). Thus, SiglecH<sup>GFP/+</sup> mice appear to be healthy and have normal kidney function in steady-state.

**SiglecH deficiency does not impact IFN-I production by pDC ex vivo**

It was shown that SiglecH has an immunomodulatory role during inflammation and viral infections (6, 9, 11). To determine whether SiglecH deficiency altered cytokine production by pDC, we measured IFN-α levels in supernatants from enriched or sort-purified pDC from WT and SiglecH<sup>GFP/+</sup> mice stimulated ex vivo with CpGA. CpGA is a synthetic TLR ligand that induces IFN-I production by pDC through TLR9 (19). pDC from WT and SiglecH<sup>GFP/+</sup> mice produced comparable amounts of IFN-α in response to CpGA (Fig. 2A, 2B), suggesting that lack of SiglecH does not strongly alter IFN-I production by pDC ex vivo. We next evaluated IFN-I responses to CpGA in vivo. WT and SiglecH<sup>GFP/+</sup> mice were injected i.v. with CpGA complexed with DOTAP, and serum IFN-α was measured 6 h later (Fig. 2C). Analyses of several mice revealed no significant differences in serum IFN-α levels between WT and SiglecH<sup>GFP/+</sup> mice. IFN-α was not detectable in serum from naive WT or SiglecH<sup>GFP/+</sup> mice (data not shown).

We next evaluated whether SiglecH deficiency influenced IFN-I production by pDC after exposure to a live virus. MCMV is sensed by pDC through TLR9 (20). Moreover, it was reported that pDC are an important and early source of IFN-I during MCMV infection (3, 20–23). Thus, we sort-purified pDC from WT and SiglecH<sup>GFP/+</sup> mice and cultured them with MCMV, pDC from both groups of mice produced similar levels of IFN-α (Fig. 2D), indicating that SiglecH deficiency did not affect IFN-I production by virus-stimulated pDC ex vivo. We next evaluated whether mice lacking SiglecH had altered IFN-I responses in vivo during MCMV infection. We found that SiglecH<sup>GFP/+</sup> mice had increased levels of systemic IFN-α compared with WT mice at 48 h p.i. (Fig. 2E), similar to a recent study (11). Taken together, these data suggest that SiglecH deficiency does not affect IFN-I secretion by pDC in response to a synthetic TLR9 ligand or virus ex vivo, but it may influence IFN-I production by pDC during viral infection in vivo.

**Enhanced cytokine responses to HSV-1 in SiglecH<sup>GFP/+</sup> mice occur in the presence and absence of pDC**

SiglecH<sup>DTR/DTR</sup> mice were reported to have increased cytokine responses to systemic HSV-1 infection (6). Corroborating these findings, SiglecH<sup>GFP/+</sup> mice also had slightly elevated levels of systemic IFN-α and proinflammatory cytokines compared with WT mice after HSV-1 infection (Fig. 3A–C). These results are consistent with an immunomodulatory role for SiglecH in antiviral responses (6, 9, 11). To determine whether this effect was due to pDC, we bred CLEC4C-DTR–Tg mice to SiglecH<sup>GFP/+</sup> and SiglecH<sup>GFP/+</sup> mice and infected them with HSV-1 in the presence or absence of pDC. We found that SiglecH<sup>GFP/+</sup> mice depleted of pDC produced more IFN-α than did pDC-depleted SiglecH<sup>GFP/+</sup> mice or pDC-depleted CLEC4C-DTR–Tg mice (Fig. 3D). In all three lines of depleted mice, there was an ~2-ng reduction in serum IFN-α levels relative to their undepleted counterparts. These findings suggest that lack of SiglecH may affect cytokine secretion by cells other than pDC during viral infection or that viral burden and/or distribution is altered in the absence of SiglecH.

**SiglecH is expressed by MZM, medullary macrophages, microglia, and progenitors of cDC and pDC**

Although SiglecH expression is mainly confined to pDC in cell suspensions from primary and secondary lymphoid organs, it was observed by microscopy that specialized macrophage subsets in the spleen and LN express SiglecH (10). Corroborating this, we found that MZM in spleen (Fig. 4A) and medullary macrophages in LN (data not shown) were SiglecH<sup>+</sup> by immunohistochemistry. In addition, we observed that SiglecH was expressed by CD4<sup>+</sup>CD11b<sup>+</sup> microglia in brain (Fig. 4B), in agreement with a recent study (24).

We reported previously that cDC are eGFP<sup>+</sup> in SiglecH<sup>GFP/+</sup> mice (3). Closer examination revealed that both CD4<sup>+</sup> and CD4<sup>+</sup>cDC in spleen were eGFP<sup>+</sup> (Fig. 5A); however, they did not express SiglecH on the surface or at the transcript level (Fig. 5B, 5C).
data not shown). These findings indicated that the SiglecH promoter was active in DC progenitors during development, consistent with a recent study (25), and that eGFP persists in differentiated or mature cDC. In the BM, there is a subset of SiglecH+ cells that has been defined as prepDC (Fig. 5C), which can differentiate into pDC and cDC in vitro and in vivo (Fig. 5D) (12, 13). Both mature pDC and prepDC expressed the pDC-specific transcription factor Tcf4/ E2-2 (Fig. 5E) (12, 26) and were eGFP+ in SiglecH-GFP mice (data not shown). Taken together, these data indicate that SiglecH is expressed by mature pDC, specialized macrophages, and progenitors of cDC and pDC.

**PrepDC and MZM are depleted in SiglecH-DTR–Tg mice**

Given that SiglecH expression is not restricted to mature pDC, we hypothesized that prepDC and MZM may also be depleted in mice that express DTR under the control of the SiglecH promoter. To test this hypothesis, we used newly generated C57BL/6 SiglecH-DTR–Tg mice that express DTR under the control of the SiglecH promoter. After DT injection, both mature pDC and prepDC were ablated in SiglecH-DTR–Tg mice (Fig. 5F). In contrast, only mature pDC were eliminated in CLEC4C-DTR–Tg mice (Fig. 5F). Furthermore, when CLEC4C-DTR–Tg mice were bred to SiglecH-GFP mice, eGFP+ cDC were not depleted after DT administration (3).

We next asked whether MZM were depleted in SiglecH-DTR–Tg mice after DT treatment. To address this, we generated C57BL/6 SiglecH-DTR–Tg BM chimeras, because normal C57BL/6 SiglecH-DTR–Tg mice do not tolerate DT very well. After 4–5 mo of rest, we injected CLEC4C-DTR–Tg mice and SiglecH-DTR–Tg chimeras with PBS or DT and harvested spleens 24–36 h later. Spleen sections were stained with Ab against the scavenger receptor MARCO, which is expressed by MZM (Fig. 6A) (27, 28). Results indicated that MZM were intact in CLEC4C-DTR–Tg mice treated with either PBS or DT. In contrast, very few MZM could be identified in spleen sections from SiglecH-DTR–Tg chimeras injected with DT. The depletion of MZM in SiglecH-DTR–Tg mice was validated in a second SiglecH-DTR–Tg line that was generated on a BALB/c background (15). Spleen sections stained for both MZM and metallophilic macrophages (MM) revealed that MZM, but not MM, were reduced in BALB/c SiglecH-DTR–Tg mice injected with DT (Fig. 6B).

**Altered bacterial uptake and increased susceptibility to LM-OVA infection in depleted SiglecH-DTR–Tg mice**

MZM are important for the clearance of apoptotic cells (29–31) and for the uptake of bacteria, such as *S. pneumoniae* (32–34). Mice lacking SIGN-R1, a receptor expressed by MZM, are more susceptible to systemic *S. pneumoniae* infection and show signs of altered bacterial distribution in the spleen upon infection (33, 34). Therefore, we hypothesized that SiglecH-DTR–Tg mice, which appear to lack MZM after DT treatment, also would exhibit altered bacterial distribution and perhaps be more susceptible to infection. To test this, we injected control or depleted CLEC4C-DTR–Tg mice and SiglecH-DTR–Tg chimeras with fluorescent-labeled *S. pneumoniae* and...
examined spleens 1 h p.i. by flow cytometry. We first confirmed depletion in mice treated with DT by staining for pDC (Fig. 7A, top panels). Next, using a variety of Abs, we evaluated which cells from each group of mice were associated with fluorescent bacteria. In all groups of mice, bacteria were mainly found among CD11b+Gr-1+F4/80^2 cells but not pDC (Fig. 7A, bottom panels and data not shown). However, we noted that, in SiglecH-DTR–Tg chimeras treated with DT, there was a 3-fold increase in the frequency of Gr-1^+ cells, presumably neutrophils, which were associated with bacteria. These findings suggested that the absence of MZM in SiglecH-DTR–Tg mice resulted in increased bacterial burden in the red pulp. To confirm this, we analyzed spleens by microscopy 1 h after injection of fluorescent-labeled *S. pneumoniae* (Fig. 7B). Very few fluorescent bacteria could be detected in CLEC4C-DTR–Tg mice injected with PBS or DT or in SiglecH-DTR–Tg chimeras injected with PBS. In contrast, SiglecH-DTR–Tg chimeras treated with DT had many bacteria located in the red pulp, as visualized by Gr-1 staining, confirming altered bacterial distribution and impaired clearance in the absence of MZM.

Given the differences in bacterial uptake and clearance between CLEC4C-DTR–Tg and SiglecH-DTR–Tg chimeras, we sought to determine whether SiglecH-DTR–Tg mice were more or less susceptible to bacterial infection after DT administration. A study by Takagi et al. (6) found that SiglecH^DTR/DTR^ mice treated with DT were more resistant to lethal infection with LM-OVA than were nondepleted mice. Thus, we performed a similar experiment in our CLEC4C-DTR–Tg mice and SiglecH-DTR–Tg chimeras. SiglecH-DTR–Tg chimeras treated with DT succumbed to LM-OVA infection faster than did mice in the other three groups (Fig. 8A). Moreover, it did not appear that lack of pDC was responsible for this phenotype, because CLEC4C-DTR–Tg mice, depleted or not of pDC, exhibited identical rates of survival and died later than did DT-treated SiglecH-DTR–Tg chimeras (Fig. 8A). Analysis of serum cytokine levels revealed that DT-treated SiglecH-DTR–Tg chimeras produced very little IL-12p70 and had exaggerated levels of TNF-α and IL-6 in their...
S. pneumoniae DTR–Tg mice. These results are consistent with a role for MZM in S. pneumoniae bacterial distribution and impaired clearance after injection of mice. Furthermore, depleted SiglecH-DTR–Tg mice showed altered phenotypes than observed in CLEC4C-DTR–Tg mice. Indeed, using depleted SiglecH-DTR-knockin or Tg mice, thus yielding stronger infection, perhaps by altering viral burden and/or distribution.

In this study, we found that SiglecH deficiency resulted in increased cytokine production by a variety of cell types during a viral infection, because PBS- and DT-treated mice had identical survival rates and similar levels of systemic cytokines. It should be noted that SiglecH–Tg mice lack SiglecH expression (6). Therefore, the findings reported by Takagi et al. (6) do not discriminate effects of SiglecH deficiency from pDC depletion. Although it has been suggested that pDC may be detrimental during Listeria infection because of their ability to produce IFN-I, studies showed that myeloid cells and CD8+ DC are important in pathogenesis as either IFN-I-producing cells or initial cellular entry points that establish productive infection (39, 40). Taken together, we conclude that the broad expression pattern of SiglecH should be considered when using SiglecH-DTR mice to evaluate pDC functions in vivo.

The authors have no financial conflicts of interest.

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