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The DC-HIL/Syndecan-4 Pathway Regulates Autoimmune Responses through Myeloid-Derived Suppressor Cells

Jin-Sung Chung, Kyoichi Tamura, 1 Hideo Akiyoshi, 2 Ponciano D. Cruz, Jr., and Kiyoshi Ariizumi

Having discovered that the dendritic cell (DC)–associated heparan sulfate proteoglycan–dependent integrin ligand (DC-HIL) receptor on APCs inhibits T cell activation by binding to syndecan-4 (SD-4) on T cells, we hypothesized that the DC-HIL/SD-4 pathway may regulate autoimmune responses. Using experimental autoimmune encephalomyelitis (EAE) as a disease model, we noted an increase in SD-4+ T cells in lymphoid organs of wild-type (WT) mice immunized for EAE. The autoimmune disease was also more severely induced (clinically, histologically, and immunophenotypically) in mice knocked out for SD-4 compared with WT cohorts. Moreover, infusion of SD-4−/− naïve T cells during EAE induction into Rag2−/− mice also led to increased severity of EAE in these animals. Similar to SD-4 on T cells, DC-HIL expression was upregulated on myeloid cells during EAE induction, among the myeloid cells examined. The critical role of DC-HIL was supported by DC-HIL+ cells from the infused MDSC preparation. Our findings indicate that transfer of MDSCs from EAE-affected WT mice into DC-HIL−/− APCs, respectively. The coinhibitory limb includes CTLA-4, programmed death-1 (PD-1), T cell Ig- and mucin domain-containing molecule 3, and T cell immunoreceptor with Ig and ITIM domains (TIGIT). Although all of these coinhibitors share the T cell inhibitory capacity, each must be somewhat disparate in function because their respective deficiencies or dysfunctions are associated with different autoimmune states.

We discovered new coinhibitors in DC–associated heparan sulfate proteoglycan–dependent integrin ligand (DC-HIL) on APCs and syndecan-4 (SD-4) on activated (but not resting) T cells (5, 6). DC-HIL belongs to the Ig receptor superfamily (95–120 kDa) expressed constitutively by epidermal Langerhans cells, DCs, macrophages, and other monocytes (7). Binding of DC-HIL to SD-4+ T cells strongly inhibits T cell activation triggered via the TCR (5, 7). Blocking such binding through soluble DC-HIL receptor or anti–SD-4 Ab augments delayed-type hypersensitivity responses (6, 8), and infusion of SD-4−/− T cells into sublethally gamma-irradiated allogeneic mice worsened acute graft-versus-host disease (9). We examined the role of the DC-HIL/SD-4 pathway in the activation of autoreactive T cells in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (10). EAE immunization induced expression of SD-4 and DC-HIL on T cells and myeloid cells, respectively. Genetic deficiency of SD-4 or DC-HIL was associated with a hyperacute EAE phenotype, and adoptive transfer studies showed SD-4−/− T cells to be responsible for this disease exacerbation. Among DC-HIL−/− myeloid cells in EAE-affected mice, CD11b+Gr1− MDSCs were the most expanded and most potent suppressors of T cell activation, and DC-HIL was proven to be the critical mediator of MDSC suppressor function.

Materials and Methods

Mice

Female 6- to 8-wk-old C57BL/6 and Rag2−/− mice (B6Cg-Rag2tm1.Cge/J) (11) were purchased from Harland Breeders (Indianapolis, IN) and Taconic Farms (Hudson, NY), respectively. SD-4−/− mice were produced by mating...
SD-4+/− mice (C57BL6 genetic background) (12). DC-HIL−/− mice were generated from embryonic stem cells derived from C57BL/6 mice backcrossed to C57BL6 mice for seven generations (J.-S. Chung, K. Tamura, P. Cruz, Jr., and K. Arizumi, submitted for publication). Controls included DC-HIL−/− or SD-4−/− wild-type (WT) from the same backcrossed generation. Following National Institutes of Health guidelines, mice were housed and cared for in a pathogen-free facility and subjected to experimental procedures approved by the Institutional Animal Care Use Center at the University of Texas Southwestern Medical Center.

Abs and immunofluorescent staining

mAbs against CD3 (145-2C11), CD4 (RM4-5), CD8 (53-6.7), CD11b (M1/70), CD11c (N418), CD19 (eBio1 D3), CD28 (37.51), F4/80 (BM8). Gr-1 (RB6-8C5), and PD-1 (J43) were purchased from eBioscience (San Diego, CA); mAbs against SD-4 (KY/8.2) were from BD Pharmingen; and secondary Abs were from Jackson ImmunoResearch Laboratories. We generated UTX103 rabbit anti–DC-HIL mAb (13).

Single-cell suspensions from spleen or peripheral lymph nodes (LN) were assayed for viability using trypan blue exclusion. When viability was <80%, live cells were enriched by Ficoll-gradient centrifugation for 20 min at room temperature (~90% for typical recovery). The cell suspension (5–10×10^6) was treated with 5 μg/ml Fe blocker (BD Pharmingen) on ice for 30 min and incubated with primary Ab (5–10 μg/ml), followed by addition of secondi-bound fluorescein-conjugated secondary Abs and incubation for 30 min and incubation with primary Ab (5–10 μg/ml). After washing, cell-bound fluorescence was analyzed by FACSCalibur (BD Biosciences) with a minimum of 10,000 events collected. Dead cells were excluded using 1 μg/ml propidium iodide.

Cell isolation

T cells, DCs, macrophages, or MDSCs were isolated from spleen of untreated or EAE-immunized WT or knockout (KO) mice. CD3+ T cells, DCs, and macrophages were isolated using a Pan-T cell isolation kit, anti-CD11c Ab microbeads, and anti-F4/80 Ab microbeads (all from Miltenyi Biotec), respectively. For MDSC isolation, spleen cells were depleted of CD19+, CD11c+, F4/80+ cells using beads conjugated with the corresponding Ab and then positively purified using anti-CD11b microbeads. Purity of all cell preparations was ~95%, as determined by flow cytometry. For depletion of DC-HIL+ MDSCs, spleen cells left after the first depletion were further depleted using biotin-binder beads (Invitrogen) precoated with anti-CD-HIL mAb or control IgG. CD11b+ cells were purified as before, with the former and latter fractions termed DC-HIL–depleted and undepleted MDSCs, respectively. The DC-HIL–depleted preparation contained only a trace number (1–3%) of DC-HIL+ cells. For isolation of CNS-infiltrating cells, EAE-sick mice (14 d postimmunization) were perfused with 48 h, parafin-embedded, thin-sectioned (6 μm), and stained with H&E according to Ehrlich (Sigma-Aldrich). Histological examination was carried out using an Olympus BH2 microscope under ×4 magnification.

Statistical analysis

Data are presented as means ± SD. Significant differences between experimental variables were determined using two-tailed Student t test, with p < 0.05 considered significant. Statistical analysis of EAE clinical scores was performed by two-way ANOVA. All data shown are representative of at least two independent experiments.

Results

EAE induction induced T cells to express SD-4

Because negative regulators of T cell function restrict development of EAE (2), we posited the DC-HIL/SD-4 pathway to be involved. We first examined expression of SD-4 by T cells in spleen and draining lymph nodes (DLNs) before and after EAE immunization, in comparison with expression of another T cell inhibitor, PD-1 (Fig. 1A, 1B). Prior to immunization, there were very few SD-4-expressing T cells in either tissue, whereas PD-1+ T cells were noted in greater numbers. After immunization, expression of both SD-4 and PD-1 rose among CD4+ and CD8+ cells, with varying kinetics that peaked on day 6, but declined slowly for spleen CD4+ and CD8+ and DLN CD4+ cells (Fig. 1A, 1B). In contrast, SD-4 expression continued to rise among DLN CD8+ cells despite PD-1 expression falling to a lower level (Fig. 1B). Thus, EAE induction induced expression of SD-4 and PD-1 on T cells, with different kinetics in CD4+ versus CD8+ cells, particularly in DLNs.

Among DC-HIL–expressing myeloid cells in spleen during induction of EAE, MDSCs are the most expanded population

We next examined frequencies of three types of myeloid cells in spleen (macrophages, DCs, and MDSCs) at different time points during EAE induction (Fig. 1C). Before immunization, all three cell types were present at very low frequencies (1–2%). Fourteen days after inducing EAE, those frequencies rose progressively (up to 10–40%), with MDSCs as the most expanded population (40%). DC-HIL was expressed by 50% of each cell type (Fig. 1D). We also examined DC-HIL and SD-4 expression by MDSCs and
T cells, respectively, isolated from the CNS of EAE-sick mice (14 d postimmunization). Of the CNS-infiltrating cells, 38% of MDSCs (representing at 30% among the total cells) expressed DC-HIL, and 44–47% of CD4+ or CD8+ T cells (minuscule fractions) expressed SD-4 (Fig. 1E). Thus, akin to expression of its ligand SD-4 on T cells, DC-HIL on myeloid cells was upregulated during EAE induction.

SD-4 inhibited EAE by negatively regulating autoreactive T cells

We next examined the functional significance of upregulated SD-4 expression on T cells during EAE induction in WT versus SD-4 KO mice immunized with MOG peptide. From 5 to 13 d postimmunization, tails of WT mice remained flaccid (median score of 1.75 on day 10), but worsened to a peak score of 2.0 on day 20, that then regressed to 1.75 by day 30 (Fig. 2A). In contrast, SD-4 KO mice developed perceptibly hardened tails as early as days 4–7, but with an insignificantly higher score of 3.0 on day 10 (p < 0.1) (Fig. 2B), worsening to 3.75 on day 30 (p < 0.001) (Fig. 2C). Histologically, the spinal cord 20 d postimmunization (Fig. 2D) showed a more intense infiltrate in KO mice (clinical score of 4) versus WT mice (score of 2). Finally, T cell activation was measured by the number of effector T cells: KO mice had markedly greater numbers of IFN-γ- or IL-17-secreting splenocytes 10 d after immunization (Fig. 2E). Thus, SD-4 gene deletion augmented EAE severity associated with significantly more autoreactive T cells.

SD-4 deletion made T cells more potent inducers of EAE disease

Because SD-4 is also expressed by cells other than T cells (e.g., fibroblasts), we questioned whether increased susceptibility of SD-4 KO mice to EAE was due to greater responsiveness of SD-4+/- T cells. We isolated T cells from naive WT or SD-4 KO mice and adoptively transferred these cells into Rag2−/− mice that lack mature T cells (11). Control Rag2−/− mice without T cells (infused only with PBS) developed minimal EAE (score of 1.5) on days 15–25 (Fig. 3A). Rag2−/− mice injected with SD-4+/+ T cells displayed similarly low disease severity that lasted longer, until day 40 (Fig. 3B). In contrast, injection of SD-4−/− T cells enhanced EAE (score of 4.5) on days 18–25 (Fig. 3C). Severity of EAE was tracked through early (day 10), middle (day 20), and later (day 30) phases (Fig. 3D). Injected SD-4−/− T cells worsened EAE only minimally during all three phases, especially because their changes were not statically significant compared with those in control Rag2−/− mice (p > 0.104). In contrast, injected SD-4−/− T cells markedly exacerbated EAE during all phases. Moreover, histology of spiral cords from these mice showed significantly more infiltrating lymphocytes compared with those of Rag2−/− mice infused with SD-4+/+ T cells (Fig. 3E). These differences ran parallel to increased numbers of IFN-γ- and/or IL-17-producing effector T cells in spleen of EAE-induced SD-4 KO mice. Frequencies of Tregs and memory T cells in the T cell preparation used for this adoptive transfer were similar between WT and KO mice (data not shown). Thus, SD-4 gene deletion augmented reactivity of T cells to MOG.

DC-HIL deletion increased susceptibility to EAE

Because DC-HIL expression rose in EAE-induced mice, we inferred that DC-HIL suppresses induction of EAE. To confirm this postulate, we compared EAE induction in WT versus DC-HIL KO mice. Note that KO mice did not exhibit any gross abnormality or developmental defect of lymphoid organs (J.-S. Chung et al., submitted for publication). WT mice manifested EAE 5–12 d after immunization, reaching a peak score of 3.0 on days 10–30 (Fig. 4A). Similar to SD-4 KO mice, DC-HIL KO mice also manifested EAE by days 3–5, peaking on days 12–25, with the disease lasting through day 40 (Fig. 4B). Average scores for DC-HIL KO mice were markedly greater than for WT mice in all phases of EAE induction (p < 0.01) (Fig. 4C). KO mice spinal cords had greater numbers of infiltrating lymphocytes (Fig. 4D), and ELISPOT assays revealed markedly more effector T cells in their spleens (Fig. 4E). Thus, DC-HIL deletion worsened EAE.

DC-HIL-expressing MDSCs are potent suppressors of T cell function in EAE

Theorizing that DC-HIL–expressing myeloid cells are the most potent T cell suppressors in EAE-afflicted mice, we purified F4/80+ macrophages, CD11c+ DCs, or CD11b+Gr-1+ MDSCs from splenocytes of WT mice 14 d after MOG immunization. Each cell...
type was assayed for T cell suppressor activity, in which increasing cell numbers were added to CFSE-labeled T cells activated by anti-CD3/CD28 Ab (Fig. 5A). T cell activation was measured by CFSE dilution assays. Neither macrophages nor DCs from EAE-sick mice inhibited T cell activation, whereas MDSCs caused suppression in a dose-dependent manner. We next addressed whether DC-HIL was responsible for MDSC suppressor function by assaying the T cell inhibitory activity of MDSCs (versus other myeloid cells) purified from EAE-affected DC-HIL KO mice (Fig. 5B). DC-HIL deletion had no effect on the suppressor activity of macrophages or DCs, but it markedly reduced MDSC suppressor function (T cell proliferation rose from 26 to 83% at the highest ratio). Results of IL-2 secretion assays were consistent with the proliferation assays (Fig. 5C). We further examined the critical role of DC-HIL in MDSC suppressor function (Fig. 6) by adding anti–DC-HIL mAb (but not control IgG) to T cell/MDSC cocultures: inclusion of the anti–DC-HIL mAb restored MSDC-inhibited T cell activation in a dose-dependent fashion as measured by CFSE proliferation assays (Fig. 6A). The same mAb had only a minuscule effect on macrophages or DCs. Similar results were obtained by IL-2 production assays (Fig. 6B). These outcomes indicate that deletion of the DC-HIL gene or

FIGURE 2. SD-4−/− mice develop significantly worse EAE. WT (A) or SD-4 KO (B) mice (n = 8) were immunized with MOG peptide and CFA on day 0 and boosted with pertussis toxin on days 0 and 2. Kinetics of EAE development were monitored by clinical score of each mouse, plotted in a graphic version. Clinical scores were: 0, no abnormality; 1, flaccid tail; 2, moderate hindlimb weakness; 3, severe hindlimb weakness; 4, complete hindlimb paralysis; and 5, quadriplegia, moribund state. (C) Clinical scores in early (day 10), middle (day 20), or late phases (day 30) were plotted on a scatter chart (median, n = 8), with statistical significance of scores (ANOVA, *p < 0.001) shown between WT and KO mice. (D) Spinal cords of mice 20 d after immunization were stained with H&E and examined under a microscope (original magnification, ×4). (E) IFN-γ– or IL-17–producing cells in spleen of WT and KO mice 10 d after immunization were enumerated by ELISPOT assay and calculated as number per spleen. All data shown are representative of three separate experiments. *p < 0.001 between WT and KO mice.

FIGURE 3. Adoptively transferring SD-4−/− T cells into Rag2−/− mice exacerbated EAE. Rag2−/− mice (n = 10) were injected i.v. with PBS alone (A), T cells isolated from naive WT (B) or SD-4 KO (C) mice, and EAE-immunized. Clinical scores (median) were assessed daily, plotted in a scatter chart, and sorted to early (day 10), middle, (day 20) or late phases (day 30) (D) with statistical significance of scores (*p < 0.01) between mice injected with WT-T cells and KO-T cells. (E) Spinal cords of mice (injected with T cells from WT or KO mice) 20 d after immunization were stained with H&E and examined under a microscope (original magnification, ×4). (F) IFN-γ– or IL-17–producing cells from spleen of mice injected with PBS, or T cells from WT or KO mice 10 d after immunization, were counted by ELISPOT assay and calculated as number per spleen. Data are representative of two independent experiments. *p < 0.001 between WT and KO T cells.
inhibition of DC-HIL activity leads to functionally defective MDSCs that may be responsible for increased severity of EAE.

**Activation of DC-HIL induced MDSC expression of INF-γ, NO, and reactive oxygen species**

Because T cell–inhibitory soluble factors are critical mediators for MDSC function, including cytokines, urea/L-ornithine produced by arginase I (15), NO (16), and reactive oxygen species (ROS) (17), we examined their influence on MDSC suppressor activity by adding specific inhibitors to cocultures of spleen cells and MDSCs isolated from EAE-affected mice. Whereas Abs neutralizing IL-10 or TGF-β1 had no effect on suppressor activity, anti–IFN-γ Ab abrogated it completely (Fig. 7A). Inhibitors for arginase I, indoleamine, or ROS had little to no effect, but inhibitors of NO synthases or inducible NO synthase, respectively, blocked suppression substantially or completely (Fig. 7B). We then addressed association of DC-HIL with expression of these soluble factors. MDSCs were cultured with immobilized anti–DC-HIL mAb (to activate DC-HIL receptor) and examined for expression of IFN-γ, NO, and ROS. Anti–DC-HIL treatment induced MDSC expression of IFN-γ 40-fold greater than did control IgG treatment, and TNF-α was also upregulated (10-fold), but there was no significant effect on IL-10 and TGF-β expression (Fig. 7C). Ab treatment also increased MDSC expression of ROS and NO, compared with controls (Fig. 7D, 7E). Both IFN-γ and NO expression were enhanced, highest on day 1, followed by a quick fall on the next day. We then addressed whether DC-HIL is critically involved in NO production by MDSCs during coculturing with T cells. MDSCs isolated from EAE-affected WT or DC-HIL KO mice were added to culture of spleen cells from WT mice activated by anti-CD3/CD28 Ab, and NO production was measured (Fig. 7F). Addition of WT MDSCs increased markedly NO production compared with spleen cells alone, whereas DC-HIL−/− MDSCs failed to produce NO. Altogether, IFN-γ and NO were critical mediators of the sup-
pressor activity of MDSCs induced in EAE-affected mice, the expression of which was induced at least through activation of DC-HIL.

To determine whether DC-HIL+ MDSCs are responsible for exacerbated EAE in DC-HIL KO mice, we purified MDSCs from spleens of WT mice immunized 14 d prior for EAE. Control mice (WT and DC-HIL KO mice injected with PBS) were also immunized in parallel and scored clinically. WT and KO mice developed EAE similar to levels found in previous experiments (Fig. 8A, 8B). Injection of undepleted MDSCs (containing 50% DC-HIL+ cells) into DC-HIL KO mice reduced disease severity compared with PBS-treated DC-HIL KO mice (2.5 median score versus 4.0, \( p < 0.01 \)) as assessed on day 10, although both disease scores were higher than for EAE-treated WT mice (2.0) (Fig. 8C). However, score differences between MDSC-injected KO and PBS-injected WT mice became smaller and eventually insignificant in later phases (2.5 versus 4.5 on day 20 with \( p = 0.6 \), and 4.0 versus 3.0 on day 30 with \( p = 0.06 \)) (Fig. 8E). In contrast, DC-HIL KO mice injected with DC-HIL-depleted MDSCs developed EAE as severely as did those treated with PBS alone (Fig. 8D). These outcomes were associated with markedly increased numbers of IFN-\( \gamma \)/IL-17–producing effector T cells (Fig. 8F). Thus, infusion of DC-HIL+ MDSCs alleviated EAE exacerbated by DC-HIL deletion, indicating that increased severity of EAE in DC-HIL KO mice was due to functionally defective MDSCs.

**Discussion**

Activation of autoreactive T cells by autoantigen-loaded APCs triggers competing costimulatory and coinhibitory linkages that determine whether the net TCR signaling is immunostimulatory or tolerogenic. Our findings indicate that the DC-HIL/SD-4 pathway is an important downregulator of autoimmune responses because genetic deficiency of either DC-HIL or SD-4 increased host susceptibility to EAE and this hyperacute phenotype could be transferred to Rag2−/− mice by infusion of SD-4+ T cells during immunization for EAE. Moreover, infusion of DC-HIL+ (but not DC-HIL−) MDSCs from EAE-affected WT mice into DC-HIL−/− mice were added to spleen cell culture and NO levels were measured. Data are representative of at least 2 separate experiments.
KO mice with EAE alleviated the autoimmune disease’s severity.

How does the DC-HIL/SD-4 pathway compare with other coinhibitory systems? CTLA-4, expressed constitutively and exclusively by T cells, is key to maintaining central and peripheral tolerance, which it implements via Tregs (15, 16). CTLA KO mice spontaneously develop lethal autoimmune disease involving multiple organs (18). PD-1 also regulates central and peripheral tolerance, and similar to CTLA-4 it does so via Tregs (19). Unlike CTLA-4, PD-1 is expressed by a wider range of cells, including T and B cells, NK T cells, DCs, other monocytes, and thymocytes (20). PD-1 KO mice spontaneously develop a lupus erythematosus–like disease, but at a later age than CTLA-4 KO mice and with milder forms of glomerulonephritis or cardiomyopathy (21). TIGIT has a similar cell expression profile as PD-1 and resembles CTLA-4 it in its ability to bind the competing ligands CD155 and CD112 expressed by APCs and nonlymphoid cells (22, 23). Whereas TIGIT KO mice do not spontaneously develop autoimmune disease, these animals manifest EAE spontaneously when crossed with MOG<sub>35-55</sub>-specific TCR transgenic 2D2 mice (23). Finally, T cell Ig- and mucin domain–containing molecule 3 is expressed by CD4<sup>+</sup> Th1, but not Th2 cells (24), and its functional blockade causes hyperacute EAE in mice (25).

SD-4 differs from the aforementioned coinhibitors by its restricted expression to only some effector/memory T cells and by the absence of direct physical interaction with the TCR. Instead SD-4, which lacks an intracellular ITIM (21), suppresses T cell activation by stimulating CD148 via its protein tyrosine phosphatase activity (26). Most importantly, the suppressor function of SD-4 is independent of Treg function. Indeed, SD-4 has never been shown to be expressed by Tregs in vivo, although it has been induced on these cells following in vitro stimulation by anti-CD3 Ab (9). DC-HIL also differs from other coinhibitory ligands by its expression on nonlymphoid cells (melanocytes, keratinocytes, and osteoblasts) and by having an intracellular ITAM (27–29). Having shown previously that crosslinking of DC-HIL receptor with specific Ab induced tyrosine phosphorylation of the ITAM (13), we hypothesized that DC-HIL activation induces MDSCs to express IFN-γ, NO, and ROS via these ITAM-linked signal pathways. We are currently studying the DC-HIL–induced signal pathway in MDSCs.

MDSCs are potent inhibitors of T cell function; their expansion in blood and tissues has been associated with cancer growth (27) and chronic inflammatory conditions, including bacterial and parasitic infections as well as autoimmune diseases (28). In particular, CD11b<sup>+</sup>Gr−1<sup>+</sup> MDSCs (including CD11b<sup>+</sup>Ly6C<sup>+</sup> cells) have been isolated from autoimmune inflammatory environments (30). Although their potent immunosuppressive function is beyond dispute, it is not clear whether they are pathologic or protective for autoimmune diseases. A pathologic role for MDSCs in EAE has been supported by studies showing that 1) blood CD11b<sup>+</sup>Ly6Ch<sup>+</sup> cells migrated into the CNS to become inflammatory APCS that activate autoreactive T cells (30, 31), and 2) anti–Gr−1<sup>+</sup> Ab-mediated depletion of CD11b<sup>+</sup>Gr−1<sup>+</sup> MDSCs reduced severity of EAE induced by Thielier’s murine encephalomyelitis virus infection in mice (32). In contrast, a protective role for MDSCs has been buttressed by the following findings: 1) blood MDSCs in naive mice were potent T cell suppressors and their loss of function promoted EAE (33) and 2) MDSCs reduced inflammation in the central nervous system by promoting T cell apoptosis (34).

Our findings favor a protective role for MDSCs in EAE because CD11b<sup>+</sup>Gr−1<sup>+</sup> MDSCs containing a subset expressing DC-HIL strongly suppressed T cell function and adoptive transfer of DC-HIL<sup>+</sup> MDSCs (from EAE-affected mice) alleviated the hyperacute EAE phenotype in DC-HIL KO mice. These outcomes are consistent with those from mouse models of inflammatory bowel disease (35), alopecia areata (36), and type 1 diabetes (37).
seemingly contradictory roles for MDSCs in autoimmunity may be due to divergent genetic backgrounds of mouse strains used, which in turn lead to disparate responses to autoantigens by different MDSC subsets (38).

Mice lacking the DC-HIL/SD-4 pathway appear to develop large expansion of the pathogenic T cells in peripheral tissues during induction of EAE. Similar expansion was noted in spleen of DC-HIL/−/− or SD-4/−/− mice following allogeneic bone marrow transplantation (C57BL/6 bone marrow cells injected into total body gamma-irradiated BALB/c mice) (9) and contact hypersensitivity induction (C57BL/6 bone marrow cells injected into total body irradiation) (10). This expansion may be caused by synergistic effects of hyperactive APCs and disabled MDSCs lacking the DC-HIL pathway. In EAE-sick mice, we speculate that T cells may be hyperactivated by DC-HIL/−/− APCs because these cells possessed 2-fold greater T cell stimulatory capacity than did WT counterparts (J.-S. Chung et al., submitted for publication). Moreover, their activation may not be suppressed efficiently by DC-HIL/−/− MDSCs, being the suppressors most expanded and most potent in EAE-sick mice. Because MDSCs migrate into inflamed tissues and other organs (e.g., spleen, LN, and kidney), T cells could be hyperactivated in many organs, particularly in regional LNs where inflammatory APCs accumulated.

Tolerogenic DCs generated from ex vivo culture have been used to vaccinate against autoimmune diseases (39). Such DCs can expand Tregs and render autoreactive T cells anergic in an Ag-specific manner (39). MDSCs may possibly be exploited in a similar manner (40) with potentially greater potency as protectors against autoimmunity, given their added ability to inhibit NK cell activity (41) and macrophage cytokine production (42), and in an Ag-nonspecific way.

In summary, in the DC-HIL/SD-4 pathway, we have uncovered a new negative regulator of autoimmune responses that works via MDSC-mediated T cell suppression. Because MDSCs are distinct targets from those of other coinhibitory pathways and because DC-HIL induces expression of T cell inhibitory factors required for MDSC suppressor activity, the DC-HIL/SD-4 pathway represents an opportune platform for studying autoimmune regulation and is a promising focus for treatment of autoimmune disease.

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Disclosures
The authors have no financial conflicts of interest.

References