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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, of IFN-γ, NO, and reactive oxygen species. Akin to SD-4+/− mice, DC-HIL−/− mice manifested exacerbated EAE. Adoptive transfer of MDSCs from EAE-affected WT mice into DC-HIL−/− mice reduced EAE severity to the level of EAE-immunized WT mice, an outcome that was precluded by depleting DC-HIL+ cells from the infused MDSC preparation. Our findings indicate that the DC-HIL/SD-4 pathway regulates autoimmune responses by mediating the T cell suppressor function of MDSCs. The Journal of Immunology, 2014, 192: 000–000.

A mong the immune system’s difficult tasks is to defend the host against microbial pathogens while controlling autoreactivity. Most autoreactive T cells are depleted (centrally) in the thymus during early development, but some escape this screening process (1) and will require suppression of their activation (peripherally) to maintain homeostasis. Cells responsible for peripheral tolerance include regulatory T cells (Tregs), tolerogenic macrophages and dendritic cells (DCs), and invariant NK T cells (2). A newly recognized player in this milieu are CD11b+Gr-1+ myeloid-derived suppressor cells (MDSCs) that can potently suppress T cell function as well as promote expansion of Tregs (3, 4).

T cell activation is regulated by costimulatory and coinhibitory ligand and receptor pairs of molecules expressed on T cells and 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 on dendritic cells (DCs) and monocytes (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.Cgen/J) (11) were purchased from Harland Breeders (Indianapolis, IN) and Taconic Farms (Hudson, NY), respectively. SD-4−/− mice were produced by mating

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Abbreviations used in this article: DC, dendritic cell; DC-HIL, dendritic cell–associated heparan sulfate proteoglycan–dependent integrin ligand; DLN, draining lymph node; EAE, experimental autoimmunne encephalomyelitis; KO, knockout; LN, lymph node; MDSC, myeloid-derived suppressor cell; MOG, myelin oligodendrocyte glycoprotein; PD-1, programmed death-1; ROS, reactive oxygen species; SD-4, syndecan-4; TIGIT, T cell immunoreceptor with Ig and ITIM domains; Treg, regulatory T cell; WT, wild-type.

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SD-4+/− mice (C57BL/6j genetic background) (12). DC-HIL−/− mice were generated from embryonic stem cells derived from C57BL/6j mice backcrossed to C57BL/6j mice for seven generations (I.-S. Chng, 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 the 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 (35-6-7), CD11b (M1/70), CD11c (N418), CD19 (eBio 1D3), 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 (KY7/8.2) 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 x 10^6) was treated with 5 μg/ml Fc blocker (BD Pharmingen) on ice for 30 min and incubated with primary Ab (5–10 μg/ml), followed by addition of secondary antibody (2.5 μg/ml). After wash, 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–CD11b microbeads, and anti-F4/80 Ab microbeads (both from Miltenyi Biotech), 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–DC-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 (~3%) of DC-HIL+ cells. For isolation of CNS-infiltrating cells, EAE-sick mice (14 d postimmunization) were perfused through the left cardiac ventricle with cold PBS. Their spinal cords were cut into small pieces and digested in 2.5 mg/ml collagenase D (Roche)/1 mg/ml DNAse I (Sigma-Aldrich) in DMEM at 37˚C for 45 min. Cells were then spun down, and washed. Single-cell suspensions from spleen or peripheral 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 immunization 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

On day 0, mice were injected s.c. with 200 μg myelin oligodendrocyte glycoprotein (MOG) peptide (MEVGWYRSPFSRVVHLNYRNGK) in CFA (Difco Laboratories) containing heat-killed Mycobacterium tuberculosis H37 RA (500 μg). On days 0 and 2, mice were injected i.p. with 200 ng pertussis toxin (Difco Laboratories) (10). Disease was assessed in an unbiased manner and scored using an established scale (10). To assess MOG–specific T cell response in EAE-induced mice, spleen cells were prepared from mice immunized 10 d prior and seeded onto ELISPOT wells at varying cell densities in the presence of MOG peptide (5 μg/ml) for 2 d. IFN-γ– or IL-17–producing cells were counted using ELISPOT assay (eBioscience).

For adoptive T cell transfer experiments, 1 x 10^7 T cells isolated from spleens of naive WT or SD-4 KO mice were injected i.v. into Rag2−/− mice (n = 10). Next day, all mice were immunized with MOG peptide/adjuvant, followed by toxin injections. Mice were examined daily for signs of disease. To assess effects of DC-HIL+ MDSCs on EAE, MDSCs were isolated from pooled spleens of EAE-sick WT mice (10 mice on day 14 after EAE immunization), depleted or undepleted of DC-HIL− cells, and then injected i.v. into DC-HIL KO mice (5 x 10^6 cells/mouse) (n = 10) that were EAE-immunized 4 d prior.

Activation of DC-HIL and soluble factors

MDSCs (5 x 10^5) isolated from day 14 postimmunization were cultured in 96-well plates (2 x 10^5 cells/well) precoated with anti–DC-HIL mAb or control IgG (10 μg/ml). After 1 or 2 d of culture, the culture supernatant and cell pellets were collected separately: the former were tested for cytokine secretion using ELISA, and the latter were assayed whole-cell extracts for NO production using the Griess method (14). MDSCs were also mixed with spleen cells (2 x 10^6 cells/well) from naive WT mice at increasing cell ratios and cultured for 2 d in wells precoated with anti–CD3/CD28 Ab (each 1 μg/ml). Cells were pooled from six wells and used for NO assay.

Histological examination

Twenty days after EAE immunization, spinal cords were dissected from three to four representative mice in each group. Specimens were fixed in 4% neutral buffered formalin for 48 h, paraffin-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 x4 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 immunization 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 node (DLN) 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 immunization induced expression of SD-4 and PD-1 on T cells, with different kinetics in CD4+ versus CD8+ cells, particularly in DLNs.

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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 spinal 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 MDSC-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 tox in 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–INF-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-

FIGURE 4. DC-HIL−/− mice developed hyperacute EAE after immunization with MOG peptide. WT (A) or DC-HIL KO (B) mice (n = 11) were immunized similarly with MOG peptide and CFA, and EAE development was monitored by scoring clinical symptoms. (C) Each clinical score on early (day 10), middle (day 20), or late phases (day 30) was plotted in a scatter chart (median) with statistical significance of scores (*p < 0.01) between WT and KO mice. Similarly, spinal cords of mice were examined histologically (H&E stain; original magnification ×4) (D), and IFN-γ– or IL-17–producing cells from spleen of WT and KO mice counted and calculated (E). Data are representative of three independent experiments. *p < 0.0001 between WT and KO mice.

FIGURE 5. Among DC-HIL–expressing myeloid cells in EAE-developing mice, MDSCs are the most potent suppressors. (A and B) Three myeloid cell subsets were purified from spleen of WT (A) or DC-HIL KO (B) mice that were EAE-immunized 14 d prior. These cells were assayed separately for T cell–suppressive activity by coculturing with CFSE-labeled WT T cells at varying cell ratios in the presence of anti-CD3/CD28 Ab. Three days after culture, the percentage of proliferated cells was examined by flow cytometry and data are shown in histograms. (C) Similar assays were performed, but with unlabeled T cells. T cell activation is shown by IL-2 production (mean ± SD, n = 3). "None" indicates T cells and Ab without myeloid cells. Data are representative of three independent experiments. *p < 0.001 between WT and KO mice.
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. 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-γ/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 was due to functionally defective MDSCs.

FIGURE 6. Anti–DC-HIL treatment abrogated T cell–suppressive activity of MDSCs. Each myeloid cell subset was purified from EAE-disease mice and cocultured with CFSE-labeled WT T cells activated by anti-CD3/CD28 Ab in the presence of anti–DC-HIL mAb or control Ab (IgG) at 25 or 50 μg/ml. Dilution of CFSE fluorescent intensity was measured by flow cytometry (A) or IL-2 production was assayed (B) (mean ± SD, n = 3). T cells alone indicates T cell culture with anti-CD3/CD28 cells but without myeloid cells. Data are representative of three independent experiments. *p < 0.001 between DC and MDSCs.

FIGURE 7. Activation of DC-HIL on MDSCs induced expression of IFN-γ, NO, and ROS. (A and B) Specific inhibitors were added separately to cocultures of spleen cells and MDSCs (1:1 cell ratio) isolated from EAE-sick mice, including anti-cytokine Ab (A) and 5 mM L-Nω-(1-iminoethyl)-l-lysine (inducible NO synthase [iNOS]), 1 mM L-NAME-hydroxyl-nor-arginine (Arg), 0.5 mM 6-(1-iminoethyl)-L-lysine (inhibitor for NO synthases), 0.2 mM 1-methyl-tryptophan (Indol), 1000 U/ml catalase (C-ROS), and 200 U/ml superoxide dismutase (S-ROS). Effects of inhibitors are expressed as percentage suppression relative to untreated coculture (set as 100%). (C–E) One or 2 d after culturing MDSCs with immobilized anti–DC-HIL mAb or control IgG, cytokine secretion (C), ROS expression (D), and NO production (E) were measured by the cells. Cytokine expression is shown by fold increase relative to IgG-treated cultures. ROS expression is shown in gray-filled (untreated MDSCs) or open (Ab-treated) histograms, with mean fluorescence intensity. (F) Varying doses of MDSCs from EAE-sick WT or 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-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 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 CD<sup>4+</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>Gr-1</sup>− MDSCs (including CD11b<sup>Ly6C</sup><small><sup>+</sup></small> 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>Ly6C</sup><small><sup>+</sup></small> cells migrated into the CNS to become inflammatory APCs that activate autoreactive T cells (30, 31), and 2) MDSCs reduced severity of EAE induced by Theiler’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>Gr-1</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 autoimmune 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 in transplantation (C57BL/6 bone marrow cells injected into total body irradiated C57BL/6 mice) (39). MDSC-mediated T cell suppression. Because MDSCs are distinct from those of other coinhibitory pathways and because DC-HIL induction of EAE. Similar expansion was noted in spleen of DC-HIL/−/− 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 vaccine 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 autoimmune disease. We thank Irene Dougherty and Megan Randolph for technical and secretarial assistance.

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Disclosures

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References


