Depleting Syndecan-4⁺ T Lymphocytes Using Toxin-Bearing Dendritic Cell-Associated Heparan Sulfate Proteoglycan-Dependent Integrin Ligand: A New Opportunity for Treating Activated T Cell-Driven Disease

Hideo Akiyoshi, Jin-Sung Chung, Mizuki Tomihari, Ponciano D. Cruz, Jr. and Kiyoshi Ariizumi

*J Immunol* 2010; 184:3554-3561; Prepublished online 22 February 2010; doi: 10.4049/jimmunol.0903250

http://www.jimmunol.org/content/184/7/3554

References
This article cites 40 articles, 13 of which you can access for free at:
http://www.jimmunol.org/content/184/7/3554.full#ref-list-1

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Depleting Syndecan-4⁺ T Lymphocytes Using Toxin-Bearing Dendritic Cell-Associated Heparan Sulfate Proteoglycan-Dependent Integrin Ligand: A New Opportunity for Treating Activated T Cell-Driven Disease

Hideo Akiyoshi,1,2 Jin-Sung Chung,1 Mizuki Tomihari,3 Ponciano D. Cruz, Jr., and Kiyoshi Ariizumi

Because syndecan-4 (SD-4) is expressed by some (but not all) T cells following activation and serves as the exclusive ligand of dendritic cell-associated heparan sulfate proteoglycan-dependent integrin ligand (DC-HIL), we envisioned the DC-HIL/SD-4 pathway to be a therapeutic target for conditions mediated by selectively activated T cells. We conjugated soluble DC-HIL receptor with the toxin saporin (SAP; DC-HIL-SAP) and showed it to bind activated (but not resting) T cells and become internalized by and deplete SD-4⁺ T cells. In hapten-sensitized mice, DC-HIL-SAP injected i.v. prior to hapten challenge led to markedly suppressed contact hypersensitivity responses that lasted 3 wk and were restricted to the hapten to which the mice were originally sensitized. Such suppression was not observed when DC-HIL-SAP was applied during sensitization. Moreover, the same infusion of DC-HIL-SAP produced almost complete disappearance of SD-4⁺ cells in haptenated skin and a 40% reduction of such cells within draining lymph nodes. Our results provide a strong rationale for exploring use of toxin-conjugated DC-HIL to treat activated T cell-driven disease in humans. The Journal of Immunology, 2010, 184: 3554–3561.

Syndecan-4 (SD-4) is expressed by some (but not all) T cells following activation and serves as the exclusive ligand of the dendritic cell-associated heparan sulfate proteoglycan-dependent integrin ligand (DC-HIL). We conjugated soluble DC-HIL receptor with the toxin saporin (SAP; DC-HIL-SAP) and showed it to bind activated (but not resting) T cells and become internalized by and deplete SD-4⁺ T cells. In hapten-sensitized mice, DC-HIL-SAP injected i.v. prior to hapten challenge led to markedly suppressed contact hypersensitivity responses that lasted 3 wk and were restricted to the hapten to which the mice were originally sensitized. Such suppression was not observed when DC-HIL-SAP was applied during sensitization. Moreover, the same infusion of DC-HIL-SAP produced almost complete disappearance of SD-4⁺ cells in haptenated skin and a 40% reduction of such cells within draining lymph nodes. Our results provide a strong rationale for exploring use of toxin-conjugated DC-HIL to treat activated T cell-driven disease in humans. The Journal of Immunology, 2010, 184: 3554–3561.

Department of Dermatology, University of Texas Southwestern Medical Center and Dermatology Section (Medical Service), Dallas Veterans Affairs Medical Center, Dallas, TX 75390

1H.A. and J.-S.C. contributed equally to this work.
2Current address: Department of Veterinary Science, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka, Japan.
3Current address: Department of Clinical Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan.

Received for publication October 5, 2009. Accepted for publication January 23, 2010.

This work was supported by National Institutes of Health Grant AI064927-05A2 and a Pilot and Feasibility Study grant from Galderma.

Address correspondence and reprint requests to Dr. Kiyoshi Ariizumi, Department of Dermatology, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390-9069. E-mail address: Kiyoshi.Ariizumi@UTSouthwestern.edu

Abbreviations used in this paper: CH, contact hypersensitivity; DC-HIL, dendritic cell-associated heparan sulfate proteoglycan-dependent integrin ligand; DLN, draining lymph node; LN, lymph node; Os, 2-phenyl-4-ethoxyethylene-5-oxazolone; PD-1, programmed cell death-1; SAP, saporin; SD-4, syndecan-4; TNCB, 2,4,6-trinitrochlorobenzene.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.0903250
Production of Fc-fused recombinant proteins

A plasmid vector (pSTB-DC-HIL-Fc) encoding the extracellular domain of DC-HIL fused to the Fc portion of human IgG1 was constructed as reported previously (19). The Fc-fusion protein (DC-HIL-Fc) was produced in COS-1 cells and purified as described previously (19). Purity of final preparations was high (19), as judged by a single band (120 kDa) in SDS-PAGE/Coomassie blue staining or in immunoblotting with goat anti-human IgG Ab or I4E anti–DC-HIL mAb (19).

Conjugation of sapoin with Fc protein

DC-HIL-Fc or human IgG1 was biotinylated using EZ-link NHS-Biotin (Pierce, Rockford, IL) following manufacturer’s recommendations. Normally, one Fc protein molecule has one to two biotin molecules. Biotinylated Fc protein was then conjugated with streptavidin-saporin (SAP) (Advanced Targeting System, San Diego, CA) by mixing them together at a molecular ratio (Fc-protein:SAP) of 5:1. Resulting SAP conjugates are referred to as DC-HIL-SAP and Ig-SAP, respectively.

Abs

mAbs against CD4 (L3T4), CD8 (53-6-7), CD19 (eBio 1D3), and CD69 (H1.2F3) were purchased from eBioscience (San Diego, CA), anti–SD-4 mAb (KY/8.2) from BD Pharmingen (San Diego, CA), anti-SAP Ab from Advanced Targeting System, and anti-human IgG Ab and secondary Ab from Jackson ImmunoResearch Laboratories (West Grove, PA).

T cell isolation and in vitro assays

CD4+ and CD8+ T cells were purified from spleen or lymph node (LN) cells of naive or immunized BALB/c mice using CD4+ and CD8+ T cell isolation kits (Miltenyi Biotec, Auburn, CA). For experiments examining binding of SAP conjugates to T cells, splenic CD4+ and CD8+ T cells (1 × 10^6/ml) were activated by immobilized anti-CD3 Ab (2 μg/ml) for 3 d in complete RPMI 1640 media. Freshly isolated (resting) or activated T cells were incubated with 10 μg/ml DC-HIL-SAP or control Ig-SAP plus 2.5 μg/ml PE–anti-human IgG Ab. After washing, cell-bound fluorescence was analyzed by FACSCalibur (BD Biosciences, San Jose, CA).

To assay the effect of DC-HIL-SAP on T cell proliferation, CD4+ or CD8+ T cells (2 × 10^5/well–plate) were cultured with immobilized anti-CD3 Ab (2 μg/ml) for 2 d, after which DC-HIL-SAP or Ig-SAP was added to culture at indicated concentrations (calculated as SAP concentration) and incubated for another 1 d. After [3H]thymidine (1 μCi/well) was pulsed for 20–22 h, cells were harvested and measured for [3H] radioactivity. In parallel, T cells were also examined for surface expression of SD-4 by flow cytometry. To confirm specificity of DC-HIL-SAP killing for SD-4 cells, we used the DO11.10 T cell line (provided by J. Kappler and P. Marrack, National Jewish Medical and Research Center, Denver, CO) that was transfected with SD-4 gene or empty vector (20). These cells (1 × 10^5) were incubated for 2 h with a SAP conjugate at indicated concentrations. After washing, treated cells were cultured in media containing human rIL-2 (50 U/ml). The next day, [3H] thymidine was pulsed for 20 h and then harvested.

To examine SD-4 expression on memory T cells, H-2Kb–associated OVA peptide (SIINFEKL, 30 μg, synthesized by Protein Chemistry Technology Center, University of Texas Southwestern Medical Center) in TiterMax Gold Adjuvant (Sigma-Aldrich, St. Louis, MO) was injected into the footpad of OT-1 transgenic mice (three mice). Seventeen days postimmunization, CD8+ T cells were purified from LN of immunized mice (pool of three mice). These T cells were then stained with FITC–anti-CD44, APC–anti-CD62L, and PE–anti–SD-4 Ab and then examined by flow cytometry.

Immunofluorescence staining

For experiments examining internalization of DC-HIL-SAP, activated CD4+ or CD8+ T cells (2 × 10^5) were incubated with 10 nM DC-HIL-SAP for 1 h on ice. After washing, cells were split into two batches: one batch was kept on ice, and the other was incubated at 37°C for 30 min. Cells were then fixed with peroxidase/Permeabilization buffer (eBioscience) for 30 min on ice and incubated with goat anti-SAP Ab, followed by fluorescent labeling with Alexa 594–secondary Ab (2 μg/ml) and with Alexa 488–secondary Ab (0.5 μg/ml). Fluorescent images were taken using a TCS-SP1 laser scanning confocal microscope (Leica Microsystems, Bannockburn, IL) at <100 magnification. Images were analyzed by the ImageJ program (National Institutes of Health, Bethesda, MD).

To examine skin-homing T cells, ear skin biopsies were procured from mice sensitized/challenged with 2-phenyl-4-ethoxymethylene-5-oxazolone (oxazolone or Ox) (day 2 postchallenge), doubly stained with rat anti-CD4 (or anti-CD8) Ab and biotinylated anti–SD-4 mAb (5 μg/ml each), and fluorescently labeled with Alexa 488 goat anti-rat IgG (2 μg/ml) and Alexa 594 streptavidin (2 μg/ml). Fluorescence images were taken using a confocal microscope and merged to analyze coexpression of SD-4 and a T cell marker. T cells doubly stained with anti-CD4 or CD8 Ab and anti–SD-4 Ab were counted using three different views.

CH assays

BALB/c mice (n = 4) were sensitized for CH on day 0 by painting 2% of Ox (Sigma-Aldrich) in acetone-oil (4:1 in volume) on shaved abdominal skin (sensitization) (25). Mice were challenged on day 6 by painting 1% Ox and solvent control to right and left ears, respectively (elicitation). In some experiments, mice were sensitized by topical application (20 μI) of 50 μg/ml PMA (dissolved in 1% DMSO/99% methanol) and challenged by 10 μI of 25 μg/ml PMA or the solvent alone (as control) (26). Thereafter, CH was assessed daily from days 7–9 by an unbiased technician who measured ear thickness and calculating changes in ear swelling (thickness of right ear minus that of control left ear) (27). Different panels of mice were injected via the tail vein with 20, 40, or 80 nM DC-HIL-SAP, Ig-SAP (as concentration of SAP), or PBS (200 μI/mice) at indicated time points.

For experiments examining the duration of DC-HIL-SAP effects, sensitized mice were injected i.v. with DC-HIL-SAP (or Ig-SAP) 3 h prior challenge. A week postinjection, mice were challenged weekly with Ox or solvent control (on right and left ears, respectively) up to four times, after which ear thickness was measured daily.

CH responses of DC-HIL-SAP–treated mice to a different contact allergen, 2,4,6-trinitrochlorobenzene (TNCB; Sigma-Aldrich), were also examined following sensitization, injection of SAP, and challenge as above. On day 6, the same mice were sensitized by epicutaneous application of 7% TNCB (solved in acetone-oil olive) to a different area of shaved abdominal skin and on day 12 challenged with 1% Ox and 1% TNCB on left and right ears, respectively, after measuring thickness of both ears (control).

Histological examination of skin and phenotyping of LN cells

Two days after painting Ox on ears of Ox-sensitized mice treated with SAP conjugates or PBS, ear skin and DLNs were procured. Ear skin was frozen, thin sectioned, and stained with H&E according to Ehrlich (Sigma-Aldrich). Histological examination was carried out under light microscopy at a magnification of ×10.

Cervical DLNs were excised and cells per LN counted and examined for frequency of CD4+ T cells, CD8+ T cells, and CD19+ B cells using respective antimaker Abs. To assay CD69 expression, LN cells (5 × 10^5) were cultured with T cells and with T cell–derived markers (SD-4 and CD69) (2.5 μg/ml each) in the presence or absence of PE–anti–CD69 mAb or PE–isotypic control hamster IgG (BD Pharmingen) (2.5 μg/ml each) and examined by flow cytometry for surface expression of CD69 in each leukocyte subpopulation.

For SD-4 expression on T cells in DLNs, sensitized mice (n = 3) were treated similarly and their DLNs excised 2 d postchallenge and pooled. CD4+ and CD8+ T cells were purified from the pooled DLN cells and stained with PE–anti–SD-4 mAb or PE–isotypic control rat IgG (2.5 μg/ml each) and examined by flow cytometry for surface expression of SD-4 on T cells.

Cytokine assay

Mice (n = 3) were treated similarly with Ox and injected i.v. with SAP conjugate (40 nM). A day postchallenge, DLNs were isolated and cultured (2 × 10^7/well) with immobilized anti-CD3 Ab (2 μg/ml) and soluble anti–CD28 Ab (2 μg/ml) for 2 d. Culture supernatant was harvested and assayed for production of IL-4, IFN-γ, IL-10, and IL-17 using mouse ELISA Ready-SET-Go kits (eBioscience).

Statistical analysis

Data are presented as means ± SD. The significance of differences between experimental variables was determined using the paired Student t test.

Results

SD-4 is expressed by effector memory T cells

Having shown previously that SD-4 (like PD-1) is expressed by T cells within DLNs of recently challenged hapten-sensitized mice, we analyzed more rigorously the spectrum of SD-4 expression in
We next assessed effects of DC-HIL-SAP on T cell activation (Fig. 2C). CD4+ or CD8+ T cells were activated by incubation with immobilized anti-CD3 Ab for 2 d and then with DC-HIL-SAP (or control Ig-SAP) for 1 d. DC-HIL-SAP blocked proliferation of CD4+ and CD8+ T cells in a dose-dependent manner, achieving 70–80% reduction at the highest dose tested (16 nM SAP). Reduced T cell proliferation was due to selective depletion of SD-4+ T cells because DC-HIL-SAP treatment markedly reduced the number of SD-4+ T cells (Fig. 2D) and because DC-HIL-SAP potently inhibited proliferation of a SD-4-transfected D011.10 T cell line (but not vector-transfected control cells) in a dose-dependent manner (Fig. 2E). These results document the ability of toxin-conjugated DC-HIL to selectively kill SD-4+ (activated) T cells.

A single infusion of DC-HIL-SAP blocks elicitation of CH

CH is an established model for T cell-mediated, delayed-type hypersensitivity, in which the effect of depleting SD-4+ T cells by DC-HIL-SAP could be tested readily in vivo (Fig. 3). BALB/c mice were sensitized to Ox on shaved abdominal skin (day 0), then challenged with Ox on ear skin (day 6). Mice were injected i.v. with DC-HIL-SAP (20 or 40 nM SAP concentration), Ig-SAP, or PBS 3 h prechallenge (Fig. 3A). Whereas PBS-injected mice developed strong ear swelling, DC-HIL-SAP–injected mice exhibited markedly reduced ear swelling (by 50% and 80% corresponding to 20 and 40 nM SAP, respectively). A dose of 80 nM of DC-HIL-SAP produced similar 80% reduction, with a similar dose of Ig-SAP causing increased toxicity (data not shown), thereby indicating 40 nM to be optimal for suppressing CH. Ig-SAP had minimal effect (<10% reduction for either SAP concentration). Histologic examination of Ox-painted ear skin in DC-HIL-SAP–injected mice versus controls documented less ear thickness and fewer infiltrating leukocytes (Fig. 3B).

The duration of unresponsiveness was assessed by rechallenging mice infused with DC-HIL-SAP (Fig. 3C) with Ox each week for 4 wk. Such mice showed persistently reduced ear swelling after second and third challenges, but regained responsiveness after a fourth challenge.

Having shown previously that infusion of DC-HIL-Fc (unconjugated with toxin) into mice impacted elicitation but not inhibiting markedly reduced ear swelling (by 50% and 80%)

FIGURE 1. Characterization of SD-4+ T cells generated in immunized mice. A, CD4+ or CD8+ T cells purified from DLNs of mice sensitized/challenged with Ox day 0 (just prior to challenge), day 1, or day 3 postchallenge were examined for coexpression of SD-4 and PD-1. B, These CD4+ T cells also were analyzed for coexpression of SD-4 and a T cell marker. C-E, Seventeen days postimmunization of OT-I transgenic mice with OVA peptide, CD8+ T cells were purified from DLNs of immunized mice and then stained with FITC–anti-CD44, APC–anti-CD62L, and PE–anti-SD-4 Ab, followed by flow cytometry. C, Dot-blot analysis of SD-4 versus CD62L is shown for CD44 high CD8+ cells. CD62L low and CD62L high fractions represent effector/memory (T EM) and central memory T cells (T CM), respectively. Frequency of SD-4+ cells for each T cell subset is expressed as %. CD62L expression by total CD44 high/CD8+ (D) or by SD-4+CD44 high/CD8+ (E) is shown in histograms. All data are representative of at least two experiments.
FIGURE 2. Effects of DC-HIL-SAP on T cells in vitro. A, Binding of DC-HIL-SAP to T cells. Splenic CD4+ or CD8+ T cells from BALB/c mice were left untreated (resting) or activated by anti-CD3 Ab (2 μg/ml) for 3 d, and then incubated with 5 μg/ml DC-HIL-SAP (open histograms) or Ig-SAP (shaded), followed by fluorescent labeling with PE–anti-human IgG Ab. Binding was measured by flow cytometry. B, Internalization. Activated CD4+ T cells were incubated with 10 nM DC-HIL-SAP for 1 h on ice. After washing, cells were either left untreated (Before) or incubated (After) at 37°C for 30 min. Cells were then fixed and fluorescently stained with anti-DC-HIL (green fluorescence) or anti-SAP Ab (red), followed by confocal microscopy. Original magnification ×40. C, Effects on T cell proliferation. Splenic CD4+ or CD8+ T cells (2 × 10^5 per well) were activated with immobilized anti-CD3 Ab (2 μg/ml) for 2 d, and then different doses of DC-HIL-SAP or Ig-SAP (shown as SAP concentration) were added to the culture. The next day, [3H]thymidine (TdR) incorporation was measured. Relative cpm to the control (0 nM or PBS) are expressed as percentages (mean ± SD; n = 3). D, Depletion of SD-4+ T cells. Similarly treated T cells (just prior to harvest for counting [3H] cpm) were examined by flow cytometry for expression of SD-4; percentage of positive cells is shown. E, Specificity to SD-4. DO11.10 T cell lines transfected with SD-4 gene (SD-4–DO11) or empty vector (DO11) were treated with indicated doses of SAP conjugates and cultured for 2 d. Proliferation was measured (mean ± SD; n = 3). Data shown are representative of at least two separate experiments.

We also examined whether DC-HIL-SAP injection reduces established ear swelling (Fig. 3E). Sensitized/challenged mice developed similar levels of ear thickness (measured day 1

FIGURE 3. Effects of DC-HIL-SAP on CH. On day 0, BALB/c mice (n = 4) were sensitized by painting 2% Ox on shaved abdominal skin. On day 6, CH was elicited in sensitized mice by painting 1% Ox or solvent control to right or left ears, respectively (Challenge). Ear thickness was measured daily from days 1–3 following challenge. A, Mice also were injected i.v. with PBS, Ig-SAP, or DC-HIL-SAP (20 or 40 nM) 3 h prechallenge. Daily change in ear thickness (10^-3 inch) was plotted for each panel. B, On day 2, ear skin specimens of mouse representative in each group were stained with H&E and examined for histology. C, One day postchallenge, mice (n = 4) were also sensitized and challenged but with a different chemical, PMA. Ear thickness was measured 1 d following challenge. Statistical significance is denoted by asterisks (*p < 0.001; **p = 0.003) as compared with ear thickness treated with control Ig-SAP. Data shown are representative of four (A) and two (B–F) independent experiments.
postchallenge), and they were injected with 40 nM DC-HIL-SAP or controls (PBS or Ig-SAP). DC-HIL-SAP (but not Ig-SAP) reduced ear thickness by 40%.

Finally, we questioned whether injection of DC-HIL-SAP also inhibits CH response to other chemicals. Mice were sensitized and challenged with PMA using a similar time schedule and injected with 40 nM SAP conjugates 3 h prechallenge (Fig. 3F). Infusion of DC-HIL-SAP reduced CH response by 55%. Altogether, these results indicate that a single injection of DC-HIL-SAP markedly suppresses elicitation of CH in sensitized mice and that this immunosuppression lasts 3 wk. Moreover, DC-HIL-SAP effectively inhibits an established CH response.

CH inhibition by DC-HIL-SAP is restricted to Ag at time of infusion

We next examined the immune reactivity of mice treated with DC-HIL-SAP to other haptens during their unresponsive state to the original hapten (Fig. 4). BALB/c mice were sensitized and challenged with Ox and treated i.v. with PBS, Ig-SAP, or DC-HIL-SAP. On the same day (day 6), mice were treated with Ox (right ear) and TNCB on right and left ears, respectively. Ear thickness was measured on day 7. Ear thickness was measured on day 1 every postchallenge (B). *p < 0.05 as compared with ear thickness of mice treated with Ig-SAP. A second experiment showed similar results.

Because significant accumulation of lymphocytes was observed in DLNs 2 d (not 1 d) postchallenge, we procured DLN at this time point and examined effects of DC-HIL-SAP on T cell phenotypes (Fig. 5). DLNs of mice treated with DC-HIL-SAP were smaller compared with controls (mice treated with PBS or Ig-SAP) (Fig. 5A). There were no significant changes in the relative proportions of CD4+, CD8+, and CD19+ cells (Fig. 5A). However, DLNs from mice treated with DC-HIL-SAP contained less numbers of CD69+ (activated) cells in all three subpopulations compared with those for

FIGURE 4. Suppression of CH by DC-HIL-SAP is restricted to hapten administrated at the time of infusion. A, Immunization protocol is depicted schematically: BALB/c mice (n = 4) were sensitized with Ox on day 0, and i.v. injected with PBS or SAP conjugate 3 h prechallenge (day 6). On the same day, mice were challenged with Ox and solvent alone on right (R-ear) and left ears (L-ear), respectively, and also sensitized to TNCB. On day 7, ear thickness was measured (1° Ox). Day 12, all mice were challenged with Ox (2° Ox) and TNCB on right and left ears, respectively. Ear thickness shown is measured on day 1 every postchallenge (B). *p < 0.05 as compared with ear thickness of mice treated with Ig-SAP. A second experiment showed similar results.

FIGURE 5. Immunological phenotypes of DLN cells from mice treated with SAP conjugates. A and B, Sensitized mice (n = 3) were i.v. injected with PBS, Ig-SAP, or DC-HIL-SAP (each 40 nM) 3 h prechallenge. Two days postchallenge with Ox, DLNs procured from treated mice were counted and expressed as cell number per DLN. Frequency (%) of CD4+, CD8+, and CD19+ B cells was measured by flow cytometry (A). DLN cells also were examined for expression of CD69 on all LN cells, CD4+, CD8+, or CD19+ cells, and frequency (%) was calculated (B). C, DLN cells from similarly treated mice (n = 3) were cultured for 2 d in the presence of anti-CD3 and anti-CD28 Ab and assayed for production of IL-4, IFN-γ, IL-10, and IL-17. As control, DLN cells from unsensitized mice (None) were also analyzed in the same manner. SD was derived from experimental values for three mice. Statistical significance is denoted by asterisks (*p < 0.001; **p = 0.14; ***p = 0.03) as compared with frequency in LNs of mice treated with control Ig-SAP. Data shown are representative of three separate experiments.
Ig-SAP–treated mice (Fig. 5B). We also examined cytokine production by DLN cells (Fig. 5C). A day after hapten challenge, DLN cells were procured and then cultured for 2 d in the presence of anti-CD3 and anti-CD28 Ab. In mice treated with DC-HIL-SAP, DLN cells produced 40% more IL-4, but secreted 40% less IFN-γ and 60% less IL-10 and IL-17 compared with controls. Thus, not only did DC-HIL-SAP decrease the number of activated T cells in DLN, but it also altered the balance of Th cytokine expression.

**DC-HIL-SAP deletes SD-4+ T cells in hapten-painted skin and DLNs**

We also examined effects of DC-HIL-SAP on SD-4+ T cells in Ox-painted ear skin. Two days after challenging sensitized mice treated with SAP conjugates (40 nM) or controls, Ox-painted ear skin biopsies were procured and stained fluorescently with anti-CD4 (or anti-CD8) Ab and anti–SD-4 Ab (Fig. 6A). Doubly positive cells were counted and averaged from three separate microscopic views (Fig. 6B). There were none to very few T cells in untreated skin (None) but many CD4+ and CD8+ T cells in Ox-painted skin injected with PBS, almost all of which were SD-4+ (Fig. 6A). Numbers of CD4+ and CD8+ T cells in skin of mice injected with Ig-SAP were similar to those of mice treated with PBS; both were reduced markedly following DC-HIL-SAP infusion (90% reduction for CD4+ and 80% for CD8+ T cells). These results indicate that a single infusion of DC-HIL-SAP depletes almost all SD-4+ T cells in Ox-challenged skin.

We also examined effects of DC-HIL-SAP on DLN cells (Fig. 7). Two days after challenging sensitized mice infused with SAP conjugates, CD4+ and CD8+ T cells were purified from DLNs and assayed for SD-4+ by flow cytometry. SD-4+ T cells comprised 3.4% of CD4+ and 3.2% of CD8+ T cells in LNs of mice treated with PBS (Fig. 7A). Mice treated with DC-HIL-SAP showed reduced frequency of SD-4+ cells: 2.8% for CD4+ and 2.6% for CD8+ cells. By contrast, there were no changes in mice treated with PBS or Ig-SAP control. Because the total number of CD4+ T cells per LN varied (5.6×10^5 cells/LN of mice treated with PBS, 5.2×10^6 cells/LN with Ig-SAP, and 3.6×10^6 cells/LN with DC-HIL-SAP), the total number of SD-4+ CD4+ T cells was 1.9×10^5 cells/LN for mice with PBS, 1.7×10^5 cells/LN with Ig-SAP, and 1.0×10^5 cells/LN with DC-HIL-SAP (Fig. 7B). For CD8+ T cells, LNs of mice treated with DC-HIL-SAP contained 3.8×10^5 SD-4+ CD8+ T cells (versus 6.8×10^5 cells for PBS and 6.3×10^5 cells for Ig-SAP). These results document the ability of a single infusion of DC-HIL-SAP to deplete by 40% CD4+ and CD8+ T cells from DLNs.

**Discussion**

Several immunotoxins and cytotoxic mAbs have been used to kill T lymphocytes in diseases in which these cells play critical pathogenic roles. A well-studied example is DAB389IL-2 (Denileukin Diftitox or Ontak), an immunotoxin created by replacing the receptor-binding domain of diphtheria toxin with the entire IL-2 molecule, that targets intermediate (CD122/CD132) and high-affinity (CD25/CD122/CD132) IL-2Rs (29–31) expressed by activated T and B cells and macrophages. Originally used to treat psoriasis, it is now approved by the U.S. Food and Drug Administration to treat cutaneous T cell lymphoma (32), a condition in which T cells overexpress high affinity IL-2R. Although useful for these diseases, the potential of DAB389IL-2 to kill all activated T lymphocytes including those in the memory pool may have debilitating consequences with respect to protective immunity. Thus, in cases in which only a restricted subpopulation of T cells are pathologic, the use of toxin-bearing DC-HIL may hold a special advantage because of its specific binding to SD-4 expressed on effector memory T cells.

CTLA-4 also has been targeted by immunotoxins because of its central role in inhibiting T cell activation (33). Recombinant anti-
CTLA single-chain fragment of variable domain conjugated with SAP was shown to effectively deplete activated T cells in MLRs and in infiltrating tumors in mice (34). By contrast, it was not effective for inhibiting established inflammatory conditions because CTLA-4 expression is restricted to early activated T cells that comprise only a small portion of T cells in secondary responses. Like DABaptop-2, anti–CTLA-4 immunotoxin was more efficacious for treating CTLA-4–expressing neoplastic cells of acute myeloid leukemia (35). We found SD-4 to be highly expressed by CD4+ T cells of patients with mycosis fungoides and Sezary syndrome (a leukemic variant of cutaneous T cell lymphoma) and showed our DC-HIL immunotoxin to strongly kill these cells (J.-S. Chung, L. H. Shiue, A. Dougherty, X. Ni, M. Duvic, P. D. Cruz, Jr., and K. Ariizumi, manuscript in preparation).

Because SD-4 and PD-1 share several features (20), including overlapping T cell expression profiles and functions, we also examined the ability of SAP-bearing PD-L1-Fc (the extracellular domain fused with the Fc portion) to suppress elicitation of CH responses. Surprisingly, it failed to suppress CH responses regardless of infusion during sensitization or challenge (data not shown). These negative outcomes were due to the inability of PD-L1-Fc to bind stably activated T cells. The biological activity of our PD-L1-Fc preparation was ascertained by the ability of its immobilized form to strongly inhibit the anti-CD3 response (36).

Thus, among coinhibitory receptors, our effort to target SD-4 is highly beneficial because a single infusion of DC-HIL-SAP mediated inflammation. Future applications targeting SD-4 may prove highly beneficial because a single infusion of DC-HIL-SAP strongly suppressed elicitation of CH response, lasting 3 wk. Because infusion of DC-HIL-SAP markedly reduces the number of CD4+ T cells in hapten-challenged skin (but less efficiently depletes these T cells in DLNs), we speculate that SD-4 may be involved in homing of T cells to skin, especially because it facilitates binding of the chemokine RANTES/CCCL5 to the specific receptor CCR5 responsible for recruiting leukocytes into inflammatory sites (37). Moreover, SD-4 was reported to bind the chemokine X. Ni, M. Duvic, P. D. Cruz, Jr., and K. Ariizumi, manuscript in preparation).

In sum, our studies document the effectiveness of DC-HIL–mediated targeting of SD-4 to alleviate a cutaneous inflammatory response in mice. This immunotherapy may benefit management of graft-versus-host disease and protection of transplanted organs from rejection because SD-4 also is expressed by cytotoxic T lymphocytes directed to allo-Ag (M. Tomihari and K. Ariizumi, unpublished observations).

Acknowledgments
We thank Irene Dougherty and Julie Yang for technical and secretarial assistance.

Disclosures
The authors have no financial conflicts of interest.

References

Downloaded from http://www.jimmunol.org/ by guest on July 26, 2017


