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Attenuation of Th1 Response in Decoy Receptor 3 Transgenic Mice

Tsui-Ling Hsu,* Ying-Yu Wu,**† Yung-Chi Chang,* Chih-Ya Yang,* Ming-Zong Lai,†
Wenlynn B. Su,* and Shie-Liang Hsieh*‡§

The soluble decoy receptor 3 (DcR3) is a member of the TNFR superfamily. Because DcR3 is up-regulated in tumor tissues and is detectable in the sera of cancer patients, it is regarded as an immunosuppressor to down-regulate immune responses. To understand the function of DcR3 in vivo, we generated transgenic mice overexpressing DcR3 systemically. In comparison with HNT-TCR (HNT) transgenic mice, up-regulation of IL-4 and IL-10 and down-regulation of IFN-γ, IL-12, and TNF-α were observed in the influenza hemagglutinin126–138 peptide-stimulated splenocytes of HNT-DcR3 double-transgenic mice. When infected with Listeria monocytogenes, DcR3 transgenic mice show attenuated expression of IFN-γ as well as increased susceptibility to infection. The Th2 cell-biased phenotype in DcR3 transgenic mice is attributed to decreased IL-2 secretion by T cells, resulting in the suppression of IL-2 dependent CD4+ T cell proliferation. This suggests that DcR3 might help tumor growth by attenuating the Th1 response and suppressing cell-mediated immunity. The Journal of Immunology, 2005, 175: 5135–5145.

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3 Abbreviations used in this paper: DcR3, decoy receptor 3; AICD, activation-induced cell death; BMDC, bone marrow-derived DC; DR3, death receptor 3; FasL, Fas ligand; HA, hemagglutinin; HKLM, heat-killed L. monocytogenes; HVEM, Herpesvirus entry mediator; LIGHT, receptor homologous to lymphotoxins that exhibits inducible expression, competes with HSV glycoprotein D for the HVEM, and is expressed by T lymphocytes; LN, lymph node; LTβR, light-chain β receptor; m, murine; PGK, phosphoglycerate kinase; PI, propidium iodide; SPC, splenocyte; TL1A, TNF-like molecule 1A.

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Isolation of CD4^+ T cells and induction of activation-induced cell death (AICD)

To isolate CD4^+ T cells, total LN cells or SPCs were collected and stained sequentially with anti-mCD4-FITC mAb (0.5 µg/10^6 cells) and goat anti-FITC MicroBeads (Miltenyi Biotec). Cells were then purified using the VarioMACS (Miltenyi Biotec) technique and eluted from LS^-MACS columns according to the supplier’s protocol. To induce AICD, CD4^+ cells, prepared from murine LN cells, were seeded onto 24-well plates (10^5 cells/ml well) precoated with anti-mTCRβ mAb (clone H57.597; 2.5 µg/0.5 ml PBS/well at 37°C overnight) and cultivated in the presence of IL-2 (500 U/ml; R&D Systems) for 5 days at 37°C in complete medium (RPMI 1640 supplemented with 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, 20 mM HEPES, and 50 µg/ml β-2-ME). Cells were repleted with a half of the initial IL-2-supplemented medium (500 U/ml) on day 3. Dead cells were removed by density gradient centrifugation (Histopaque 1083; Sigma-Aldrich) at 500 x g for 20 min at 25°C, whereas live cells (density of 10^9/ml) were restimulated with plate-bound anti-mTCRβ mAb for 24 h. The percentages of apoptotic cells were determined by annexin V-FITC/propidium iodide (PI) double staining. To block AICD, receptor-Fc fusion proteins (5 µg/ml) or control IgG1 (5 µg/ml; Sigma-Aldrich) were added to the cell culture at the time of restimulation.

Flow cytometric analysis

For cell surface staining, 5 x 10^6 cells were preincubated with rat anti-mCD16/32 mAb (1 µg/ml) at 4°C for 10 min in 50 µl of FACS staining/washing buffer (1% (v/v) FCS and 0.1% NaN₃ in PBS) to prevent nonspecific Ab binding through the FcR. Cells were also incubated with fluorochrome-conjugated Ag-specific mAbs at 4°C for 20 min, followed by washing twice with 1 ml of FACS staining/washing buffer. Those cells stained with unlabelled or biotinylated mAbs were additionally incubated with fluorochrome-conjugated secondary Ab at 4°C for 20 min. After washing twice with 1 ml of FACS staining/washing buffer, cells were fixed with 1% (v/v) paraformaldehyde/PBS for 30 min at 4°C, then subjected to flow cytometric analysis. Intracellular cytokine staining was performed as vendor’s suggestion (BD Biosciences). All samples were analyzed with FACS Calibur (BD Biosciences) using CellQuest software (BD Biosciences).

Cytokine RT-PCR and ELISA

To detect cytokine transcripts, total RNA was extracted from stimulated cells using RNAzol B (Iso-Tex Diagnostics) and reverse transcribed using a Prostar first-strand RT-PCR kit (Stratagene). Specific primer pairs provided by Dr. C.-P. Hu (Veterans General Hospital, Taipei, Taiwan) and Dr. S.-L. Chang (Tzu Chi University, Hualien, Taiwan) were used to amplify target cytokines. Supernatants from stimulated cells were collected and stored at −80°C until cytokine detection by ELISA. Mouse IL-4, IL-10, and IFN-γ ELISA kits were purchased from Pierce Endogen; mouse IL-12 and TNF-α ELISA kits were purchased from R&D Systems; the mouse IL-2 ELISA kit was purchased from BioSource. Procedures were conducted as suggested by the manufacturers.

Stimulation of HNT CD4^+ T cells

The CD4^+ T cells from HNT mice were activated by incubation with the antigenic peptide hemagglutinin (HA)^126-134 (HNTNGVTACASH; synthesized by Sigma-Aldrich and dissolved in PBS) or the control peptide OVA^257-264 (ISQAVHAAHAEINEAGR) in vitro. Briefly, 2 x 10^5 SPCs/ml were suspended in complete medium and stimulated with 10 µg/ml peptide for 6 h (for cytokine RT-PCR) or 3 days (for cytokine ELISA) in the presence or the absence of human IL-2 (500 U/ml; R&D Systems). Alternatively, purified CD4^+ T cells (10^6 cells/ml/well in 24-well plates) were stimulated with immobilized anti-mTCRβ mAb (2.5 µg/ml 0.5 ml PBS/well at 37°C overnight) and cultivated for 3 days at 37°C in complete medium. Supernatants were collected to determine the levels of IFN-γ and IL-4 by ELISA.

Listeria infection and restimulation of mesenteric LN cells

Eight- to 12-wk-old wild-type or Dr3 transgenic mice were infected with sublethal doses of L. monocytogenes (250,000 CFU/mouse) by i.p. injection. After 5 days, 2 x 10^5/ml mesenteric lymphocytes were restimulated with 10^6 CFU/ml heat-killed L. monocytogenes (HKLM) in 24-well plates. Cytokine concentrations were determined using a Cytometric Bead Array kit (BD Biosciences). IL-12 and TNF-α levels were significantly higher in Dr3 transgenic mice than in wild-type mice at 48 h post-infection.
bacterial culture were spread out on trypticase soy broth agar (Difco Laboratories) plates. The plates were incubated at 37°C for 16 h to determine the number of colonies. To determine the survival rate of infection, mice were infected with lethal doses (10^6 CFU/mouse) of L. monocytogenes by i.p. injection. The survival rates of wild-type and DcR3 mice were checked daily for 20 days.

**Cell proliferation assay**

To measure cell proliferation by [3H]thymidine incorporation assay, 2 × 10^7/200 μl of SPCs were stimulated with HA126–138 or OVA323–339 peptide (10 μg/ml) in U-bottom, 96-well microtiter plates for 3 days. [3H]Thymidine (0.5 μCi/well; PerkinElmer) was added to each well, and the stimulated SPCs were incubated for an additional 16 h. Cells were collected using a cell harvester (Skatron), and the incorporated radioactivity was measured using a beta counter (model LS5801; Beckman Coulter). To detect the level of cell division in stimulated CD4^+ T cells, 5 × 10^5/ml SPCs were incubated with 5 μM CFSE (Molecular Probes) for 5 min at room temperature in PBS, followed by three washes with 5% (v/v) FCS/PBS. CFSE-labeled SPCs were stimulated with peptide for 3 days. CD4^+ T cells were distinguished from SPCs by staining with anti-CD4-allophycocyanin, and the CFSE profiles of proliferating CD4^+ T cells were analyzed with FACSCalibur and CellQuest software.

**Surface plasmon resonance**

Association and dissociation rates of the interaction between human DcR3Fc and mouse FasL, LIGHT, and TL1A were determined by surface plasmon resonance (SPR) in a BIAcore X biomolecular interaction analysis system. Human IgG1 (50 μg/ml) was first immobilized on flow channel 1 of a CM5 sensor chip as the blank to determine the bulk effect of injection itself, whereas DcR3Fc (50 μg/ml) was immobilized on flow channel 2 by amine coupling at pH 5.0. The sensor surface was equilibrated by PBS, and sensograms were collected at 25°C at a flow rate of 30 μl/min. A 180-μl injection of mouse FasL, LIGHT, or TL1A was passed over the sensor surface. After the association phase, 60 s of dissociation data were collected. The sensor surface was regenerated after each cycle with a 15-s regeneration step. The association and dissociation rate constants of the interaction between human DcR3Fc and mouse FasL, LIGHT, and TL1A were calculated.

**Statistical evaluation**

Values are expressed as the mean ± SEM of at least three experiments. One-way ANOVA and t tests were used to assess the statistical significance of the differences, with a value of p < 0.05 considered statistically significant.

**Results**

**Generation and characterization of DcR3 transgenic mice**

To produce a transgene construct, a cDNA encoding full-length human DcR3 was amplified by PCR and subcloned into the PGK-Neo^8-bpa vector (19) in place of the neomycin-resistant gene (Neo^8). A transgene encoding DcR3 under the control of the PGK promoter (PGK-DcR3) was excised from the vector and microinjected into FVB-fertilized eggs. To screen for insertion of the transgene into the mouse genome, we performed PCR using primers that anneal to the PGK promoter and the DcR3 coding region. As shown in Fig. 1A, a product of the expected size (320 bp) was amplified from the genomic DNA of transgenic mice. To assay for the DcR3 transcript, total RNA extracted from peripheral blood cells of both DcR3 transgenic-positive and -negative mice was subjected to RT-PCR. A DNA fragment with predicted size was amplified from the cDNA template in four of five founder mice that were positive for the PGK-DcR3 transgene (Fig. 1B). We also examined the expression of DcR3 protein in cell lysates prepared from SPCs of both DcR3 transgenic and wild-type littermates by immunoblotting. As shown in Fig. 1C, a 33-kDa protein was observed in cell lysates from DcR3 transgenic, but not wild-type, mice. Because DcR3 is detectable in the sera of certain cancer patients (8), we next performed a sandwich ELISA to determine serum DcR3 levels. As shown in Fig. 1D, the average concentration of DcR3 in all DcR3 transgenic mice tested was 4.7 ng/ml; no protein was detected in the sera of nontransgenic littersmates. Therefore, both intra- and extracellular DcR3 could be detected in DcR3 transgenic mice. Taking these results together, DcR3 transgenic mice, as defined by genomic PCR screening, are able to express both DcR3 RNA transcript and DcR3 protein.

To study Ag-specific T cell responses in the subsequent experiments, DcR3 transgenic mice on an FVB background were backcrossed with HNT-TCR (HNT) transgenic mice on a BALB/c background. The latter carry rearranged TCRα and TCRγ transgenes that recognize influenza HA peptide (HA126–138) in the context of I-A^d (22). Screening of HNT transgenic mice was performed by flow cytometry, as described in Materials and Methods.

**DcR3 transgenic mice show inhibition of AICD**

To understand whether DcR3 expressed in transgenic mice is functional, we examined the extent of cell apoptosis in AICD, which is mediated by FasL, one of the ligands of DcR3. To address this question, CD4^+LN cells prepared from wild-type or DcR3 transgenic mice were stimulated with anti-mouse TCRβ mAb to induce AICD, followed by annexin V-FITC/PI double staining to detect the percentages of apoptotic cells. In the nontransgenic littermates, the percentages of annexin V-FITC^+/PI^+ and annexin V-FITC^−/PI^− CD4^+ LN cells were 46 and 15%, respectively, 24 h after restimulation in control mice (Fig. 1E, lower panel). In contrast, the percentages of annexin V-FITC^+/PI^+ and annexin V-FITC^−/PI^− cells were only 12 and 5%, respectively, for DcR3 transgenic mice treated under the same conditions (Fig. 1E, upper panel). Exogenous DcR3Fc and FasFc recombinant proteins have similar inhibitory effects on cell apoptosis, whereas LTβR.Fc and IgG1 do not inhibit AICD. In this study we have shown that endogenous DcR3 expressed in transgenic mice, like recombinant DcR3Fc and FasFc (12), can inhibit cell death. Therefore, we conclude that DcR3 is an effective inhibitor of AICD in vivo.

**DcR3 transgenic mice show an attenuated Th1 and polarized Th2 character upon stimulation**

We have shown previously, in both human and murine systems, that DcR3Fc-treated DCs induce CD4^+ T cells to differentiate into a Th2 phenotype (12, 13). To examine the possibility that DcR3 skews the immune system toward a Th2-predominant response when it is expressed systemically in vivo, SPCs prepared from HNT-DCR3 and HNT mice were treated with HA126–138 peptide, which specifically stimulates HNT-bearing CD4^+ T cells when presented by I-A^d on APCs (22). Subsequent analysis of cytokine profiles by RT-PCR revealed that the expressions of IL-2 (77%), IL-3 (85%), IL-12 p40 (64%), IFN-γ (49%), and TNF-α (50%) were down-regulated in HNT-DcR3 SPCs, whereas the expressions of IL-4 (163%), IL-10 (152%), and IL-13 (139%) were up-regulated (Fig. 2A). Because IL-4, IL-10, and IL-13 are characteristic of a Th2-polarized immune response, whereas IL-12 p40, IFN-γ, and TNF-α are representative cytokines of a Th1 response, this observation suggests that HNT-DcR3 SPCs become biased toward a Th2 response upon HA126–138 peptide stimulation. To test this hypothesis, supernatants from cultured SPCs stimulated with HA126–138 peptide for 3 days were subjected to cytokine ELISA. In accord with the results of RT-PCR, the secretion of IL-4 (p = 0.003) and that of IL-10 (p = 0.015) were significantly up-regulated, whereas production of IL-2 (p = 0.045), IFN-γ (p = 0.048), TNF-α (p = 0.036), and IL-12 p40 (p = 0.042) was down-regulated, in HNT-DcR3 SPCs (Fig. 2B). Similar results were observed when HA126–138 peptide-primed.
SPCs were restimulated with immobilized anti-mouse TCRβ mAb (Fig. 2C).

To determine whether the down-regulation of IFN-γ and the up-regulation of IL-4 were due to the APCs of HNT-DcR3 transgenic SPCs, we next investigated IFN-γ and IL-4 secretion by purified CD4+ T cells stimulated with anti-mTCRβ mAb. As shown in Fig. 2D, CD4+ T cells purified from HNT and HNT-DcR3 SPCs showed equal amounts of IFN-γ and IL-4 secretion. These data suggested that APCs of HNT-DcR3 transgenic mice were crucial for the polarized Th2 response.
FIGURE 2. Cytokine profiles of stimulated-HNT-DcR3 or HNT SPCs. 

A, Cytokine RT-PCR. cDNAs were reverse transcribed from the total RNA of HA126–138 peptide-stimulated SPCs, isolated from HNT and HNT-DcR3 mice. PCR products were fractionated on 1.5% agarose gels. The intensity of each PCR product was quantified by ImageQuant software (Amersham Biosciences) and normalized against β2-microglobulin. The relative intensity of each PCR product was calculated by dividing the volume of the PCR product amplified from HNT or HNT-DcR3 SPCs by that amplified from HNT SPCs. One set of representative data from three experiments is shown.

B, Cytokine ELISA. Supernatants collected from 3-day cultures of HA126–138 peptide-stimulated SPCs were assayed by ELISA kits. The data presented in this study were obtained by pairing the results for HNT and HNT-DcR3 mice from six independent experiments. The p values, calculated by unpaired t tests, are indicated in each panel.

C, IFN-γ and IL-4 ELISA of restimulated SPCs. SPCs were stimulated with HA126–138 peptide for 3 days, followed by restimulation with plate-bound anti-mTCRβ mAb (H57.597) for 20 h. Supernatants were collected for ELISA. The data presented in this study were obtained by pairing the results for HNT and HNT-DcR3 mice from four independent experiments. The p values, calculated by unpaired t tests, are indicated in each panel.

D, Secretion of IFN-γ and IL-4 by anti-mTCRβ mAb-stimulated CD4+ T cells. Purified CD4+ T cells were stimulated with immobilized anti-mTCRβ mAb as described in Materials and Methods. The amounts of IFN-γ and IL-4 were measured by ELISA.
FIGURE 3. Stimulation of HNT-DcR3 and HNT mice in the presence or the absence of IL-2. A and D. Intracellular cytokine staining. SPCs were restimulated with plate-bound anti-mTCRβ mAb for 6, 15, or 24 h after 3 days of HA126–138 peptide stimulation in the absence (A) or the presence (D) of human IL-2 (500 U/ml). Cells were triple stained with anti-mIFN-γ-FITC/anti-mIL-4-PE/anti-mCD4-allophycocyanin and subjected to flow cytometric analysis. The cell populations presented in this study are CD4+ T cells; the percentage of positively stained cells is indicated in each quadrant plot. One set of representative data from three experiments is shown. B. [3H]thymidine incorporation assay. SPCs prepared from HNT and HNT-DcR3 mice were stimulated with HA126–138 peptide or OVA323–329 peptide or were left untreated (CTRL) for 3 days, followed by the addition of [3H]thymidine (0.5 μCi/well) for 16 h before harvesting. The data presented in this figure were obtained from two HNT (HNT-1 and -2) and two HNT-DcR3 (HNT-DcR3-1 and -2) mice. *p < 0.05 compared with both HNT-1 and HNT-2 controls, by paired t test. C, CFSE labeling assay. CFSE-labeled (Figure legend continues)
Suppression of IL-2-dependent CD4\(^+\) T cell proliferation in DcR3 transgenic mice

The decreased IFN-\(\gamma\) secretion described above might due to the lower percentage of IFN-\(\gamma\)-producing CD4\(^+\) T cells or to a reduction in the amount of IFN-\(\gamma\) secreted by CD4\(^+\) T cells. Intracellular cytokine (IFN-\(\gamma\) and IL-4) staining was performed to address this question. Compared with cells from HNT mice, decreased percentages of IFN-\(\gamma\)-expressing CD4\(^+\) T cells (24 vs 35% at 6 and 15 h; 35 vs 51% at 24 h) and an increased percentage of IL-4 expressing CD4\(^+\) T cells (8 vs 4% at 6 and 15 h) CD4\(^+\) T cells were observed for HNT-DcR3 mice (Fig. 3A). Moreover, compared with HNT littermates, the proliferation of HA\(_{126-138}\) peptide-stimulated CD4\(^+\) T cells in HNT-DcR3 transgenic mice was suppressed, as shown by \(^{3}H\)thymidine incorporation (Fig. 3B) and flow cytometric analysis of CFSE-labeled CD4\(^+\) T cells (80 vs 69%; Fig. 3C). Because the secretion of IL-2 decreased under the same conditions (Fig. 2), we tested whether exogenous IL-2 could restore IFN-\(\gamma\) expression in CD4\(^+\) T cells. In the untreated group, the proportions of IFN-\(\gamma\)-expressing CD4\(^+\) T cells in HNT-DcR3 and HNT mice were 24 vs 35% at 15 h and 35 vs 51% at 24 h (Fig. 3A), whereas treatment with IL-2 increased the percentages of IFN-\(\gamma\)-expressing CD4\(^+\) T cells (50 vs 54% at 15 h; 58 vs 61% at 24 h) in HNT-DcR3 and HNT mice and significantly increased the ratio of IFN-\(\gamma\)-expressing cells in the former compared with the latter (Fig. 3D). It is interesting to note that IL-2 had no obvious effect on the ratio of IL-4-secreting CD4\(^+\) T cells between HNT-DcR3 and HNT mice (from 8 vs 4% to 9 vs 5% at 15 h in IL-2-treated samples). In accordance with the measurements of intracellular IFN-\(\gamma\) and IL-4, the levels of IFN-\(\gamma\) secreted by HA\(_{126-138}\) peptide-stimulated HNT and HNT-DcR3 SPCs were similar after the addition of exogenous IL-2, whereas the differences in IL-4 secretion between stimulated HNT and HNT-DcR3 SPCs remained significant in the presence of exogenous IL-2 (Fig. 3E).

FIGURE 4. DcR3 transgenic mice infected with L. monocytogenes show attenuated induction of IFN-\(\gamma\). A, ELISA of IFN-\(\gamma\). Mesenteric LN cells of infected wild-type (WT) or DcR3 transgenic (DcR3) mice were restimulated with HKLM for 24 h. Culture supernatants were collected and subjected to ELISA. *, p < 0.05 compared with WT control, by paired t test. One representative experiment of three is shown. B, Detection of intracellular IFN-\(\gamma\), Mesenteric LN cells were restimulated with HKLM for 15 or 24 h before cell harvest and subsequent staining. CD4\(^+\) lymphocytes were gated for analysis, and the percentage of IFN-\(\gamma\)-expressing CD4\(^+\) T cells is indicated in each quadrant plot. One representative experiment of three is shown. C, DcR3 transgenic mice are more susceptible to L. monocytogenes infection. WT (\(n = 12\)) and DcR3 transgenic (\(n = 12\)) mice were injected with lethal doses of L. monocytogenes (10\(^6\) CFU/mouse) i.p. The number of survivors was checked daily for 20 days.
To investigate whether a Th2-biased phenotype could be observed in HNT-DCR3 transgenic mice and whether this might affect the outcome of a host immune reaction to pathogen invasion, we examined the level of cytokine expression in DC R3 transgenic mice after infection with L. monocytogenes, an intracellular bacteria that typically induces a strong Th1 response in vivo (23, 24). DcR3-BALB/c mice and nontransgenic littermates were infected with sublethal doses of L. monocytogenes i.p.; mesenteric LN cells from infected mice were then restimulated with HKLM to assess the L. monocytogenes-induced immune response. The amounts of IFN-γ and IL-4 in the culture supernatant were measured by ELISA. As shown in Fig. 4A, IFN-γ secretion was significantly suppressed in the HKLM-restimulated mesenteric LN cells of DcR3-BALB/c mice (0.9 ng/ml), compared with that in wild-type littermates (12 ng/ml). However, the level of IL-4 was too low to be detected under the same conditions. From this observation, it is obvious that IFN-γ secretion is suppressed not only in HNT-DCR3 CD4+ T cells after HA126–138 peptide stimulation, but also in DcR3-BALB/c T cells stimulated with L. monocytogenes.

To test whether the decreased secretion of IFN-γ was due to lower responsiveness of CD4+ T cells to L. monocytogenes infection in DcR3-BALB/c mice, the intracellular expression levels of IFN-γ and IL-4 were measured in mesenteric LN cells isolated from L. monocytogenes-infected mice (Fig. 4B). Compared with nontransgenic littermates, the percentage of IFN-γ-expressing CD4+ T cells in DcR3-BALB/c mice was decreased (13 vs 9% at 15 h; 35 vs 10% at 24 h). However, the expression of IL-4 was not detected by intracellular cytokine staining. This also supports the argument that DcR3 skews the immune response toward a Th2 phenotype by suppressing IFN-γ-secreting CD4+ T cell proliferation.

We asked whether decreased IFN-γ expression correlated with the susceptibility to L. monocytogenes infection. To answer this question, mice were injected with lethal doses (106 CFU/mouse) of L. monocytogenes i.p. to study survival rates for 20 days. As shown in Fig. 4C, 83% of L. monocytogenes-infected DcR3-BALB/c mice died within 7 days. In contrast, 50% of nontransgenic littermates were still alive on day 7. The survival rates of L. monocytogenes-infected mice did not change up to 20 days after infection. Thus, the increased susceptibility of DcR3 transgenic mice to L. monocytogenes infection correlated with the reduced percentage of IFN-γ-expressing CD4+ T cells in L. monocytogenes-infected DcR3 mice.

**Exogenous TL1A restores DcR3-mediated cytokine secretion**

DcR3 has been shown to neutralize the biological effects of three ligands: LIGHT, TL1A, and FasL. Both LIGHT and TL1A have been shown act as costimulators of T cell proliferation and to enhance IFN-γ secretion (4, 20, 25); therefore, we tested whether the Th2-biasing effects of DcR3 occur via the neutralization of endogenous FasL, LIGHT, and TL1A. To address this question, we studied the effects of recombinant Fas.Fc, DcR3.Fc, and DR3.Fc on cytokine secretion from T cells. As shown in Fig. 5A, DcR3.Fc and DR3.Fc, but not Fas.Fc, had similar effects, causing down-regulation of IFN-γ secretion (300 vs 125 ng/ml) and up-regulation of IL-4 secretion (145 vs 170 ng/ml) compared with untreated controls (Fig. 5A) in HNT

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**FIGURE 5.** Expression IFN-γ and IL-4 in receptor.Fc- or ligand-treated SPCs. A, Cytokine secretion of recombinant receptor.Fc-treated HNT SPCs. SPCs prepared from HNT mice were stimulated with HA126–138 peptide for 3 days in the presence of 3 μg/ml Fas.Fc, DcR3.Fc, or DR3.Fc. Supernatants were harvested and subjected to ELISA. *p < 0.05 compared with the control group (none), by paired t test. One representative set of data from three experiments is shown. B, Cytokine secretion by recombinant ligand-treated HNT-DCR3 SPCs. SPCs prepared from HNT-DCR3 mice were stimulated with HA126–138 peptide for 3 days in the presence of 1 μg/ml LIGHT or TL1A. Supernatants were collected and subjected to ELISA. One representative set of data from three experiments is shown.
SPCs. Because DcR3 binds to both LIGHT and TL1A, the observed effects might be attributed to the neutralization of endogenous TL1A and/or LIGHT. To address this question, recombinant LIGHT and TL1A were added to HA126–138 peptide-stimulated SPCs of HNT-DcR3 transgenic mice. As shown in Fig. 5B, TL1A, but not LIGHT, was able to restore IFN-γ secretion. This suggests that decreased secretion of IFN-γ is mediated by the inhibition of endogenous TL1A in HNT-DcR3 transgenic mice. This is in accordance with the previous observation that TL1A, but not LIGHT, can enhance T cell responsiveness to IL-2, so the neutralization of TL1A in HNT-DcR3 transgenic mice impairs IL-2-dependent proliferation of Th1 cells, thus reducing the total amount of IFN-γ. This argument is also supported by the effect of DR3, which binds only TL1A, to suppress IFN-γ secretion and up-regulate IL-4 secretion.

**Binding affinity of human DcR3 for murine FasL, LIGHT, and TL1A**

Because the human DcR3 ortholog is not found in mouse genome, understanding of the interaction between human DcR3 and murine ligands (mFasL, mLIGHT, and mTL1A) is crucial to reveal the mechanism of DcR3-mediated attenuation of the Th1 response in DcR3 transgenic mice. To answer this question, a surface plasmon resonance technique was applied to determine the affinity between human DcR3.Fc and recombinant mFasL, mLIGHT, and mTL1A, respectively. As shown in Fig. 6 and Table I, DcR3 has a higher affinity to mTL1A (54 ± 26 nM) and mFasL (179 ± 46 nM), whereas its affinity to mLIGHT (375 ± 158 nM) is much lower. From the results shown above, we conclude that the attenuated Th1 response is due to the neutralization of mTL1A, thus inhibiting IL-2 secretion and reducing Th1 cell proliferation in DcR3 transgenic mice.

**Discussion**

DcR3 has been reported to be a potent modulator of host immunity (7, 26, 27), as well as being able to enhance the differentiation of osteoclasts (16) and angiogenesis (11). DcR3-treated dendritic cells skew host immunity toward a Th2-predominant response in both human (12) and murine (13) model systems, and overexpression of DcR3 in the pancreas inhibits the onset of type I diabetes (17), a Th1-mediated autoimmune disease. However, the systemic effects of DcR3 and the mechanism by which it promotes a Th2-predominant response have not been addressed.

The Th2-prone response, observed in DcR3 transgenic mice, apparently occurs through the suppression of IL-2 secretion, thereby impairing the proliferation of Th1 cells, which rely on IL-2

![Figure 6](http://www.jimmunol.org/)

**FIGURE 6.** Kinetic analysis for the interactions of mFasL, mLIGHT, and mTL1A with DcR3.Fc. Human IgG1 was first immobilized on flow channel 1 of a CM5 sensor chip as the blank to determine the bulk effect of injection itself, whereas DcR3.Fc was immobilized on flow channel 2 for analysis of its kinetic interaction with murine ligands. Murine ligand, as the analyte, was injected at doses of 250–2000 nM. The interactions of DcR3.Fc with mFasL (A), mLIGHT (B), and mTL1A (C) were analyzed by surface plasmon resonance using a BIAcore X.

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<th>Ligand</th>
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<td>mFasL</td>
<td>9.89 ± 0.32</td>
<td>0.32</td>
<td>31.30 ± 19.6</td>
</tr>
<tr>
<td>mTL1A</td>
<td>1.79 ± 0.51</td>
<td>2.14 ± 1.89</td>
<td>0.054 ± 0.033</td>
</tr>
<tr>
<td>mLIGHT</td>
<td>179 ± 46</td>
<td>54 ± 26</td>
<td>375 ± 158</td>
</tr>
</tbody>
</table>

*Values are the mean ± SD of three measurements over a ligand concentration range of 250–2000 nM. $K_a$, association rate constant; $K_d$, dissociation rate constant; $K_D$, equilibrium dissociation constant ($K_d/K_a$). The measured $K_D$ represents the avidity between bivalent DcR3.Fc and each ligand, and would not reflect the true affinities in vivo.*
as a growth factor. This is supported by the observation that addition of exogenous IL-2 abolishes DcR3-mediated Th1 attenuation.

The neutralization of TL1A and LIGHT by DcR3 might also be important in biasing the T cell response. Both LIGHT (20) and TL1A (4, 25) have been reported to be costimulators of T cell proliferation and IFN-γ secretion in studies with human cell culture system. In nontransgenic mice, the addition of DcR3.Fc and DR3.Fc proteins can inhibit IFN-γ secretion. Moreover, the addition of TL1A increases IFN-γ secretion of HNT/DcR3 SPCs significantly, whereas LIGHT has little effect on IFN-γ secretion under the same conditions. Furthermore, the kinetic analysis of the interactions between DcR3.Fc and murine ligands (mFasL, mLIGHT, and mTL1A) revealed that DcR3 had the highest binding affinity to mTL1A and had the lowest affinity to mLIGHT. This indicates that TL1A is the major molecule affected by DcR3 in transgenic mice. It has also been reported that TL1A acts as a costimulator that increases IL-2 responsiveness and secretion of proinflammatory cytokines both in vitro and in vivo (4). Therefore, it is reasonable to speculate that the DcR3-mediated effect occurs via the neutralization of endogenous TL1A during T cell activation. The lack of TL1A-mediated costimulation imparis responsiveness to IL-2, thus suppressing the proliferation of Th1 (IFN-γ-positive) cells, which depend on IL-2 as their growth factor.

The fact that DcR3 is such a potent immunomodulator makes it a good candidate for immunotherapy. Recently, we have successfully used DcR3.Fc to treat type I diabetes in NOD mice (13). Because the onset of this condition is attributed to the activity of autoreactive Th1 cells (28), the effectiveness of this treatment supports the idea that DcR3.Fc might ameliorate diabetes by inducing Th2 polarization. Moreover, we have found that human DcR3.Fc is able to modulate the surface marker expression as well as the cytokine secretion profile of murine BMDCs; the effects are similar to those seen for human CD14+ monocyte-derived dendritic cells, and DcR3.Fc-treated murine BMDCs also induce an increased IL-4/IFN-γ ratio when co-cultivated with T cells. To directly investigate the therapeutic potential of DcR3 in preventing diabetes, transgenic mice that overexpress DcR3 in their pancreatic β cells were generated (17), and transgenic DcR3 was shown to protect mice from autoimmune and cyclophosphamide-induced diabetes in a dose-dependent manner. The observation that transgenic islets have a higher transplantation success rate and a longer survival period than controls clearly demonstrates the possibility of using DcR3 as a therapeutic agent.

Because DcR3 is overexpressed in various cancers, with expression levels being linked to tumor status, it is possible that DcR3 may enable tumor cells to evade immune surveillance by neutralizing FasL- and LIGHT-induced cytotoxicity and blockade of LIGHT- and TL1A-induced T cell costimulation. Moreover, DcR3 has been implicated in the enhancement of tumor growth through neutralizing TL1A, thereby enhancing angiogenesis in HUVECs in vitro and inducing angiogenesis in vivo (11). In combination with its ability to suppress Th1 activities, resulting in a Th2-predominant response, this may allow DcR3 to promote tumor progression through both local and systemic actions. However, there is no evidence that DcR3 is directly involved in tumor genesis, because the incidence rates of tumor formation in DcR3 transgenic mice and nontransgenic littermates are similar up to 12 mo of age (data not shown). Therefore, understanding the pathological actions and the regulation of DcR3 is important in developing new strategies for tumor therapy. It would be very interesting to test whether the neutralization of DcR3 secretion by tumors might enhance the efficacy of chemotherapy or immunotherapy in the future.

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References


