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Immunological Effects of Transgenic Constitutive Expression of the Type 1 Sphingosine 1-Phosphate Receptor by Mouse Lymphocytes

Markus H. Gräler,* Mei-Chuan Huang,‡ Susan Watson,‡ and Edward J. Goetzl†‡

The type 1 sphingosine 1-phosphate (SIP1) G protein-coupled receptor (SIP1) normally transduces SIP effects on lymph node (LN) egress and tissue migration of naive lymphocytes. We now show that persistent expression of SIP1 by lymphocytes of SIP1- transgenic (Tg) mice suppresses delayed-type hypersensitivity and results in production of significantly more IgE Ab and less IgG2 Ab than in wild-type (wt) mice. Wt host LN homing of 51Cr-labeled T cells from SIP1-Tg mice was only 30–40% of that for wt T cells. Adoptive-transfer of dye-labeled activated T cells from SIP1-Tg mice into wt mice resulted in 2.2-fold more in blood and 60% less in LNs than for activated wt T cells after 1 day. Proliferative responses of stimulated T cells from SIP1-Tg mouse were also 10–34% of those for wt T cells. Disordered cellular and humoral immunity of SIP1-Tg mice thus may be attributable to both altered T cell traffic and depressed T cell functions, suggesting that SIP1-specific agonists may represent a novel therapeutic approach to autoimmune and transplant rejection. The Journal of Immunology, 2005, 174: 1997–2003.

Sphingosine 1-phosphate (SIP) is a bioactive lysophospholipid ligand for five G protein-coupled receptors (GPCRs), designated SIP1–5 (1–6). SIP1 and SIP2 are the predominant SIP receptors expressed by all naive lymphocytes, and both are rapidly down-regulated after lymphocyte activation (7). Lymphocyte SIP1 transduces SIP chemotaxis and SIP inhibition of chemokine chemotaxis, and is required in vivo for thymocyte egress and tissue migration of naive lymphocytes from secondary lymphoid organs (SLOs) into blood (8–10). The SIP-SIP1 chemotactic signal is lost in activated lymphocytes due to down-regulation of SIP1, but SIP1-R expression recovers some days after in vivo activation of T cells (10). Such recovery of the SIP-SIP1 axis in T cells, which may be attributable to progressive differentiation, consequently induces egress of functionally specialized lymphocytes from SLOs into lymph, blood, and tissues. The immunosuppressive compound FTY720 inhibits lymphocyte traffic by down-regulating the expression of SIP1 required for their egress from SLOs (10, 11).

SIP is the natural ligand for SIP1, that is present in plasma and serum at high nanomolar to low micromolar concentrations (8, 18). Therefore an increase in the systemic SIP concentration or the level of expression of SIP1 may alter the range of functional responses of lymphocytes, as well as their lymphoid tissue distribution. SIP1 is a widely expressed GPCR, which also has critical roles in endothelial cell and cardiomyocyte survival and functions (1, 19). Stimulation of SIP1 on endothelial cells increases cell-to-cell contact and the resultant endovascular barrier by inducing adherens junction assembly (20), which may contribute to inhibition of thymocyte and lymphocyte movements. Thus SIP-SIP1 signals also may regulate lymphocyte traffic by effects on nonlymphoid cells.

We now show that transgenic (Tg) constitutive expression of SIP1 on lymphocytes modulates the migration, lymphoid tissue distribution, and proliferation of activated lymphocytes, and consequently suppresses cutaneous delayed-type hypersensitivity (DTH) reactions and significantly alters Ab responses to TNP Ag challenge with skewed isotype specificity.

Materials and Methods

Tg construct and microinjection

A blunt-end fragment of the coding region of human SIP1-R cDNA was cloned into the Smal site of an improved version of human CD2 minigene-based Bluescript vector, kindly provided by Dr. S. Hedrick (University of California, San Diego, CA (21)). After sequencings the construct (ELIM Biopharmaceuticals), the minigene was cut out of the vector with SalI and NotI (New England Biolabs), purified and injected into C57BL/6 × DBA/2 F2 hybrid embryos (Transgenic Core Facility of the Cancer Center, University of California, San Francisco, CA). Tail DNA of Tg pups was tested by real-time PCR and slot blot techniques. Tg mice and control human SIP1-R negative littermates were studied between 8 and 14 wk of age. All experiments were conducted according to protocols approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco.

Real-time PCR

Mouse and human SIP1 cDNA and human Tg SIP1-R DNA were quantified by real-time PCR as described (11). Each run was standardized with...
the respective detection of 1 ng of genomic DNA from C57BL/6 wild-type (wt) mice or from the human T cell line Jurkat (American Type Culture Collection, TIB-152), and each sample was normalized to the expression of either hypoxanthine guanine phosphoribosyl transferase cDNA or GAPDH genomic DNA. Conditions were as follows: 1X Taq Polymerase buffer, 0.5 U of Taq polymerase (Invitrogen Life Technologies), 10 mM MgCl2, 100 µM dNTPs (Invitrogen Life Technologies), 100 nM primer, 25 nM probe, 40 nM Rox (Integrated DNA Technologies, Coralville, IA), 20 ng of template-cDNA or 1 ng of template-genomic DNA. The PCR program was as follows: 1 cycle of 4:30 min at 94°C, 40 cycles of 30 s at 94 °C and 1 min at 60°C. Total RNA was isolated with TRIzol (Invitrogen Life Technologies), treated with DNase I (Invitrogen Life Technologies), and transcribed with the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen Life Technologies).

Slot blot analysis
The coding region of the human S1P1-R cDNA was cloned with HindIII and Xbal into the pcDNA3.1+ expression vector (Invitrogen Life Technologies) and cut with Xbal. This construct was used to generate a digoxigenin-labeled probe with the DIG RNA Labeling Kit (Roche Molecular Biochemicals) using the kit T7 polymerase. Two nanograms of genomic DNA were blotted on Hybond N+ nylon membranes (Amersham Biosciences), and blots were hybridized and detected using the DIG Wash and Block Buffer Set and the DIG Nucleic Acid Detection Kit (Roche Molecular Biochemicals).

Western blot analysis
Membrane proteins extracted from 5 × 10⁶ T cells were separated in a 10% polyacrylamide gel, blotted onto a polyvinylidene fluoride membrane, and developed with polyclonal rabbit anti-human S1P1, (ExAlpha) and monoclonal rat anti-mouse CD44 (BD Biosciences) Abs, as described (22).

Isolation and activation of T cells
Mouse CD4 and CD8 T cells were isolated from splenocytes using metallic beads bearing the respective mAbs (Miltenyi Biotec). Suspensions of 5 × 10⁶ purified T cells in 2 ml of RPMI 1640–10% FCS (HyClone) according to the manufacturer's protocol (25).

Transwell chemotaxis assay
Chemotaxis of mouse splenocytes was quantified as described (23), using 12-well Transwell chambers with 5-µm pore size filters (Costar) coated with 100 µg/ml collagen type IV (Sigma-Aldrich) overnight at 4°C. Filter chambers were washed twice with 500 µl of PBS, air-dried, and filled with 100 µl of suspensions of 1 × 10⁶ cells/ml in RPMI 1640 including 25 mM HEPEs, pH 7.4 (University of California, San Francisco, Cell Culture Facility, San Francisco, CA), 0.1% fatty acid-free BSA (Calbiochem), and 1X penicillin/streptomycin (Invitrogen Life Technologies). Bottom compartments were filled with 600 µl of the same medium containing different concentrations of S1P (Sigma-Aldrich). Cells that migrated to the bottom chamber in 4 h at 37°C in 5% CO₂ in air were counted in a Neubauer hemocytometer (Fisher Scientific).

Flow cytometry analysis
Cell staining and analysis were performed according to standard protocols (24). FITC-, R-PE-, allophycocyanin-, or PerCP-conjugated anti-mouse CD3-, CD4-, CD8-, CD19-, and CD69 Ab (BD Biosciences)-stained cells were analyzed using FACSCalibur (BD Immunocytometry Systems).

Enumeration of leukocyte subpopulations in blood
Leukocyte counts were determined in blood samples collected from the retro-orbital venous sinus using a Hemavet 850 Mascot model blood cell counter (CDC Technologies) according to the manufacturer’s protocol (25).

Quantification of plasma Ig concentrations
Two dilutions of plasma from the retro-orbital blood samples were analyzed with ELISA kits for total IgG (Cygnus Technologies), IgG1, IgG2a, IgA, and IgM (Bethyl Laboratories), and IgE (Crystal Chemical) (25).

Results
Generation of CD2-S1P₁-Rtg mice
cDNA encoding the human S1P₁-R was cloned into an improved version of the human CD2 mimigenase-based vector (Fig. 1a), which...
promotes T cell-specific and copy number-dependent expression of gene products (21). Microinjection of the construct in C57BL/6 × DBA/2 F1 hybrid embryos resulted in four positive founders, that were identified by real-time PCR and slot blots of genomic tail DNA. Mating of the highest expressors of each generation resulted in Tg S1P1 mice, bearing ~35 copies of the CD2-S1P1 transgene, in generation E5. Tg expression of human S1P1 by lymphocytes

Real-time PCR revealed high levels of expression of endogenous mouse S1P1-R in naive T and B cells of both wt and human S1P1-R Tg mice (Fig. 1b). Ex vivo activation of CD4 and CD8 T cells with immobilized anti-CD3 and anti-CD28 Abs. Significantly greater responses for S1P1-R Tg mice than for wt mice in blood, but not in LNs and spleen (Fig. 3c) and of CD4 and CD8 T cells (Fig. 3d) isolated from LNs, spleen, and blood of both S1P1-R Tg mice and wt mice were determined by flow cytometry analysis. A significantly higher number of CD3 T cells was found for S1P1-R Tg mice than for wt mice in blood, but not in LNs and spleen (Fig. 3c). wtCD3 T cells and CD19 B cells each constituted ~25% of total blood leukocytes, whereas S1P1 Tg CD3 T cells

Relative and absolute levels of lymphocytes in blood and immune organs in vivo

The S1P-S1P1-R axis regulates thymocyte egression and lymphocyte egression from SLOs in mice (9, 10). Therefore, the distribution of lymphocytes and lymphocyte subsets was analyzed in blood and lymphoid organs to determine whether higher expression of the human S1P1-R transgene modulates lymphocyte trafficking in the naive state. No statistically significant changes were observed in absolute or relative blood lymphocyte counts of S1P1-R Tg mice compared with wt mice (Fig. 3, a and b). Blood from both sets of mice had identical average counts per microliter and percentages of total leukocytes, lymphocytes, and neutrophils. Relative counts of CD3 T cells and CD19 B cells (Fig. 3c) and of CD4 and CD8 T cells (Fig. 3d) isolated from LNs, spleen, and blood of both S1P1-R Tg mice and wt mice were determined by flow cytometry analysis. A significantly higher number of CD3 T cells was found for S1P1-R Tg mice than for wt mice in blood, but not in LNs and spleen (Fig. 3c). wtCD3 T cells and CD19 B cells each constituted ~25% of total blood leukocytes, whereas S1P1 Tg CD3 T cells

Ex vivo T cell chemotaxis to S1P

CD4 and CD8 T cells migrate optimally to 10 nM S1P ex vivo (7). Higher expression of total S1P1-R by lymphocytes of S1P1-R Tg mice increased chemotaxis of naive CD4 T cells to 10 nM S1P when compared with that of wt mice (Fig. 2a). However, the most significant difference between wt mice and S1P1-R Tg mice was observed after ex vivo activation with immobilized anti-CD3 and anti-CD28 mAbs. Whereas activated CD4 and CD8 T cells from wt mice almost completely lost their migratory response to a range of concentrations of S1P, activated CD4 and CD8 T cells from S1P1-R Tg mice still migrated chemotactically to S1P with optimal responses at 10 nM S1P (Fig. 2b).
were nearly 40% of total blood leukocytes (Fig. 3c). There were no differences between Tg and wt mice in CD19 B cells. Similar CD4 and CD8 T cell ratios were observed in LNs, spleen, and blood of S1P1-R Tg mice and wt mice (Fig. 3d). The overall numbers of lymphocytes in S1P1-Tg and wt mice also were similar. Age-matched S1P1-Tg and wt mice had similar sizes and weights of spleens and LNs.

Altered plasma IgE concentration and anti-TNP Ab responses in S1P1-R Tg mice

In their naive state, Tg and wt mice had similar plasma levels of IgG1, IgG2a, total IgG, IgA, and IgM, but significantly elevated plasma concentration of IgE (Fig. 4a). After challenging immunized mice with TNP-KLH, anti-TNP-specific IgG1 Ab levels increased to similar levels in plasma of both S1P1-R Tg mouse and wt mice (Fig. 4b). Anti-TNP-specific IgG2b Ab levels in wt mice rose ~30-fold at 21 days after primary immunization with TNP-KLH (7 days after the boosting dose), whereas those in S1P1-R Tg mice increased only 5-fold at the same time (Fig. 4b). The lower TNP-specific IgG2b levels in TNP-KLH challenged S1P1-R Tg mouse plasma was also reflected partially in lower levels of anti-TNP-specific total IgG in S1P1-R Tg mice compared with wt mice (Fig. 4b). The anti-TNP-specific IgE Ab level was significantly
greater in S1P1-R Tg mice at 14 and 21 days after primary TNP-KLH immunization than in wt mice (Fig. 4).

**Impaired DTH response in S1P1-R Tg mice**

T cell-mediated DTH responses were assessed by ear reactions to contact with DNFB or by footpad responses to intradermal NP OSu, with measurements of swelling relative to control sites at 24 and 48 h after the respective challenges. Both methods revealed a significantly reduced DTH response in S1P1-R Tg mice compared with that in wt mice (Fig. 5).

To further investigate the observed differences between wt mice and S1P1-R Tg mice in their DTH response, cervical LNs were taken from the challenged right side and the left control side 24 h after DNFB contact-challenge of mouse ears. Lymphocyte subsets from these LNs were subsequently identified with anti-CD4, anti-CD8, and anti-CD19 Abs and stained with anti-CD69 Abs as one marker of their state of acute activation. CD4 and CD8 T cells as well as B cells taken from LNs of the control side of wt and S1P1-R Tg mice showed no or little expression of CD69 reflecting their naive unchallenged status (Fig. 5b, insets). In wt mice, 50% of CD4 T cells and 41% of CD8 T cells from cervical LNs of the challenged side expressed high levels of the CD69 marker of early lymphocyte activation. Significantly fewer LN T cells from challenged S1P1-R Tg mice expressed a high level of CD69. These differences in CD69 expression are predominately T cell selective, as the relative number of CD69-high B cells as well as their level of CD69 expression was similar in S1P1-R Tg mice and wt mice (Fig. 5).

**Modulation of T cell trafficking in vivo**

The effects of persistent expression of S1P1 by activated as well as naive Tg T cells on their in vivo trafficking was examined in two models.
activation in wt mice (7). T cells with down-regulated S1P1 GPCRs show reduced chemotactic responses to S1P, diminished S1P inhibition of chemotaxis to chemokines, and decreased S1P inhibition of other T cell functions ex vivo (18). Down-regulation of S1P1 blocks thymocyte exitation and egress of lymphocytes from SLOs in vivo (9, 10). FTY720, a spongino-line-like compound, and its phosphorylated form induce lymphopenia and are immunosuppressive in rodents and humans by down-regulating S1P1 on lymphocytes (11). To further define the immunological functions of S1P1, we developed human S1P1 Tg mice with CD2-S1P1 mini-gene copy numbers that are high enough to maintain expression of S1P1 GPCRs on activated CD4 and CD8 T cells at a level similar to that of naive wt T cells (Figs. 1, a–c, and 2, a–c). Because expression of the S1P1 Tg transcript in naive B-cells is only 6–8% of that observed in T cells (Fig. 1c), the immune phenotype of S1P1-Tg mice is considered to be influenced predominantly by altered T cell functions. However, contributions of possibly increased expression of S1P1 by dendritic cells are not excluded at present.

Tg S1P1 expression by naive T cells had no or little effect on most aspects of basal immunity, including total and relative lymphocyte counts in blood (Fig. 3, a and b), CD4 and CD8 T cell counts and ratios in LNs, spleen, and blood (Fig. 3d), and all Ig levels except IgE in plasma (Fig. 4a). S1P is present in blood at concentrations high enough to nearly completely occupy S1P1 GPCRs, but the level of saturation of S1P1 is predicted to be lower in tissues (12–14). The S1P-S1P1 GPCR axis has two distinct effects on T cell migration, including direct chemotactic stimulation by low nanomolar S1P and suppression of chemotaxis to chemokines by high nanomolar to low nanomolar S1P typical of blood (7, 8). Therefore S1P may elicit thymocyte exitation and lymphocyte egress from SLOs by acting as a chemoaattractant and also retain lymphocytes in blood by suppressing chemotaxis to chemokines generated in SLOs and nonlymphoid tissues (11). The ex vivo chemotactic responses of naive CD4 T cells from S1P1 Tg mice to 10 nM S1P were greater than those of the CD4 T cells from wt mice (Fig. 2a). Enhancement of both migration effects of S1P by increased levels of S1P1 in S1P1-R Tg mice may explain the higher levels of CD3 T cells in blood of S1P1-Tg mice (Fig. 3c).

Significant differences were observed between the mean plasma IgE level in wt and S1P1-R Tg mice, and between isotype-selective Ab responses of wt mice and S1P1-R Tg mice (Fig. 4a). Immunization with TNP-KLH resulted in a 6- to 8-fold lower production of IgG2b and a 2.5-fold higher generation of IgE Ab levels in S1P1-R Tg mice than wt mice, without significant changes in IgG1 levels compared with wt mice (Fig. 4b). IgG1 and IgE are known as Th2 isotypes which are important for immune-mediated inflammation and hypersensitivity, whereas IgG2a, IgG2b, and IgG3 are considered Th1 isotypes that mediate resistance to microbial infections (27). The immune response to TNP-KLH is therefore skewed toward a Th2 response with increased levels of IgE and reduced concentrations of IgG2b compared with wt mice (Fig. 4b). Studies in progress will define the cytokine and transcriptional mechanisms underlying the Th2-shift elicited by the S1P1-S1P1 GPCR axis.

The dramatically diminished DTH response of S1P1-R Tg mice compared with wt mice also may reflect enhancement of S1P1-mediated direct chemotaxis of T cells into lymph and blood, and greater inhibition of chemotaxis of T cells to chemokines at sites of DTH (Fig. 5a). Diminished DTH also may reflect the suppressed proliferation of S1P1-R Tg T cells relative to that of wt T cells (Fig. 8). DTH reactions are known to be mediated principally by Th1 cells (28). Examination of lymphocytes from cervical LNs of DNFB-challenged S1P1 GPCR-Tg mice showed a lesser state of activation than those from wt mice, as determined by their level of Fig. 8. Diminished proliferative responses of T cells from S1P1-Tg mice. Each column and bar depicts the mean ± SD of the results of analyses of three different preparations of T cells. Results of Student's t test: *, p < 0.01.
expression of CD69, but this was not CD4 T cell-specific (Fig. 5b). Activation of CD4 and CD8 T cells from challenged cervical LNs in S1P1-GPCR Tg mice was significantly reduced, whereas B cells were not suppressed or only slightly affected (Fig. 5b). This result correlates with the more prominent expression of the S1P1 GPCR transgene in T cells compared with B cells (Fig. 1c), but other indices of T cell activation less directly susceptible to regulation by S1P than CD69 must be examined as well.

S1P-GPCR expression by T cells thus is critical both for T cell migration to S1P and S1P inhibition of T cell chemotaxis to chemokines, both of which are increased by a higher level of S1P1 on activated S1P1-R Tg T cells (7, 10). Expression of the human S1P1-R Tg maintains the migratory responsiveness of both CD4 and CD8 T cells to S1P after activation, whereas activation-induced down-regulation of the endogenous mouse S1P1-R transcript renders corresponding T cells from wt mice unresponsive to S1P (Fig. 2b). The persistent responsiveness to S1P of activated S1P1-R Tg T cells may contribute to both decreased entry into LNs, as a result of maintenance of S1P inhibition of chemotaxis to chemokines (Fig. 6), and increased entry into the circulation by enhanced chemotactic responsiveness to chemokines. Activated T cells from S1P1-R Tg mice that were labeled with either CFSE or Snarl and adoptively transferred into wt mice were twice as frequent in blood as their corresponding wt T cells at 20 h (Fig. 7). The same tendency was seen in spleen, although to a lesser extent. In contrast, lower numbers of T cells were recovered from LNs of S1P1-R Tg mice compared with that of T cells from wt mice also as is yet unexplained (Fig. 8). S1P suppresses T cell proliferation through both S1P1 and S1P3 receptors (18). When the level of expression of S1P1 is elevated, any endogenous S1P is capable of transmitting proliferation inhibitory effects on T cells. Whether separate subsets of CD4 T cells generate and respond to S1P must be examined as well.

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The S1P-S1P1-GPCR system is emerging as a potent lipid mediator complex capable of effectively regulating T cell responses to chemokines and thereby trafficking through LNs and along lymphatic pathways, as well as intrinsic T cell immune activities such as proliferation. This system is far more prominent than any of the eicosanoid-type lipid mediators (29) as a regulatory factor for T cells and through its effects on T cells has a broad influence on cellular and humoral immunity.

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References