Sudden Onset of Colitis After Ablation of Secretin-Expressing Lymphocytes in Transgenic Mice

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Though secretin mRNA was demonstrated in mouse lymphoid organs, its role in the immune system is unknown. Here, secretin gene-expressing cells were ablated by ganciclovir infusion in mice transgenic for the rat secretin promoter (Sec) directing the expression of herpesvirus thymidine kinase (Sec-HSVTK). Thymus, spleen, blood, and colon were investigated by histology. Lymphoid cells were extracted and quantified, and $CD19^+$ B-cells and $CD3^+$, $CD103^+$, $CD4^+$, and $CD8^+$ T-cells were analyzed by flow cytometry. Protein extracts from spleen and thymus were assayed for secretin by Western blotting, and isolated lymphocytes were investigated for HSVTK, secretin, and secretin receptor (Sec-R) mRNA by reverse transcription–polymerase chain reaction (RT-PCR). Ablation of secretin-expressing cells produced severe colitis with morphological features similar to those observed in graft-*versus*-host (GVH) disease. Profound lymphoid depletion was observed in spleen, thymus, and peripheral blood. The relative percentage of B- and T-cell subsets were unaffected. Analysis of colonic lymphocytes revealed a marked depletion of $CD4^+$ T lymphocytes. Colitis and lymphoid depletion were not reversed by secretin cotreatment. Immunoblot analysis of protein extracts from spleen and thymus identified secretin-like immmunoreactant. RT-PCR of lymphocyte mRNA from spleen and thymus identified secretin and secretin receptor transcripts. We conclude that GVH-like colitis in ganciclovir-treated Sec-HSVTK mice arises from depletion of secretin gene–expressing lymphoid cells and not from the failure of secretin production. Exp Biol Med 229:826–834, 2004

Key words: secretin; ablation; thymus; spleen; Sec-HSVTK; colitis; cytometry

Introduction

In most mammalian species, the small intestine is the major source of the hormone secretin (1). The production of secretin is restricted to the S-cell of the small intestine, a highly specialized cell type of the diffuse endocrine system of the gut (2, 3). Secretin cells are also present to lesser extent in the colon and developing pancreas (4, 5). In addition, RNA protection assays revealed secretin transcripts in the spleen and thymus of mouse (5).

We previously described the inducible ablation of secretin cells in transgenic mice expressing herpes simplex virus thymidine kinase (HSVTK) under control of 1.6 kb of the 5′ flanking sequence of rat secretin gene (Sec) (6). HSVTK expression in transgenic mice is nontoxic. However, the viral enzyme renders HSVTK-expressing cells sensitive to the nucleoside analog ganciclovir, inducing conditional cell ablation in replicating cells. In Sec-HSVTK mice, almost the entire population of secretin-producing cells of the small intestine was deleted after 5 days of ganciclovir treatment (6). This previous study focused on the small intestine to demonstrate the existence of a secretin-expressing multipotent endocrine progenitor cell and to establish the lineage relationship with other enteroendocrine cells. Sec-HSVTK mice also developed colitis and lymphoid organ defects. The current investigation reports the complex effect of the ablation of secretin gene–expressing cell in the colon and lymphoid organs of Sec-HSVTK transgenic mice.

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Materials and Methods

Transgenic Mice Generation. The details regarding the transgenic mice generation and breeding have previously been described (6). Sec-HSVTK mice of no. 995 lineage were used. Mice were bred on a CD1 background and kept in standard cages according to the current European law for animal practice.

Ablation Procedure. Sec-HSVTK mice ($n = 78$) and controls ($n = 22$) of 8–14 weeks of age, of about 33 g weight (mean, 33.4 ± 5.8 g) were treated with ganciclovir (Citovene, Sintex, Palo Alto, CA) for 5 days (7) as detailed (6). Controls consisted either of age- and sex-matched transgenic mice treated with saline or non-transgenic CD-1 mice treated with ganciclovir. Fifteen different experiments were performed with a mean of 6 mice per experiment. Mice were investigated daily for clinical signs of disease with special reference to colitis. Food and water consumption as well as production and characteristics of stools were assessed and reported daily. Some transgenic mice and controls were treated simultaneously with ganciclovir and synthetic human secretin (1 mg/kg/day) (Sigma Chemicals, Milan, Italy). Such chronic administration of secretin proved not to cause any significant change in mice (8, 9). The double treatment was obtained by the simultaneous implant of two minipumps. At the end of the treatment, transgenic mice and controls were weighed and immediately sacrificed by cervical dislocation after light Avertin anesthesia for further studies.

Histology and Immunohistochemistry. Samples of about 2-cm each in length from duodenum, ileum, terminal ileum (within 3 cm from the cecum), and colon were dissected, the thymus and the spleen weighed, fixed by immersion in Bouin's solution (for peptide hormones immunohistochemistry only) or 10% formalin for 6-8 hrs at room temperature, and processed into paraffin. Serial sections (3-5 μm) were stained with hematoxylin and eosin for conventional histology, periodic acid–Schiff (PAS)/alcian blue for mucins, and immunohistochemical tests for secretin and other hormones with pertinent specificity tests as described (5). For quantitative analysis of colon secretin cells, tissue samples correctly oriented along the longitudinal axis were evaluated in six CD1 mice. Secretin immunoreactive cells were counted per linear millimeter of mucosa as previously described (6, 10). A total of 41 mm of mucosa was evaluated for a mean value of 6.8 ± 3.5 mm per mouse (range, 1.6-10.5 mm).

Preparation of Cell Suspension. Cell suspensions were obtained from peripheral blood, spleen, thymus, and colon of 2-5 mice per experiment. Mice were randomly chosen in both transgenic and control groups. Peripheral blood lymphocytes from heparinized venous blood were isolated by a 1077 density gradient (Histopaque, SIGMA, Poole, Dorset, UK). Spleen and thymus were dispersed into single-cell suspension in RPMI 1640 medium by gentle pressing through stainless steel mesh and were washed by centrifuging at 300 *g* for 5 mins. Lymphocytes were separated by Histopaque preparation as for blood. Lymphocytes from the epithelium and lamina propria of the colon were isolated by a modification of the method of Davies and Parrot (11). In brief, 1-2-cm colon samples were washed with RPMI 1640, transferred in 25 ml of RPMI 1640 with nonessential amino acids, sodium pyruvate, L-glutamine, gentamicin, penicillin, streptomycin, HEPES buffer with 2% fetal calf serum (FCS; GIBCO, BRL Paisley, Renfrewshire, UK), and 1 m*M* dithiothreitol (DTT) and were incubated at 37°C for 30 mins with gentle stirring. The samples were transferred in 50-ml centrifuge tubes, vigorously shaken for 15 secs, and filtered through 60-mm nylon mesh to separate supernatant lymphocytes. Tissue fragments were returned to the digestion flasks, and the process was repeated twice. Colonic lymphocytes from three different incubations were pooled, washed, and suspended in RPMI media with 10% FCS and placed on discontinuous 40%–70% Histopaque gradients at 600 *g* for 30 mins. Cells from the 40%–70% interface were collected, washed in PBS, checked for purity at light microscopy, and used in phenotypic assays (see below). Lymphoid cells from all preparations were counted in Burker chamber.

Cytometry. Lymphocytes were suspended in 0.1% NaN_3 PBS with 10% normal mouse serum at a concentration of 0.5 to 1×10^5 cells/ml before antibody staining and flow or static (CD19 only) cytometry. The following monoclonal antibodies were used: rat anti-mouse CD19 R-phycoerytrin conjugate and rat anti-mouse CD3 fluorescein conjugate (Southern Biotechnology Associates, Birmingham, AL), rat anti-mouse CD8a (Ly-2), CD4 (L3T4), CD3 mononuclear complex and anti $integrin_{IEL}$ chain (CD103) (Pharmingen, San Diego, CA), and fluorescein rabbit anti-rat IgG (Vector Laboratories, Burlingame, USA). Monoclonal antibodies were added to cell suspensions at 4°C and incubated for 20–30 mins. Samples were then washed in PBS and run on a Facscan flow cytometer (Partec PAS, DAKO, Glostrup, Denmark). Single and multiple parameter analysis using dot plots and histogram overlay with corresponding statistics were used.

Semiquantitative Reverse Transcription–Polymerase Chain Reaction (RT-PCR) for Secretin/HSVTK Message. Total RNA from lymphocytes isolated from spleen, thymus, intestine, and blood of Sec-HSVTK mice was extracted using RnazolB (Biotex Laboratory Inc., Houston, TX) according to the manufacturer's instructions. RNA was checked on 1.5% agarose gel and quantified by spectrophotometer. Amplification of glyceraldehyde-6-phosphate dehydrogenase (GADPH) was used as RNA purity control (not shown). For semiquantitative PCR, 1 γ of RNA was used to synthesize cDNA with gene-specific primers and Superscript RT (Invitrogen, Carlsbad, CA) for reverse transcription. Sample quantization was performed using the Amplifluor Universal Amplification and Detection System with end-point detection and Bcl-2 as control (Intergen Company, Oxford, UK). PCR reactions were performed

according to the manufacturer's protocol in 25 ml final volume under the following conditions: 4 mins denaturation at 95°C, 40 cycles of 15 secs at 95°C, 20 secs at 54°C, 40 secs at 72°C, and final 4 mins extension step at 72°C. HSVTK was amplified with primers designed on HSVTK sequence (GenBank acc. no. J02224, www.ncbi.hlm.nih.gov): forward 5′actgaacctgaccgtacagccttgtagaagcgcgtat3′, reverse 5′ccgtctatataaacccgcagt3′. Mouse secretin was amplified with primers designed on mRNA sequence (acc. no. X73580) forward 5′actgaacctgaccgtacacagacggaatgttca ccag3′, reverse 3′agtgttcgaccacagcaag3′. Bcl-2 was amplified with kit primers. The standard curve was prepared following the manufacturer's instructions, processing serial dilutions of positive target control DNA (Bcl2) in our sample PCR conditions. PCR reactions were checked on 3% agarose gel (2% Nusieve, 1% agarose) and visualized by ethidium bromide. Band intensity was assessed using Bio-Rad Gel Doc 2000 and measured by the area under its intensity profile curve (trace quantity); units were intensity/millimeter. The standard curve was designed plotting on *y* axis the trace quantity and on *x* axis the log10 of known concentrations of Bcl-2 dilutions. Quantization of samples was made by interpolation on the Bcl-2 standard curve.

Western Blotting for Secretin. Lymphocytes extracted from CD1 mouse thymus, spleen, and from secretin-expressing STC-1 cells (12) were homogenized in PBS 1x containing PMSF 1 m*M* and pepstatin 2 mg/ml. Equal amounts of protein and human synthetic secretin (Sigma Chemicals) were separated by Tricine SDS-PAGE (13) and blotted onto nitrocellulose paper (Schleicher & Schuell, Milan, Italy) by electrotransfer at 0.6 A at room temperature for 1 hr in standard tris-glycine buffer containing 20% methanol. Protein concentration was determined by Bicinchonic Acid Protein Assay Kit (Sigma Chemicals). Western blot analysis was performed according to standard methods using the anti-secretin primary antibody (5) at 1:50,000 dilution and alkaline phosphatase–coupled secondary antibodies (14).

RT-PCR for Mouse Secretin Receptor. Total RNA from lymphocytes isolated from CD1 and Sec-HSVTK mouse thymus, spleen, gut, and blood was extracted as described above. Two primer pairs (inner and outer) in the 3′ mouse secretin receptor region (GenBank acc. no. AI595950, www.ncbi.hlm.nih.gov) were designed: outer primer pair, forward 5′gaaagaccaaatcccaggcc3′, reverse 5′attgtcttcgccttctcccc3′; inner primer pair, forward 5′ggaagctcagacctcgggg3′, reverse 5′gttctttgaattggccctggg3′. RT was carried-out in 15-μl volume consisting of 1x RT buffer, 10 units of M-MLV reverse transcriptase (Ambion, Austin, TX), 8 units of RNAse inhibitor (Boehringer, Mannheim, Germany), 1 m*M* each dNTP (Boehringer), 1 m*M* outer reverse primer, and 250 ng of RNA. Reaction was performed at 68°C for 3 mins, 37°C for 1 hr, and 95°C for 5 mins. As negative control, the same reaction mix was used with sterile H_2O for the RNA sample. One fifth of total cDNA preparation/RT reaction was used as template for a PCR reaction using the inner primer pair at these conditions: 95°C for 1 min followed by 35 cycles of 95°C for 40 secs, 56°C for 1 min 30 secs, 72°C for 2 mins, and a final extension of 72°C for 10 mins. RT-PCR products were directly sequenced using the Amplicycle Sequencing Kit, Big Dye terminator (Perkin-Elmer, Boston, MA) and analyzed by ABI PRISM 377 automated sequencer (Perkin-Elmer).

Statistical Analysis. Continuous variables are reported as median, quartiles, interquartile range (IQR), and compared across groups by the nonparametric Mann Whitney *U* test (for 2 groups) or by Kruskall-Wallis test (nonparametric analysis of variance for >2 groups). For *post hoc* comparisons, *P* values are to be interpreted after applying Bonferroni correction for multiple tests bias. All tests were two-sided. A *P* value of ≤ 0.05 was considered statistically significant. Stata 7.0 (Stata Corporation, College Station, TX) was used for computation.

Results

Ganciclovir Treatment of Sec-HSVTK Mice Results in Weight Loss and Severe Colitis. Five days of ganciclovir treatment resulted in a ~20% weight loss in transgenic mice [median weight loss (25th to 75th interquartile range, IQR) 5.1 g (4–6.2) *versus* 0.0 (0.0–2.0) in controls, $P < 0.005$)]. Sec-HSVTK mice appeared unhealthy, displaying opaque fur and maintaining an antalgic posture in the cages where only few formed stools were observed. All treated transgenic mice were equally affected. At autopsy, the abdominal cavity of Sec-HSVTK mice showed a prominent colon, often displacing the ileum (Fig. 1A). The colon was uniformly dilated at opening with almost no formed stool and liquid content (Fig. 1B).

At histology, the gastrointestinal tract of ganciclovir-treated Sec-HSVTK transgenic mice showed no significant structural abnormality (see Ref. 6 for secretin cell depletion in the ileum) except for the colon, where the mucosal architecture was severely disrupted by inflammation (Figs. 1C–E). The colitis was not transmural (i.e., was limited to the mucosa only) and involved both the proximal and the distal colon. Various degrees of gland derangement with apoptotic bodies and "crypt explosion" were observed, often with complete disappearance of the glands, though with no significant ulceration and with presence of a limiting epithelial layer (Figs. 1C, D, and F). Interstitial inflammatory cells were dramatically increased with rare PAS-positive mucous (not shown) or endocrine cells (see as an example 5HT-producing cells in Fig. 1F) to mark the gland remnants in the lamina propria. Residual endocrine cells were also observed in the limiting epithelial layer (Fig. 2). Edema and mononuclear inflammatory cells, sometimes organized in lymphoid aggregates, characterized the lamina propria. Rare, if any, granulocytes were also observed,

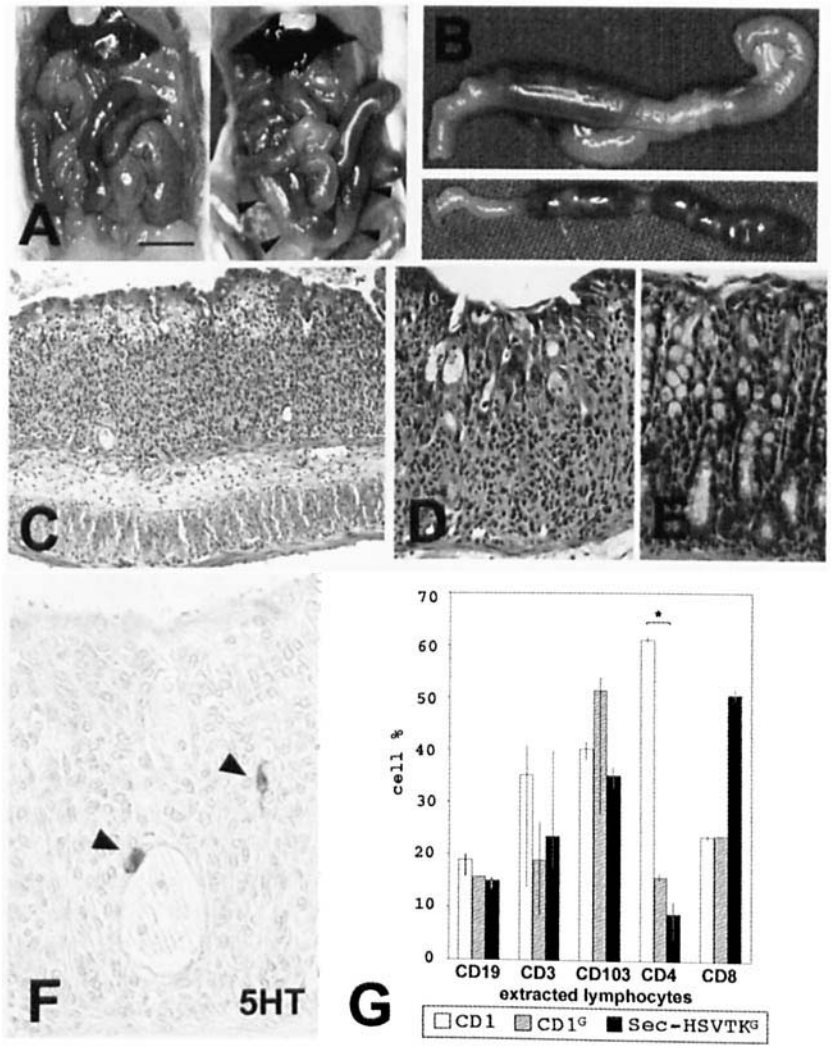

Figure 1. The effect of ganciclovir treatment in the colon of Sec-HSVTK mice and controls. (A) General view of the abdominal cavity in CD1 (left) and transgenic mouse (right). In a transgenic mouse, the colon is dilated (arrowheads), displacing the small intestine; scale bar = 1 cm). (B) The isolated colon of a Sec-HSVTK mouse (upper) is dilated and edematous with low amount of stool; compare with the thin and transparent aspect of the colon with several formed stools in a CD1 control (lower); scale bar = 1 cm. (C and D) Histology of Sec-HSVTK mouse (C, low power, and D, high power). Note the inflammatory infiltrate effacing the mucosal structure though not affecting the muscolaris propria layer (C, hematoxylin and eosin; magnification ×100). At high power (D, hematoxylin and eosin; magnification ×150), the inflammatory infiltrate is mostly composed of lymphocytes, sometimes invading gland crypts (e.g., right upper part) to be compared with the normal colon mucosal architecture in control (E, hematoxylin and eosin; magnification ×250). (G) 5-hydroxytryptamine (5-HT)–producing enteroendocrine cells (arrowheads) are present within the inflamed mucosa to mark gland remnants, one of which (center) abnormally dilated (immunoperoxidase, hematoxylin counterstain; magnification ×350). (F) Percent proportional distribution of B- and T-cell lymphocytes extracted from the colon of controls (ganciclovir untreated and treated CD1) and ganciclovir-treated transgenic mice. Open bars show results in CD1 mice, striped bars ganciclovir-treated CD1 mice, and black bars ganciclovir-treated transgenic mice. Results shown as median values with 25th and 75th interquartile range (vertical line) from two experiments (two to three mice per experiment); *Statistically significant difference observed between treated transgenic mice and both two other groups ($P \leq 0.05$).

sometimes as cryptic microabscesses. Controls showed no significant changes.

The lymphoid population was extracted from the inflamed colon mucosa of Sec-HSVTK mice and controls and analyzed for B and T subpopulations. At *post hoc* comparison between treated transgenic mice and the two other groups [two sample Wilcoxon rank-sum (Mann-Whitney test)], extracted $CD19^+$ B-cells showed similar percent distribution in both groups, similar to $CD3^+$ and $CD103^+$ T-cells (Fig. 1G). By converse, the percent distribution of extracted $CD4^+$ T-cells was almost halved [median value, 9 (3.3–11.5) vs. 16.3 (15.4–61.1), $P < 0.05$]. $CD8^+$ T-cells percent distribution was increased in treated transgenic mice and barely missed a statistically significant difference ($P = 0.07$)(Fig. 1G).

To assess whether secretin could limit or abolish the effects of ganciclovir treatment, transgenic mice were treated with the simultaneous infusion of ganciclovir and secretin. Doubly treated transgenic mice, however, showed no significant difference in weight loss [median weight loss, 5 g (2.1–7) vs. 5.9 (4.5–7.2), $P = 0.3$] and colon histology when compared to ganciclovir-only–treated transgenic mice.

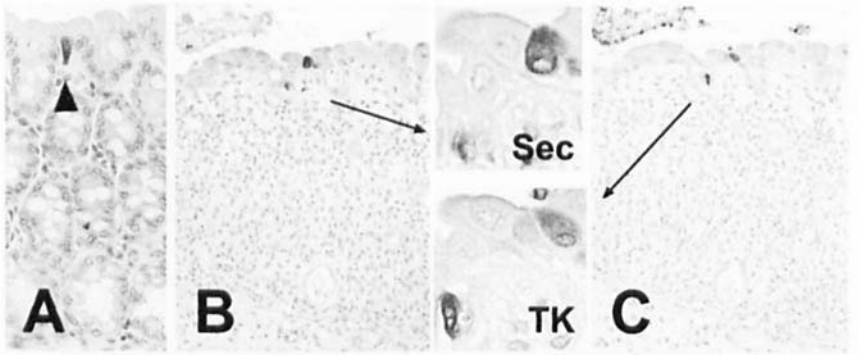

Figure 2. Secretin-immunoreactive cells in the colon mucosa of mouse. One discrete secretin-immunoreactive cell (arrowhead) is stained in the upper part of the normal mucosa in control (A) (immunoperoxidase, hematoxylin counterstain, magnification × 250). Few remaining secretin-immunoreactive cells are stained in the limiting mucosal layer of the inflamed mucosa in Sec-HSVTK mice after ganciclovir treatment (B); secretin cells coexpress the transgene-encoded protein HSVTK (C) in consecutive sections (×100); note the presence of residual gland crypts within the inflamed lamina propria (lower part of the micrograph). In the middle (arrows), the immunoreactive cells are detailed at high power for comparison (Sec, secretin; TK, HSVTK; magnification ×400).

To demonstrate the site of secretin expression in the colon, immunohistochemical tests were performed in control and transgenic mice (Fig. 2). Secretin immunoreactivity was restricted to rare epithelial, endocrine cells of the upper part of the glands (Fig. 2A), more frequently seen in the proximal colon. Only occasional (less than 1 out of 10) secretin cells were seen in the crypt. The quantitative analysis revealed a mean value of 0.9 ± 0.5 secretin cell per linear millimeter of mucosa (range, 0.2–1.5). In transgenic mice, secretin cells proved to coexpress HSVTK (Figs. 2B and C).

Ganciclovir Treatment of Sec-HSVTK Mice Results in Lymphoid Depletion. In the blood, all mice showed a comparable amount of red cells ($3.8 \times 10^6/mm^3$ in both treated transgenic mice and controls), and the number of circulating lymphocytes was dramatically reduced in treated Sec-HSVTK mice [median, $2.8 \times 10^3/mm^3$ (2.3–3.2) vs. 6.9 (6.2–7.5), $P < 0.005$] (Fig. 3A). The percent distribution of B-cells was unchanged, whereas it was abnormal for T-cells. $CD3^+$ T-cells were significantly reduced [median, 35.1 (31.7–50.7) vs. 88.2 (7.7–93.4), $P < 0.05$], $CD103^+$ were increased [median, 43 (39.5–44.8) vs. 29.1 (26.1–39.3), $P < 0.05$], whereas $CD4^+$ and $CD8^+$ remained unchanged (Fig. 3A).

The spleen of ganciclovir-treated transgenic mice was macroscopically reduced in size (Fig. 3B), and the weight was almost halved when compared to controls [median, 0.06 g (0.05–0.08) vs. 0.15 g (0.13–0.16) of controls, $P < 0.005$]. At histology, the white pulp appeared significantly reduced (not shown), and the number of extracted lymphoid cells was dramatically reduced [median, 12.3×10^6/g tissue (6.4–15.9) vs. 86.2 (84.2–90), $P < 0.005$] (Fig. 3B). The percent distribution of B- and T-cells was unchanged except for increased $CD103^+$ cells [median, 97.6 (97.3–99.3) vs. 70.7 (60.7–84.3), $P = 0.05$].

The thymus of treated transgenic mice showed no appreciable weight difference [median 0.031 g (0.03–0.04) vs. 0.032 g (0.03–0.034)]. At histology, a marked lymphoid depletion was sometimes observed (not shown), though the extracted lymphoid cells were dramatically reduced in number [median, 16.4×10^6/g tissue (13.1–17.7) vs. 39 (34–48.3), $P < 0.005$] (Fig. 3C). The percent distribution of B- and T-cells was unchanged except for increased $CD103^+$ cells [median, 88.3 (84.9–93.6) vs. 69.5 (63.9–76.2), $P < 0.05$].

Secretin RNA is Present in Thymus and Spleen Lymphocytes. Secretin transcripts of the expected size (~176 bp) were observed by RT-PCR in CD-1 duodenum (control) and in lymphocytes extracted from the spleen and thymus of Sec-HSVTK mice (Fig. 4). No amplified fragment was observed for lymphocytes from the intestine and blood. Comparable amplicons of ~598 bp of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GADPH*) were observed for all samples (not shown). Semiquantitative assessment of HSVTK and secretin messages in lymphocytes from both organs revealed slightly higher values for HSVTK message in the thymus as compared to the spleen, comparable secretin messages, and a consistent HSVTK/secretin message ratio of ~1:5 (Table 1).

Secretin-like Immunoreactant Is Present in Thymus and Spleen. Secretin-like immunoreactant was observed in crude protein extracts of thymus and spleen of CD-1 mice by Western blotting (Fig. 5A). The band was similar to that observed for extracts of secretin-producing STC-1 cells, showed a higher molecular weight than the human synthetic secretin (~3.5 kDa), and migrated between 10–14 kDa, thus corresponding to the expected weight-size of rat pro-secretin (~13.2 kDa) (15, 16).

Secretin-Receptor RNA Is Present in Thymus and Spleen Lymphocytes. Mouse secretin-receptor transcripts of the expected size (~341 bp) were observed by RT-PCR in CD1 mouse stomach (positive control) and in lymphocytes extracted from the spleen and thymus of both CD-1 and Sec-HSVTK mice (Fig. 5B). No amplified fragment was seen in isolated lymphocyte from the intestine and blood. Direct sequencing confirmed that the amplifica-

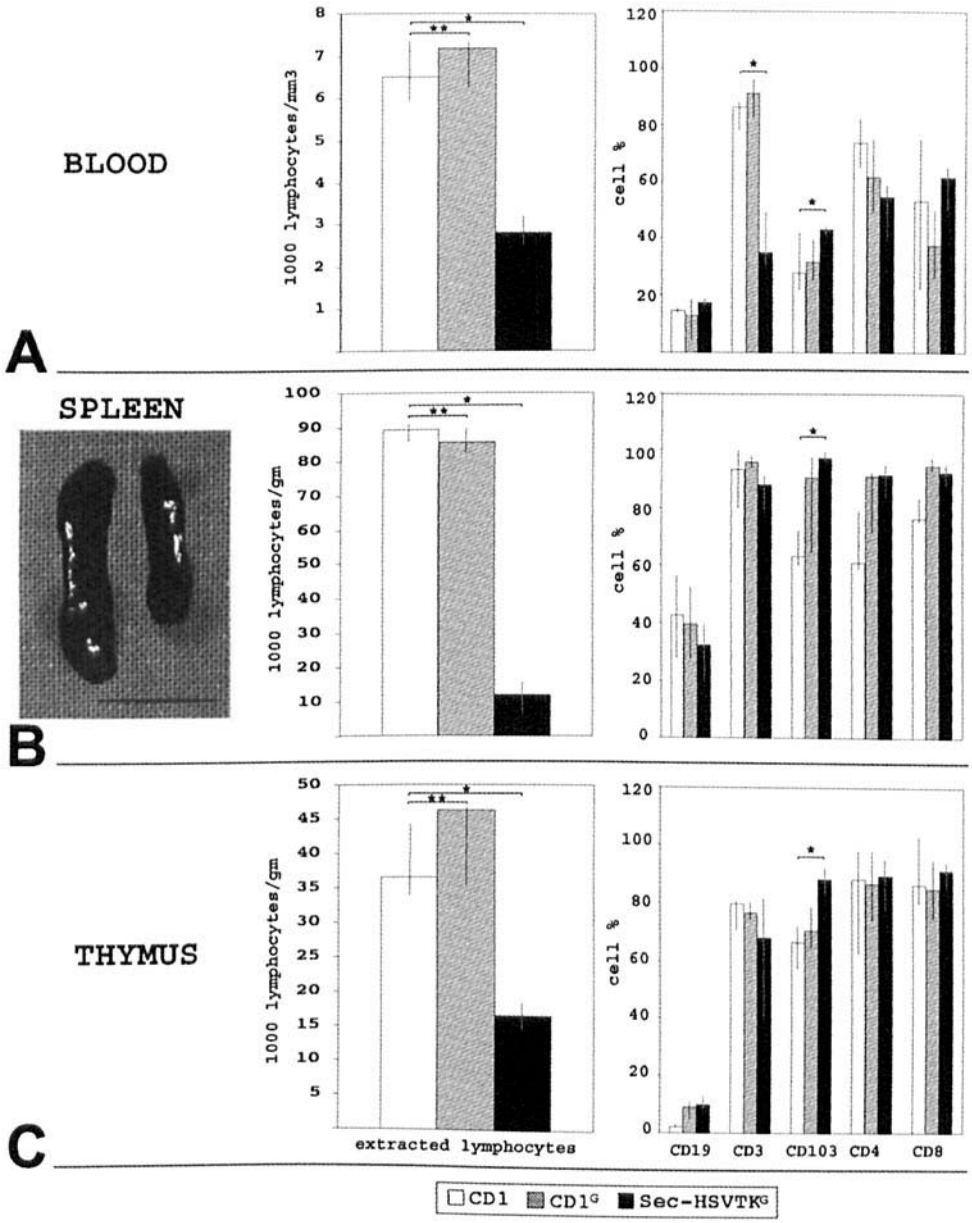

Figure 3. The effect of ganciclovir treatment in lymphoid cells isolated from blood (A), spleen (B), and thymus (C) in Sec-HSVTK mice and controls. Lymphocyte counts are described in the left graph and percent distribution of $CD19^+$ B-cells and $CD3^+$, $CD103^+$, $CD4^+$, and $CD8^+$ T-cells in the right graph. Open bars show results in CD1 mice, striped bars ganciclovir-treated CD1 mice, and black bars ganciclovir-treated transgenic mice. Results are shown as median values with 25th and 75th interquartile range (vertical line) from three to four experiments (two to four mice per experiment); *Statistically significant difference observed between treated transgenic mice and both of two other groups ($P \leq 0.05$); **no statistically significant difference ($P > 0.05$). (B) Macroscopical view of the spleen in control (left) and transgenic mice (right) after ganciclovir treatment; scale bar = 1 cm.

tion product corresponded to the mouse secretin receptor cDNA.

Discussion

We previously demonstrated that ganciclovir treatment efficiently depleted almost all secretin cells of the small intestine in Sec-HSVTK transgenic mice (6). Other enteroendocrine cell types were also depleted to some extent, presumably due to low levels of secretin-HSVTK expression. Here, we report that ablation of secretin gene-expressing cells in Sec-HSVTK transgenic mice induced profound lymphoid cell depletion associated with severe colitis. We also identified the presence of secretin protein and secretin gene transcripts in lymphocytes of spleen and thymus. Our results indicate that the ablation of secretin-expressing lymphocytes has a potent impact on spleen, thymus, and colon.

The 5′ region of rat secretin gene efficiently delivered reporter transcripts to thymus and spleen in previous, different transgenic experiments (5). Sec-SV40 large T

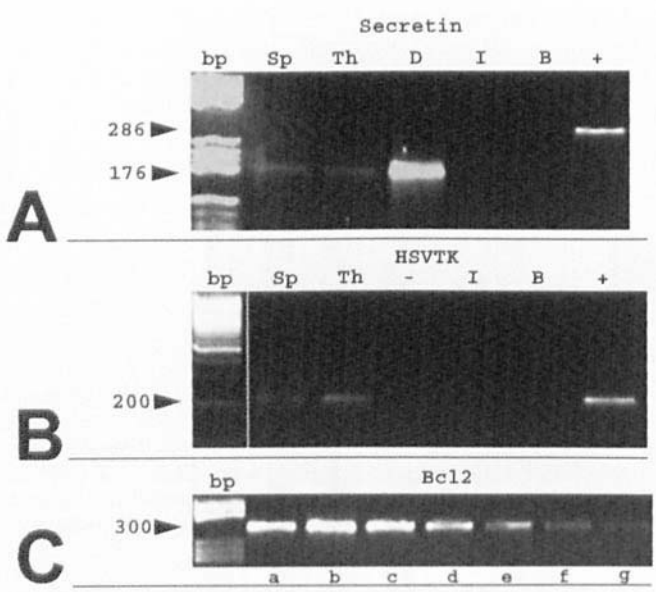

Figure 4. Secretin and HSVTK transcripts in lymphocytes of Sec-HSVTK mice. (A) RT-PCR for secretin gene. In Sec-HSVTK mice, comparable bands of ~176 bp are seen for lymphocytes isolated from spleen (Sp) and thymus (Th) but not from large intestine and blood ("I" and "B," respectively). A band of comparable size is present for the whole duodenal mucosa RNA from CD1 mouse (D, RNA control), whereas a band of ~286 bp is observed for DNA (+, positive control); base pair ladder (bp). (B) RT-PCR for HSVTK transgene. Comparable bands of ~200 bp are seen for lymphocytes isolated from spleen (Sp) and thymus (Th) but not from large intestine and blood ("I" and "B," respectively); Sec-HSVTK genomic DNA positive control (+); negative control (–). (C) Example of Bcl2 message (bands of ~300 bp) amplified from kit DNA at the different cDNA concentration values of 5×10^6 copies/μl (Lane a), 10^6 (Lane b), 5×10^5 (Lane c), 10^5 (Lane d), 5×10^4 (Lane e), 10^4 (Lane f), and 5×10^3 used to build a reference concentration curve.

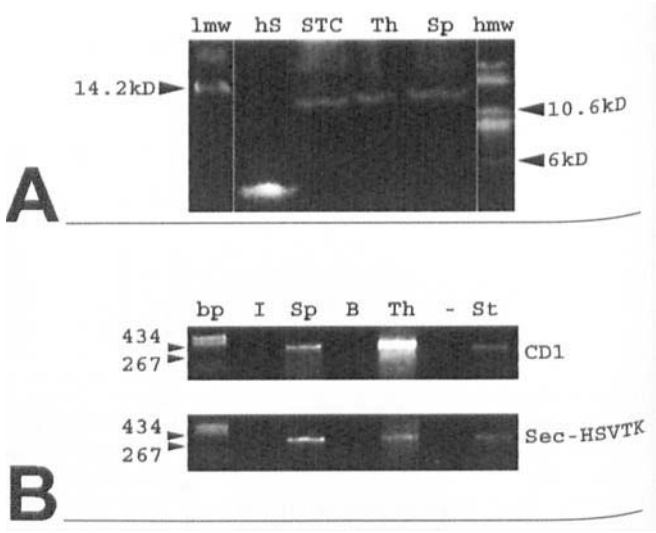

Figure 5. Secretin and secretin-receptor expression in mouse lymphoid tissues. (A) Western blot for secretin. Human synthetic secretin-28 (hS) displays a band of about 3 kDa, whereas protein crude extracts of secretin-producing mouse STC-1 cells (STC), CD1 thymus (T), and spleen (S) display bands between 12 and 14 kDa corresponding to secretin-71; low-molecular-weight size markers (lmw), high-molecular-weight size markers (hmw). (B) RT-PCR for mouse secretin receptor from CD1 (upper) and Sec-HSVTK (lower) lymphocytes. Comparable bands of about ~341 bp are seen for lymphocytes isolated from spleen (Sp), thymus (Th), and for the whole gastric mucosa (St, positive control) but not for lymphocytes isolated from large intestine and blood ("I" and "B," respectively); base pair ladder (bp).

antigen (Tag) (Sec-Tag) mice developed splenic and thymic tumors composed of Tag-positive, proliferating lymphoid elements (5, 17). Here, the ganciclovir treatment in Sec-HSVTK mice rapidly resulted in lymphoid depletion thymus and spleen. This indicates that ablation of secretin gene-expressing cell was taking place in both organs and that the transgene-targeted cells resided in their lymphoid compartment. A concurrent "bystander effect" could also be taken into account to explain the extent of lymphoid depletion in these organs. The fact that the relative percent proportion of B and T lymphocytes was relatively unaffected as compared to controls indicates that ablation equally struck both B and T cell lineages resulting in an overall severe lymphopenia. This finding suggests that low levels of secretin-gene expression takes place in targeted lympocytes of both T and B lineages. Whether this may occur in a specific lymphocyte subset or in a common lymphoid precursor remains to be elucidated.

Table 1. Semiquantitative Analysis of Secretin and HSVTK Message[a]

	HSVTK (copies/μl)	SEC (copies/μl)	HSVTK/SEC ratio
Spleen	4906 ± 4177	3561 ± 3551	1:4
Thymus	7849 ± 4749	4750 ± 3690	1:6
Duodenum	—	565,053 ± 154,881	—

[a] HSVTK, message for HSVTK transgene; SEC, message for secretin gene; HSVTK/SEC, ratio calculated for means; spleen and thymus, data derived from isolated lymphocytes of transgenic mice; duodenum, data derived from CD1 whole duodenum RNA. Mean values ± SD from three separate sets of experiments.

Indeed, the secretin gene is expressed at low levels in lymphoid cells of both spleen and thymus. Our semi-quantitative RT-PCR data identified the presence of secretin gene transcripts in spleen and thymus lymphocytes, with slight overexpression of HSVTK message in transgenic mice. Also, secretin-like immunoreactivity was observed in protein extracts of spleen and thymus of both normal and transgenic mice. In addition, we demonstrated the presence of secretin gene receptor mRNA in lymphoid cells extracted from both organs. Secretin and its receptor genes are therefore expressed in lymphoid cells of mouse spleen and thymus, providing ground for a potential autocrine loop. Other gut hormones (including VIP, somatostatin, substance P, ghrelin, and so forth) and their receptors have been demonstrated in lymphoid cells of both the thymus and the spleen (18–23). Their function(s) remains largely speculative, though in the thymus, hormones are possibly involved in T-cell differentiation and maturation (24, 25).

Because the secretin gene is expressed in the colon (5), the colitis of ganciclovir-treated Sec-HSVTK transgenic

mice could be the direct toxic effect of ganciclovir on colonic mucosa. Indeed, severe mucosal changes were described after ganciclovir treatment in mice expressing HSVTK in other sites of the gut, namely in the stomach (26) and the small intestine (27). In both reports, however, this effect was elicited after a longer ganciclovir treatment (2 weeks) and in absence of concurrent abnormality of other lymphoid organs. Here, the sudden onset after only 5 days of treatment and the histological features of colitis similar to those observed in graft-*versus*-host (GVH) disease (28–31) suggest an immunological mechanism at its basis. In particular, the observed "spared" enteroendocrine cells to mark the crypt remnants is typical of GVH colitis in man (32) and indicates that the enteroendocrine cells are somehow immune from T-cell–mediated attack. It is unclear whether this phenomenon is due to the fact that gut endocrine cells do not express Class II major histocompatibility complex (MHC) antigens. Of note, bowel inflammatory autoimmune disease may be associated with splenic hypoplasia/hypofunction and abnormal thymic function (33–38). In keeping with this hypothesis is the fact that we could not reverse the ablation effects in Sec-HSVTK mice by infusing high doses of synthetic secretin in parallel with ganciclovir. This finding indicates that the absence of secretin is *per se* irrelevant in the genesis of colitis. Alternatively, we cannot exclude that the ablation of a multipotent secretin-expressing cell profoundly impairs the normal turnover of the colon epithelium resulting in severe colitis.

The reduced presence of $CD4^+$ T-cells observed in the colon of ganciclovir-treated transgenic mice is consistent with the recent observation of a specific $CD4^+$ T subset capable of inhibiting antigen-specific immune response in the colon (39). A potential direct effect of ganciclovir on $CD4^+$ T-subset cannot be excluded. Nonetheless, it could be speculated that the GVH-like colitis in ganciclovir-treated Sec-HSVTK mice might result from unbalanced T-cell subsets of the colon mucosa. Alternatively, the profound lymphoid depletion may somehow impair the colonic immune barrier, hampering the bacterial flora tolerance. The relative absence of granulocyte in the inflamed colon mucosa of ganciclovir-treated Sec-HSVTK mice may not support this hypothesis. However, manipulation of the immune system results in various degrees of nonsuppurative colitis in mice (40), the host microbial component significantly affecting their phenotype (41). On this line, the common mouse gut pathogen *Helicobacter hepaticus* was demonstrated as determinant of colitis in immunodeficient mice (42), with $CD4^+$ T-cells as key effectors (43, 44). We were unable to detect *H. hepaticus* in mice from our colonies (not shown); however, the potential role of some undetermined component of the enteric flora for the development of colitis in lymphopenic Sec-HSVTK mice cannot be ruled out.

The severe lymphopenia observed in lymphoid organs of treated transgenic mice would support the view that low levels of secretin-gene expression is relevant in the maturation of various lymphoid lineages. On this line, the fact that CD103 expression (normally restricted to gut intraepithelial lymphocytes, IEL, and present only as a minor subset in lymphoid organs) was overall increased in blood, thymus, and spleen T cells and unchanged in the colon suggests such tissues as dialoging reservoirs of a common lymphocyte population involved in the immunological tolerance in the colon. In addition, this may be reflected by the relative increase in $CD8^+$ T-lymphocytes observed in the colon (see Fig. 1G).

In conclusion, our findings indicate that the secretin gene is actively expressed and transcribed in lymphocytes of two major lymphoid organs of the mouse: the spleen and the thymus. The association of lymphoid depletion and colitis observed in ganciclovir-treated Sec-HSVTK mice would support the hypothesis that secretin gene–expressing lymphocytes may be implicated in the immune control at colonic level. By the light of our data, it could be hypothesized that the severe systemic lymphopenia induced in Sec-HSVTK mice might trigger an immunological mechanism responsible for the development of colitis, likely involving colonic CD4+ T-cells and the colonic flora as key actors.

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