Specific overexpression of IL-7 in the intestinal mucosa: the role in intestinal intraepithelial lymphocyte development

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Yang H, Madison B, Gumucio DL, Teitelbaum DH. Specific overexpression of IL-7 in the intestinal mucosa: the role in intestinal intraepithelial lymphocyte development. Am J Physiol Gastrointest Liver Physiol 294: G1421–G1430, 2008. First published April 10, 2008; doi:10.1152/ajpgi.00060.2008.—IL-7 plays a crucial role in regulating immune response and EC growth in the epithelial layer of the intestine. It has been shown that IL-7R are expressed on the surface of CD4+CD8−, CD4+,8−, and CD4−,8+ T cells, but not in CD4+,8+ double-positive T cells (32). More recently, a study from our group has found that IL-7R were identified on both αβ-TCR+IEL and γδ-TCR+IEL subtypes. Exogenous IL-7 administration resulted in a significant expansion in the αβ-TCR+IEL subpopulation (49). These studies suggest that IL-7 plays a critical role in regulating T cell homeostasis in peripheral lymphoid tissues. IL-7 is produced by EC in the thymus and intestinal mucosa (8, 9, 37, 39). Recent studies have demonstrated that close interaction between mucosal lymphocytes and intestinal EC are crucial in regulating immune response and EC growth in the intestinal mucosa (37, 45, 49). Several studies have indicated that EC may play an important role in mucosal immune responses by helping to regulate IEL (11, 50). We have also demonstrated that there is a close physical proximity of EC-derived IL-7 and the IEL (49). IEL are a distinct population of T lymphocytes that are located at the basolateral side of EC in the intestinal epithelium. It has been shown that IL-7 expression of naive T cells is almost completely abolished, and the lifespan of naive T cells is greatly reduced (34). In vitro, in the absence of any other stimulus, IL-7 has been shown to induce proliferation of freshly isolated T cells in a dose-dependent fashion (1, 41). It has been shown that systemic overexpression of IL-7R in a transgenic mouse model increased survival of AKR/J early thymocytes and can contribute to thymocyte proliferation (19). Recently, our laboratory has shown that exogenous IL-7 administration significantly affected IEL phenotype and led to an increase in IEL number, as well as changes in IEL function. Cytokine expression changed significantly in both αβ-TCR+IEL and γδ-TCR+IEL subtypes. Exogenous IL-7 administration resulted in a significant expansion in the αβ-TCR+IEL subpopulation (49). These studies suggest that IL-7 plays a critical role in regulating T cell homeostasis in peripheral lymphoid tissues.

IL-7 IS A MEMBER OF THE gamma chain-dependent (γc) family of cytokines, which share a common receptor γc component and include IL-2, IL-7, IL-9, and IL-15. These cytokines strongly influence T cell development and function (12, 38) and signal T cell activation (35). In vivo only IL-7, and not other γc family members (e.g., IL-4 or IL-15), has been found to be essential for homeostatic proliferation of naive peripheral T cells (34). IL-7 is produced by thymic and intestinal epithelial cells (EC) (9, 37, 39), and in turn IL-7 receptors (IL-7R) have been detected on the surface of thymocytes and intestinal intraepithelial lymphocytes (IEL) (37, 50). Administration of IL-7 has been demonstrated to enhance both peripheral T cells and IEL numbers and function (9, 49). Kenai et al. (15) found that anti-IL-7 antibody treatment disturbed the induction of αβ-T cell receptor (TCR)+ T cells after athymic nude mice were implanted with fetal thymocytes. In the absence of IL-7, homeostatic proliferation of naive T cells is almost completely abolished, and the lifespan of naive T cells is greatly reduced (34). In vitro, in the absence of any other stimulus, IL-7 has been shown to induce proliferation of freshly isolated T cells in a dose-dependent fashion (1, 41). It has been shown that systemic overexpression of IL-7R in a transgenic mouse model increased survival of AKR/J early thymocytes and can contribute to thymocyte proliferation (19). Recently, our laboratory has shown that exogenous IL-7 administration significantly affected IEL phenotype and led to an increase in IEL number, as well as changes in IEL function. Cytokine expression changed significantly in both αβ-TCR+IEL and γδ-TCR+IEL subtypes. Exogenous IL-7 administration resulted in a significant expansion in the αβ-TCR+IEL subpopulation (49). These studies suggest that IL-7 plays a critical role in regulating T cell homeostasis in peripheral lymphoid tissues.

IL-7 is produced by EC in the thymus and intestinal mucosa (8, 9, 37, 39). Recent studies have demonstrated that close interaction between mucosal lymphocytes and intestinal EC are crucial in regulating immune response and EC growth in the intestinal mucosa (37, 45, 49). Several studies have indicated that EC may play an important role in mucosal immune responses by helping to regulate IEL (11, 50). We have also demonstrated that there is a close physical proximity of EC-derived IL-7 and the IEL (49). IEL are a distinct population of T lymphocytes that are located at the basolateral side of EC in the epithelial layer of the intestine. It has been shown that IL-7R are expressed on the surface of CD4−CD8−, CD4+,8−, and CD4−,8+ T cells, but not in CD4+,8+ double-positive T cells (32). More recently, a study from our group has found that IL-7R were identified on both αβ-TCR+IEL and γδ-TCR+IEL subpopulations (50). IL-7 knockout and IL-7 receptor knockout mice show distinct declines in absolute numbers of thymocytes and IEL (24). In an IEL culture model, IL-7 added to media significantly prevented the spontaneous apoptosis of IEL by decreasing caspase activity and preventing the decline in Bcl-2 expression (43).

Previous studies have examined IL-7 given either by systemic administration or systemic overexpression of IL-7 or IL-7R (38, 49) (19). Such studies, however, may not permit one to gain a complete understanding of the role that intestinal EC-derived IL-7 has on the local IEL populations and how the IEL might be modulated by local IL-7 production. To better understand the role intestinal EC-derived IL-7 has in directing transgenic; interleukin-7; mouse; epithelial cells

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IEL lineage and function, we established an intestinal EC-specific overexpressing IL-7 transgenic mouse model by using a 12.4-kb region from the mouse villin gene to direct intestinal overexpression of IL-7. We hypothesized that intestinal EC specific IL-7 overexpression would significantly affect the neighboring IEL phenotype and function.

METHODS

Creation of IL-7 Transgenic Mice

Development of a p12.4KVill-IL-7 transgene. We utilized a previously characterized 12.4-kb murine villin promoter/enhancer fragment to drive the expression of an IL-7 cDNA exclusively in the epithelium of the large and small intestine (23). Since the 12.4-kb region contains at least a portion of a locus control region, 70–80% of founder mice were expected to express the transgene in the small and large intestine.

The full-length sequence of mouse IL-7 cDNA (generously donated by Dr. Jay Bream, National Institute of Arthritis and Musculoskeletal and Skin Diseases) was cloned behind the 12.4-kb villin promoter/enhancer. The final clone used for transgenesis (P12.4KVILL-IL-7) was confirmed by restriction digestion and sequence analysis (across the complete IL-7 and into the villin sequences).

Generation of IL-7-transgenic mice. The transgene, containing villin regulatory sequences linked to IL-7, was prepared by digestion of P12.4KVILL-IL-7 with PmeI (Fig. 1). After purification, transgene DNA was injected into the pronuclei of fertilized ova (C57BL/6J × SJL/J) by the University of Michigan Transgenic Animal Core. Founders were identified by PCR amplification of tail DNA with primers specific for the transgene (forward primer ACAGGCACTAGGGAGCCAATG; reverse primer TCCTGTCATTTGTCCAATTC). Founders were then mated to C57BL/6J mice for the creation of F1 hybrids. All mice were bred and housed under clean specific pathogen-free conditions. Transgenic lines were maintained by crossing transgenic mice with C57BL/6J mice; transgenic animals were identified by PCR analysis of their tail DNA.

Sample Harvesting

All mice were euthanized at 8 wk of age, and serum and intestine, spleen, kidney, and liver tissues were harvested.

IEL and LPL isolation from small intestine. Cells were isolated as previously described (17). Briefly, the small bowel was placed in tissue culture medium (RPMI 1640 with 10% FCS; Life Technologies, Gaithersburg, MD). Mesenteric fat and Peyer’s patches were removed. The intestine was cut into 5-mm pieces, washed three times in an IEL extraction buffer (1 mM EDTA, 1 mM DTT in PBS), and incubated in the same buffer with continuous brisk stirring at 37°C for 20 min. The supernatants containing released sloughed EC and IEL were stored on ice. The remaining tissue pieces were incubated three times for 45 min each with RPMI 1640 containing collagenase (40 U/ml), 5% FBS, glutamine, and an antibiotic mixture at 37°C (20).

Supernatants containing lamina propria lymphocytes (LPL) from each incubation were pooled on ice.

Supernatants containing IEL and EC were then filtered through a glass wool column, and IEL and EC were separated by using magnetic beads conjugated with antibody to CD3 (BioMag SelectaPure antimouse CD3 antibody particles, Polyscience, Warrington, PA). Cells bound to beads were considered purified IEL; the EC remained in the supernatant. Flow cytometry confirmed purity of sorted cells, which was greater than 99%.

Supernatant containing LPL (after collagenase incubation) were filtered through a glass wool column. Suspensions were centrifuged, the pellets were resuspended in 40% Percoll (Pharmacia, Piscataway, NJ), and the cell suspensions were overlaid on 75% Percoll. After centrifugation for 20 min at 600 g at 25°C, viable lymphocytes were recovered from the 40%/75% interface and washed in RPMI 1640 with 5% FBS, glutamine, and the antibiotic mixture (20).

Purification of IEL subpopulations. Some IEL specimens were further separated between αβ-TCR+ and γδ-TCR− subpopulations by using biotinylated beads conjugated to monoclonal antibody as previously described (48). The purity of samples was confirmed by flow cytometry and compared with nonseparated IEL samples. Briefly, IEL were stained either with anti-αβ-TCR+ antibody conjugated with fluorescein isothiocyanate (FITC) (PharMingen, San Diego, CA) or with anti-γδ-TCR− antibody conjugated with FITC (PharMingen), followed by incubation with magnetic beads conjugated with antibody to FITC (Polyscience, Warrington, PA). Cells bound to beads were considered purified αβ-TCR+IEL or γδ-TCR− IEL and were processed for RNA isolation.

Isolation of lymphocytes from spleen and thymus. Thymocytes and splenocytes were individually isolated by passage through a stainless steel mesh grid in sterilized RPMI 1640. Red blood cells were lysed with red blood cell lysis buffer for splenocytes, washed twice in 10% FCS/RPMI 1640, and counted.

Reverse Transcriptase-Polymerase Chain Reaction

Isolation of total RNA. A guanidinium isothiocyanate-chloroform extraction method was used. Total RNA from isolated EC or IEL, or subtype IEL, LPL, thymocytes, splenocytes, and a piece of liver or kidney tissues were extracted by using Trizol reagent (Life Technologies) according to the manufacturer’s directions.

RT-PCR. Poly-A tailed mRNA was reversed transcribed into complementary DNA by adding total cellular RNA to the following reaction mixture: PCR Nucleotide Mix (Invitrogen, Carlsbad, CA), M-MLV Reverse Transcriptase (Invitrogen), oligo(dT)12–18 Primer (Invitrogen), and RNase Inhibitor (40 U/μl, Roche Diagnostics, Mannheim, Germany). DEPC-treated H2O was added to yield the appropriate final concentration. For quantification of the IL-7 mRNA (including transgenic IL-7 mRNA) expression study, IL-7 primers were designed by using sequence from the encodable sequence of mouse IL-7 cDNA; forward primer: TGCCCGAATAATGAACCAAGAGGAGCCAATG; reverse primer CAACCTCTCCAAGTTATGAACCA.

GTAG. For transgenic IL-7 gene detection, the forward primer was designed from exon-1, CAACTTCCTAAGATCTCCCAGGTG, and the reverse primer was designed from IL-7 cDNA, GTTCTCTGTCATTITGCTCAATTC. Primers for other cytokines, keratinocyte growth factor (KGF), and ligands were designed by use of proprietary software (Lasergene 6; DNA Star, Madison, WI). Samples were incubated at 40°C for 70 min, and the reaction was then stopped by incubating at 95°C for 3 min. PCR reaction was run. Thermal cycler settings were optimized to insure products were in the logarithmic phase of production. The PCR products were run out on a 2% agarose gel. Quantification of cDNA product was completed using a Kodak 1D image quantification software (Kodak, Rochester, NY), and target PCR products were compared by normalizing each sample to the production of β-actin.

Fig. 1. P12.4KVill vector was modified by ligation the IL-7 gene in a site beyond the villi promoter region. The 2 sites (KpnI and XhoI) where the IL-7 sequence will be inserted are labeled into P12.4KVill vector. The two sites to be cut by PmeI for release of the entire construct are also labeled. After selection of an appropriately stable clone as determined by size and sequence, the p12.4KVILL + IL-7 construct will be released and injected into pronuclei of fertilized ova to create this IL-7-transgenic mouse.
Immunoblot Analysis for EC-Derived IL-7 Expression

Briefly, isolated EC were homogenized on ice in lysis buffer (42). Protein determination was performed by using a Micro BCA Protein Assay Kit (Pierce, Rockford, IL). Approximately 40 μg of total protein in loading buffer was loaded per lane in a SDS-polyacrylamide gel (13%) and separated by electrophoresis. Proteins were then transferred to a polyvinylidene difluoride membrane (Bio-Rad Laboratories, Hercules, CA). The membrane was blocked with blocking solution (Zymed Laboratories, South San Francisco, CA) and probed with goat anti-mouse biotinylated-IL-7 antibody (R&D Systems, Minneapolis, MN) (0.15 μg/ml in blocking solution) for 1 h. Bound antibodies were exposed to a streptavidin-horseradish peroxidase conjugate (1:10,000, Zymed Laboratories), detected on X-ray film. Blots were then stripped and reprobed with monoclonal mouse anti-β-actin antibody (1:8,000 in blocking solution; Sigma, St. Louis, MO) to confirm equal loading of protein. The peroxidase-conjugated second antibody was goat anti-mouse IgG (1.8,000 in blocking solution; Invitrogen). Quantification of results was performed using Kodak 1D image quantification software (Kodak). Thus results of immunoblots are expressed as the relative expression of IL-7 to β-actin expression.

IL-7 ELISA Detection

Recombinant mouse IL-7, anti-mouse IL-7 antibody, and biotinylated anti-IL-7 antibody were purchased from R&D Systems. Methods utilized are similar to those previously described (7, 48). Briefly, anti-IL-7 antibody was coated to the ELISA plate, and IL-7 levels were measured by ELISA by use of matched-pair antibodies. Serum IL-7 levels were determined graphically by using standard curves generated with recombinant mouse IL-7.

Flow Cytometric Analysis

Lymphoid phenotype was studied by fluorescent-labeled antibody staining detected with three-color flow cytometry. Monoclonal antibodies (BD PharMingen, San Diego, CA), consisting of anti-CD4, CD8α, CD8β, αβ-TCR and γδ-TCR, CD44, as well as CD69 antibodies, were used to examine the IEL subsets. Acquisition and analysis were performed on a FACSCalibur (Becton-Dickinson, San Jose, CA) using CellQuest software (Becton-Dickinson). The IEL population was gated on the basis of forward- and side-scatter characteristics. Quantification of each subpopulation was based on its percentage of the gated IEL population.

In Vivo IEL Proliferation Study

BrdU administration. Mice were provided with water ad libitum with 0.6 mg/ml 5-bromo-2-deoxyuridine (BrdU; Sigma) for 7 days as described by Tough and Sprent (36). After this 7-day period of BrdU labeling, mice were given unsupplemented water for an additional 14 days and then analyzed.

Intracellular BrdU staining analysis. Cell staining for BrdU utilized a BrdU Flow Kit (BD Pharmingen, CA), as directed by the manufacturer. Briefly, isolated cells were suspended in FACSC buffer and cell surface was stained with appropriate phycoerythrin (PE)-conjugated antibody at 4°C for 30 min. Cells were then washed twice and resuspended, and Cytofix/Cytoperom (BrdU flow staining kit, BD Pharmingen) was added for 20 min at 4°C. The cells were washed, resuspended with Cytoperom plus buffer, and incubated for 10 min, washed with Perm/Wash buffer, resuspended with Cytofix/Cytoperom, incubated for 5 min, resuspended with diluted DNase buffer (provided with kit), and incubated for 1 h. Cells were washed with Perm/Wash buffer, and PE-conjugated antibody to BrdU was added at 4°C for 30 min. Cells were washed twice and resuspended in staining buffer prior to flow cytometry. Acquisition and analysis were performed on a FACSCalibur (Becton-Dickinson) using CellQuest software (Becton-Dickinson). The IEL population (gated on the basis of forward- and side-scatter characteristics) was quantified for each subpopulation on the basis of its percentage of the gated IEL population.

Histological Analysis

Segments 0.5 cm long of jejunum and ileum were fixed in 10% formaldehyde for histological sectioning. Tissues were then dehydrated with ethanol and embedded in paraffin. Sections were cut and stained with hematoxylin-eosin. The villus height and width and crypt depth were measured by use of a calibrated micrometer. Each measurement of the crypt and villus dimensions consists of the mean of seven different fields.

Data Analysis

Data are expressed as means ± SD. Cytokine expression and flow cytometric results were analyzed by paired t-tests, with significance being defined as P < 0.05.

RESULTS

Intestinal EC-Derived IL-7 Expression in Transgenic Mice

Villin is expressed in all EC of the small and large bowel and along the entire crypt-villus axis, with an increasing gradient from crypt to tip (25). It has been shown that the 12.4-kb fragment of the mouse villin promoter is reliable and reproducible to drive reporter gene expression in the intestine in a pattern that closely resembles that of the endogenous mouse gene (23). This 12.4-kb regulatory promoter is largely specific for the intestinal epithelium (23). RT-PCR showed EC-derived IL-7 expression to be expanded exclusively in the intestine. IL-7 mRNA expression was significantly higher in transgenic mice than wild-type mice (Fig. 2, A and B), and no transgenic expression of IL-7 mRNA was seen in thymus, kidney, spleen, or liver tissues in transgenic IL-7vill mice (data not shown).

To clarify whether IL-7 overexpression in the intestinal EC of transgenic mice led to local overproduction of IL-7 protein, Western blot was used to detect EC-derived IL-7 expression in the jejunum and ileum of IL-7vill transgenic mice. IL-7 expression was significantly higher in both jejunum and ileum compared with that of wild-type mice (Fig. 2, C and D). IL-7 expression increased nearly 3-fold in jejunum and 3.3-fold in ileum compared with wild-type mice. Furthermore, this overexpression did not extend beyond the gastrointestinal tract; serum IL-7 levels were undetectable in both IL-7vill mice and wild-type mice by ELISA detection.

Effect of EC Specific Overexpression of IL-7 on the IEL

Numbers of IEL are increased in transgenic mice. Numbers of IEL in IL-7vill mice were significantly (P < 0.05) expanded compared with wild-type mice. The number of IEL in transgenic mice increased threefold compared with wild-type mice (Fig. 3A). IEL phenotype significantly changed in IL-7vill mice. To better understand the changes occurring in the IEL in transgenic mice, phenotype analysis was performed with flow cytometry. Several changes were identified in the surface phenotype of IL-7vill mice compared with wild-type mice (Fig. 4). Both CD4+, CD8− and CD4+, CD8+ IEL subpopulations were significantly higher in transgenic mice (Fig. 3B) (P < 0.05). The CD4+, CD8− IEL numbers increased 16.8-fold and CD4+, CD8+ IEL numbers increased 8.1-fold in IL-7vill mice compared with wild-type mice (Fig. 3B). The number of...
CD8αβ+ IEL (i.e., CD8αβ heterozygote portion of CD8+ cells) was also significantly increased in the IL-7vill mice compared with wild-type mice (P < 0.05; Fig. 3B).

TCR subpopulations were also studied. The relative percentage of the αβ-TCR+ IEL population significantly increased in IL-7vill mice compared with wild-type mice (Fig. 4C), as well as the absolute number (17.1 ± 2.1 × 10⁶) of harvested αβ-TCR+ IEL compared with wild-type mice (2.7 ± 0.6 × 10⁶). The percentage of the αβ-TCR+ IEL increased twofold compared with wild-type mice, whereas it was interesting to note that the percentage of γδ-TCR+ IEL population was significantly lower in IL-7vill mice compared with wild-type mice.
mice (Fig. 4C); however, this decline was relative in respect to all IEL, and there was no significant difference of the absolute number of isolated γδ-TCR+ IEL between IL-7vill mice and wild-type mice (4.2 ± 1.1×10⁶ vs. 5.4 ± 1.2×10⁶, P > 0.05).

IEL maturation and activation changes in IL-7vill mice. CD44 was used as a marker of IEL maturation (26). CD4⁺, CD44⁺ IEL were significantly increased in IL-7vill mice compared with wild-type mice (38.4 ± 5.9 vs. 6.5 ± 1.7%, respectively) (Fig. 5A); representing a 15.2-fold increase in IL-7vill mice compared with wild-type mice (Fig. 3B). CD8⁺, CD44⁺ IEL also significantly increased (P < 0.05) in transgenic mice, representing a 1.9-fold increase compared with wild-type mice (Fig. 3B). Surface expression of CD69 was used as a marker of T cell activation (31) and was noted to be significantly altered in IL-7vill compared with wild-type mice (Fig. 5B). The CD4⁺, CD69⁺ IEL number increased nearly 19.7-fold in IL-7vill mice (Fig. 3B) whereas CD8⁺, CD69⁺ IEL number actually only increased twofold compared with wild-type mice (Fig. 3B).

IEL IL-7 receptor expression. IL-7R expression has been detected on IEL and LPL (37, 50). It has been shown that the IL-7/IL-7R-dependent signaling pathway plays a crucial role in regulating the immune response in the intestinal mucosa (44, 50). To determine whether local overproduction of IL-7 affects the neighboring IEL through the IL-7/IL-7R dependent signaling pathway, IEL IL-7R mRNA expression was studied. IL-7R mRNA expression in both αβ-TCR⁺ IEL and γδ-TCR⁺ IEL subtypes were significantly increased compared with wild-type mice (Fig. 6). The αβ-TCR⁺ IEL subtype IL-7R mRNA expression increased 92.2%, and the γδ-TCR⁺ IEL IL-7R mRNA expression increased 53.4% compared with wild-type mice (Fig. 6).

Functional Changes in the IEL

Increased IEL proliferation in transgenic mice. Because of the profound increase in IEL number and phenotypic changes in IL-7vill mice, we next investigated alterations in proliferation in various IEL subtypes. The results in Fig. 7 demonstrate that the IEL from IL-7vill mice have a dramatic increase in the proportion of cells entering the cell cycle compared with IEL from wild-type mice. More specifically, IEL proliferation in the CD4⁺ IEL was 18.5 ± 1.2% in IL-7vill mice compared with 2.1 ± 0.4% in wild-type mice (n = 6 each group, P < 0.05) (Fig. 7A). CD8⁺ IEL proliferation was 27.9 ± 6.9% in IL-7vill mice compared with 8.5 ± 0.2% in wild-type mice (P < 0.05; Fig. 7B). Similarly, the percentage of proliferating αβ-TCR⁺ IEL was significantly higher in transgenic mice (Fig. 7C). Concomitant with the observed decline in the relative number of γδ-TCR⁺ IEL; however, no significant difference in γδ-TCR⁺ IEL proliferation was noted between transgenic and wild-type mice (Fig. 7D).

Changes in cytokine expressions in αβ-TCR⁺ and γδ-TCR⁺ IEL. A panel of cytokines was selected that could contribute to both T cell activation as well as a downregulation of T cell activity (28, 40). TNF-α expression significantly increased in both αβ-TCR⁺ and γδ-TCR⁺ IEL populations in IL-7vill mice compared with wild-type mice (P < 0.05; Fig. 8). Because the
IEL is a major source of IFN-γ (16, 46), IEL-derived IFN-γ mRNA expression was also studied and was noted to be significantly increased in both wild-type mice, but either αβ-TCR+ or γδ-TCR+ IEL-derived IL-2 and IL-4 mRNA expression did not significantly change in IL-7vill mice (\( n = 6 \)) compared with control mice (\( n = 6 \)). Values (means ± SD) are expressed as the ratio of target cytokine vs. expression of β-actin; *\( P < 0.05 \).

Histological Morphology in IL-7 Transgenic Mice

IL-7vill mice were noted to have profoundly altered crypt and villus structures (Fig. 10A). Villus height in IL-7vill mice was significantly higher than in wild-type mice. Crypt depth in IL-7vill mice was significantly deeper than in wild-type mice (Fig. 10B). One of the most dramatic changes in our transgenic mice was a significant increase in the width of the villi (Fig. 10B). It appeared that this marked increase in villus width was due to a marked expansion of lymphocytes in the underlying LPL population. We therefore investigated the associated alterations in the LPL of transgenic mice.

Fig. 9. Changes in keratinocyte growth factor (KGF) mRNA expression in γδ-TCR+ IEL. Results of RT-PCR are expressed as a ratio of KGF to β-actin mRNA expression. Expression of KGF mRNA was markedly increased in IL-7vill mice (\( n = 6 \)) compared with control mice (\( n = 6 \)), *\( P < 0.05 \).
The number of LPL increased significantly in IL-7vill mice crypt-villus structure, and not just the epithelial mucosal lining. IL-7 may influence lymphoid development within the entire lamina propria Changes in IL-7vill Mice

EC-specific overexpression of IL-7 also induced a significant expansion of LPL numbers and suggested that EC-derived IL-7 may influence lymphoid development within the entire crypt-villus structure, and not just the epithelial mucosal lining. The number of LPL increased significantly in IL-7vill mice compared with wild-type mice ($P < 0.05$; Fig. 11A). Numbers of LPL increased threefold in IL-7 transgenic mice compared with wild-type mice. B cells (as detected with CD19) were not significantly different between IL-7 transgenic mice and wild-type mice (data not shown). Significant changes were also seen in the LPL phenotype. The percentages of CD3+CD4+CD8+; CD3+CD4+CD44+ and CD3+CD4+CD69+ subtypes significantly increased in transgenic mice compared with wild-type mice ($P < 0.05$) (Fig. 11B).

Systemic Lymphoid Populations

To assess whether local overexpression of IL-7 affected other lymphoid populations, thymocytes and splenocytes were studied. Overexpression of IL-7 did not result in any significant changes in thymocyte or splenocyte phenotype. There was also no significant difference in the number of thymocytes or splenocytes between transgenic and wild-type mice (data not shown). These results indicate that IL-7 overexpression by EC had no effect on systemic lymphoid populations. Thus the changes induced by EC-derived IL-7 were due to the direct effect on the mucosal level and not due to secondary changes in other lymphoid populations that might be a source of some portion of the IEL (i.e., thymic-derived IEL).

Mechanisms for IEL Recruitment to the Mucosal Epithelium

Although we demonstrated that the IEL are rapidly proliferating in our IL-7vill mice, we also noted a marked increase in TCR-$\alpha$-$\beta^{+}$ IEL; this cell population could come from extraintestinal sources, such as the thymus (13, 21, 29). Therefore, we wanted to examine whether the expansion of the IEL in these mice might be influenced by changes in the expression of the IEL integrin $\alpha_{E}\beta_{7}$, which is a critical population for homing of lymphocytes to the mucosa. Interestingly, both $\alpha_{E}$-$\beta$-TCR$^{+}$ and $\gamma_{D}$-TCR$^{+}$ IEL integrin $\alpha_{E}\beta_{7}$ expression increased significantly compared with controls (Table 1). TCR-$\alpha$-$\beta^{+}$ IEL integrin $\alpha_{E}\beta_{7}$ expression was also noted to be much higher than in the $\gamma_{D}$-IEL in IL-7vill mice, as well as has previously been observed in wild-type mice. The ligand of CD103 (E$\beta_{7}$) is E-cadherin; therefore, we next investigated whether the marked overexpression of the integrin $\alpha_{E}\beta_{7}$ resulted in any changes to E-cadherin expression. In fact, we noted a 50% increase in E-cadherin expression in our transgenic mice, suggesting that local IL-7 overexpression contributes to IEL expansion not just by increased proliferation, but by the recruitment of lymphocytes to the epithelium.

DISCUSSION

In this study, we found that EC-specific IL-7 overexpression in our IL-7 transgenic mice significantly affected IEL phenotype and function and resulted in a significant expansion of the IEL population. IEL functional changes included a significant increase in IEL proliferation and alterations in cytokine expression for both $\alpha$-$\beta$-TCR$^{+}$ and $\gamma_{D}$-TCR$^{+}$ subtypes. In addition to the IEL, the intestinal LPL population was also seen to be significantly expanded, and a significant change in the proportion of LPL subtypes was observed. Finally, systemic lymphoid populations did not change in the IL-7vill transgenic mice, which supported the concept that local overexpression of IL-7 resulted in a restricted area of action to the intestinal mucosa and submucosa.

Villin is a cytoskeletal protein that is produced by EC and is expressed along the brush border. Because villin is also expressed in the proliferative stem cells of the intestinal crypts cells, it is believed to be an early marker of EC from the remainder of the digestive tract (27). In the adult, the concentration of villin increases as cells move from the crypt to the tip of the villi (25). The 12.4-kb regulatory fragment of villin we used has been shown to be largely specific for the intestinal epithelium (22, 23). This villin promoter/enhancer has been shown to allow for the creation of a number of transgenic mouse models with robust overexpression of several genes ($\beta$-galactosidase, Cre recombinase, green fluorescent protein, and Hedgehog interacting protein) (23). In our IL-7vill mice...
The CD3- compared with wild-type mice, CD3 was used as a marker of T maturation. CD69 was used as a T activation marker.

of the LPL phenotypes. CD3 was used as a T cell marker, and CD44 was used as a hallmark for the differentiation of CD44+ T cells. The expression was compared in transgenic mice with wild-type mice.

Table 1. Alteration of CD103 (α4β7 mRNA) and E-cadherin in IL-7vill mice

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Number (IEL)</th>
<th>α4β7 mRNA (TCR-αβ*) EIEL</th>
<th>α4β7 mRNA (TCR-γδ+ EIEL)</th>
<th>E-Cadherin mRNA</th>
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<tbody>
<tr>
<td>Wild-type</td>
<td>4.8±1.2</td>
<td>261±76</td>
<td>61.8±15</td>
<td>92.9±30.5</td>
</tr>
<tr>
<td>IL-7vill</td>
<td>14.6±4.5*</td>
<td>2263±446*</td>
<td>1134±257*</td>
<td>140.6±39.8*</td>
</tr>
</tbody>
</table>

Results are means±SD. IEL, intraepithelial lymphocytes. *P < 0.05, using ANOVA compared to wild-type group.

Recent studies have demonstrated that interactions between mucosal lymphocytes and intestinal EC are crucial in regulating the immune response in the intestinal mucosa (33, 37, 45, 46). Several studies have indicated that EC may play an important role in mucosal immune responses by helping to regulate IEL phenotype and function (11, 37). Watanabe et al. (37) demonstrate that intestinal EC express IL-7. IL-7 has been shown to play a crucial role in controlling T cell development and homeostasis (4), and this is supported by the wide range of IEL subpopulations that express IL-7R (18, 37, 44). IL-7 knockout and IL-7R knockout mice show distinct declines in absolute numbers of thymocytes and IEL (including an absence of γδ-TCR + IEL) (24). Using the intestinal fatty acid binding protein promoter, Laky et al. (18) were able to reconstitute IL-7 expression in IL-7 knockout mice, and this low level of expression was sufficient for the development of the extrathymic IEL. This demonstrates the essential role that the local expression of IL-7 has on the development of the mucosal immune system. Exogenous administration of IL-7 resulted in a significant increase in CD8αβ+ IEL. Exogenous IL-7 also resulted in a similar stimulation of LPL growth (24, 49). In a more elegant fashion, Watanabe et al. (38) showed that the systemic overexpression of IL-7 with an SRα promoter demonstrated an expansion of lymphoid populations and the development of chronic colitis. One particular distinguishing feature of our particular model is that the overexpression of IL-7 is predominantly in the small bowel and progressively decreases in the colon, so that we may not observe the degree of colitic changes that was identified by Watanabe et al.

The functional relevance of altered IL-7 expression in the mucosal epithelium is illustrated in a recent study by our laboratory that showed TPN administration resulted in a significant decrease in EC-derived IL-7 expression (53). Additionally, exogenous IL-7 administration to TPN mice significantly attenuated TPN-associated IEL changes (50). The precise etiology of these TPN-associated changes in IL-7 is uncertain. It is difficult to determine whether the action of the γδ-TCR+, compared with less than 2% of peripheral blood lymphocytes (16). The function of the IEL appears to very important in maintaining the intestinal barrier and gut immunity, as well as regulating intestinal growth (regulating EC proliferation and apoptosis) (45, 46, 48, 51). Alterations in the luminal environment may produce significant changes in the IEL. A previous study from our group has shown that administration of total parenteral nutrition (TPN) in mice (the complete absence of luminal nutrients) led to a significant decline in the CD4+, CD8+, CD44+, and CD8αβ+ IEL subpopulations, as well as a nearly complete loss of the CD8αβ+ IEL population. TPN also led to a significant change in the expression of a variety of cytokines (16, 45). This change in IEL function resulted in a loss of epithelial barrier function and intestinal mucosal atrophy (47, 48, 52). The mechanism that caused these IEL changes is unknown.

this 12.4-kb villin/IL-7 transgene was only detected from small and large intestinal epithelial tissue and was not detected in thymocytes, splenocytes, and kidney and hepatic tissues. Immune blot results showed that IL-7 protein expression in both jejunum and ileum in IL-7vill mice was significantly higher than in wild-type mice. Furthermore, the expression was confined to the local EC expression as, similar to wild-type mice; serum IL-7 from transgenic IL-7 mice was undetectable. IL-7 is predominantly in the small bowel and progressively decreases in the colon, so that we may not observe the degree of colitic changes that was identified by Watanabe et al.

The predominant IEL subpopulation is CD8+, CD4− (70–85% of the total in mice) (10). IEL in mice also comprise a large number of γδ-TCR+ cells, and ∼50% of IEL are

![Graph A](image1)

![Graph B](image2)
exogenous IL-7 was due to an effect on the thymus or directly on the intestinal mucosa. Using intestinal specific overexpressing IL-7 transgenic mice has greatly facilitated our understanding of the role of EC-derived IL-7 on the neighboring IEL. This allows one to detect the action of IL-7 in a more local or paracrine fashion, as opposed to systemic (or endocrine) action of this cytokine. In our present study, the IEL population was significantly expanded, consisting of an increase in the CD4+ and CD8αβ+, as well as αβ-TCR+IEL subtypes. The increase in IEL subtypes is disproportionately biased to an expansion of the CD4+ IEL population, which increased ninefold, compared with CD8+IEL, which increased fourfold compared with wild-type mice. A bias toward the expansion of the αβ-TCR+IEL over the γδ-TCR+IEL subpopulation was also noted. This appears consistent with a greater colocalization of IL-7 expression the αβ-TCR+ group of IEL (49). Our observation of an increase in IEL proliferation, as well as a marked increase in CD103 (integrin αxβ2), suggests that the expansion of the IEL population was due to both a local increase in IEL growth as well as a recruitment of extraintestinal lymphocytes to the epithelium.

Interestingly, the LPL population was also expanded in our transgenic mice. The fact that we did not find any significant changes in the systemic lymphoid population in our IL-7vill transgenic mice shows that the critical action of this local (paracrine) expression of IL-7 was confined to the formation of the IEL and LPL populations and suggests a potential functional role of EC-derived IL-7 to act on the underlying LPL population. Studies suggest that IEL do not spend their entire cycle in the epithelium but recirculate into the lamina propria after activation, although this movement occurs at a much slower rate than for most T cells (3, 11). Those studies may explain the fact that LPL population was also found to be significantly expanded in our transgenic mice. It is also possible that this expansion of the LPL might be due to a direct effect of the IL-7 acting below the basement membrane of the epithelium on the T cell population of the LPL. Although many of these changes may potentially have been due to increased nutritional intake in the transgenic group, this appears unlikely. In fact, the measured amount of chow consumed was not different between transgenic and wild-type mice. Furthermore, body weights were actually about lower for the IL-7vill transgenic mice, compared with wild-types (20.49 ± 0.81 vs. 22.91 ± 0.87 g, at 8 wk of life for transgenic vs. wild-type, respectively), suggesting that the increase in lymphoid populations was not due to increased food intake.

IL-7 has been shown to enhance peripheral T cell expression of IL-4 (2, 6). Jiang and McGee (14) found that EC-derived IL-7 could enhance human peripheral T cell IL-4 secretion but not IFN-γ in a cell culture model. A recent study from our group showed that exogenous IL-7 administration to wild-type healthy mice can upregulate both αβ-TCR+ and γδ-TCR+IEL-derived IL-2 and IL-10 expression. As well, IL-7 was also able to increase the expression of γδ-TCR+IEL-derived KGF expression. IL-7 had no effect on the expression of IL-4 or IL-6 (49). In this present study, both αβ-TCR+ and γδ-TCR+IEL-derived cytokine expressions were studied. Both IFN-γ and TNF-α expression were noted to be significantly increased in IL-7vill transgenic mice compared with wild-type mice. Interestingly, γδ-TCR+IEL-derived KGF expression was also noted to increase in our transgenic mice. This latter finding is interesting, in that although IL-7 is critical for the formation of γδ-TCR+IEL, IL-7 overexpression had little action on expanding the γδ-TCR+IEL population but was able to influence its functional expression of both cytokines and growth factors. We did not find any significant change in IL-2 or IL-4 expression between IL-7vill and wild-type mice. It is possible that exogenous administration of IL-7 may stimulate extramucosal lymphoid populations that may account for the observed increase in IEL-derived IL-2, a finding that did not occur in our IL-7vill transgenic mouse model. A previous study of our laboratory has shown that there is a close physical cross-communication between EC-derived IL-7 and the neighboring IEL via the IL-7R (49). Taken together, our data suggest that cell-to-cell interactions between EC and IEL, via IL-7/IL-7R, are an important mode of communication for the maintenance and activation of the IEL.

In conclusion, this study demonstrated that intestinal specific IL-7 overexpression significantly affected IEL and LPL populations and significantly changed IEL function, resulting in overexpansion of the CD4+, CD8αβ+ IEL, and αβ-TCR+IEL subtypes, as well as an increase CD4+ IEL maturation and activation. The results of this study confirm a close physical and functional cross-communication between EC-derived IL-7 and the mucosal lymphoid population.

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REFERENCES

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