The T3^b gene promoter directs intestinal epithelial cell-specific expression in transgenic mice

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Abstract Although a few promoters that direct intestinal epithelial cell-specific expression in transgenic animals have been reported, they are not necessarily appropriate for transgenic studies in terms of activity and tissue specificity. Here, we examined the tissue specificity of transgene expression directed by the 2.8-kb promoter region of the T3^b gene, which encodes one of the non-classical major histocompatibility complex class I molecules. The transgene was expressed exclusively in the epithelial cells of the small and large intestines at high levels. The results indicate that the T3^b promoter is useful for directing transgene expression specifically in intestinal epithelial cells.

Key words: Tissue-specific promoter; Inflammatory bowel disease; Interleukin-12; Transgenic mouse

1. Introduction

Inflammatory bowel disease (IBD), i.e. Crohn's disease or ulcerative colitis, is an idiopathic chronic disease. Although many studies have shown abnormalities of the mucosal immune responses in IBD, its precise pathogenesis has not been elucidated. Recently, various animal models of chronic intestinal inflammation have been reported [1], such as interleukin (IL)-2 [2], IL-10 [3], and αβ TCR knockout mice [4], or gp39 (CD40 ligand) [5], and IL-7 transgenic mice [6]. Experimental colitis models, such as TNBS (2,4,6-trinitrobenzensulfonic acid)-induced colitis, have also been reported [7]. Studies of human IBD have implicated cytokines in the disease process, consistent with results from animal models [8–10]. To further investigate the roles of various cytokines in the inflammatory tissues, it would be useful to express in the intestinal epithelium various transgenes that might modulate the local immune reaction and to examine their effects on the development of IBD. Transcriptional promoters that can direct transgene expression specific to large intestinal epithelial cells are needed for such experiments. However, only a few promoters have been reported.

The T3^b gene encodes one of the thymic leukemic antigens, which are non-classical major histocompatibility complex (MHC) class I molecules, in the C57BL/6 mouse strain [11–13]. It has been shown to express strongly in the epithelial cells of the intestine of normal C57BL/6 mice. In the present study, we examined the tissue specificity of transgene expression driven by a 2.8-kb sequence that includes the promoter and upstream non-coding region of the mouse T3^b gene. The expression of the transgene was examined by Northern blot analysis, RT-PCR, and immunohistochemistry. The results show that this 2.8-kb promoter region is sufficient to drive high levels of transgene expression that is restricted to the epithelial cells of the small and large intestines. Thus, this promoter region includes the *cis*-acting elements necessary to recapitulate the tissue-specific expression pattern of the endogenous T3^b gene.

IL-12 is a heterodimeric cytokine composed of two disulfide-linked subunits, p40 and p35. It is produced by macrophages and antigen-presenting cells [14]. The p35 subunit of IL-12 is expressed in many cell types, whereas the p40 subunit (IL-12 p40) is inducible, can bind to the IL-12 receptor (and does so especially well in a homodimeric form), and blocks IL-12 from binding to the receptor. IL-12 p40 is considered to be a natural regulator of IL-12 activity [15,16]. Recent reports have shown that IL-12 is a key cytokine for Th1 cell development in organ-specific autoimmune diseases [17]. In Crohn's disease, IL-12 production in the inflammatory site has been studied [18,19]. However, the precise role of IL-12 in IBD has not been clarified. To study this issue, transgenic mice overexpressing IL-12 p40 in the intestinal epithelial cells would be very useful. The present study demonstrates that the T3^b promoter can be used to control transgene expression in the small and large intestinal mucosa for such purposes.

2. Materials and methods

2.1. DNA construct

The SAP-IL-12 p40 plasmid contained the human SAP (serum amyloid P component) promoter, the IL-12 p40 cDNA with 17 bp of upstream and 10 bp of downstream non-coding sequences in addition to the approximately 1-kb complete IL-12 p40 coding sequence, and rabbit β-globin gene sequences from the second exon to the polyadenylation signal [20]. The T3^b-IL-12 p40 transgene was constructed by replacing the SAP promoter of the SAP-IL-12 p40 plasmid with the 2.8-kb *Sph*I-*Hin*dIII T3^b promoter region obtained from the T3^b gene cloned into the pUC18 plasmid [11]. Finally, a 6.0-kb *Sph*I-*Xho*I T3^b-IL-12 p40 DNA fragment was purified and used for microinjection.

2.2. Generation of T3^b-IL-12 p40 transgenic mice

BDF1, C57BL/6J, and MCH-ICR mice purchased from CLEA Japan Inc. (Tokyo, Japan) were used throughout this study. Mice were maintained under specific pathogen-free conditions in the animal facility at Osaka University Medical School. The transgene was micro-

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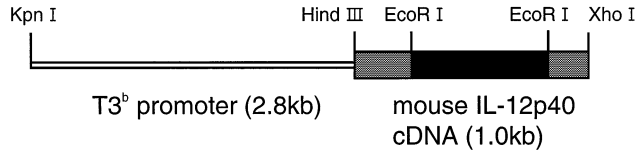

Fig. 1. The T3^b-IL-12 p40 transgene construct. The T3^b-IL-12 p40 transgene contained the T3^b promoter, IL-12 p40 cDNA, and the rabbit β-globin gene sequences from the second exon to the polyadenylation signal.

injected into the pronuclei of BDF1 fertilized eggs as described. For screening founder mice, tail DNA was isolated by the SDS-proteinase K method. Founders were genotyped by PCR using specific primers for the transgene. The following oligonucleotides were used as a forward primer, 5′-GCTGGTTATTGTGCTGCTTC-3′ and a backward primer, 5′-GGTTCACTGTTTCTCCAGGG-3′. The PCR conditions were 95°C for 1 min, 60°C for 2 min, 72°C for 2 min, and 28 cycles at 94°C for 1 min, 58°C for 1 min, 72°C for 1 min followed by an extension for 10 min at 72°C.

2.3. Southern and Northern blot analysis

Mouse tail DNA was isolated and quantified using a spectrophotometer. DNA (1.5 μg) was digested with *Hin*dIII, fractionated by electrophoresis on 0.8% agarose gels, and blotted onto Hybond N membranes (Amersham Corp., Arlington Heights, IL, USA). The IL-12 p40 cDNA was used as the probe. Probe binding was detected by enhanced chemiluminescence using Gene Images (Amersham), according to the supplier's instruction.

Total RNA was isolated from homogenized tissue by the guanidinium isothiocyanate/CsCl method. RNA was quantified using a spectrophotometer, and 10 μg of total RNA was fractionated by electrophoresis on 0.9% agarose gels made with formaldehyde and blotted onto Hybond N membranes. IL-12 p40 cDNA and human β-actin gene DNA (Wako Pure Chemicals, Tokyo, Japan) were used as probes. Probe detection was performed using the Gene Images system.

2.4. PCR detection of IL-12 p40 mRNA

RNA was reverse-transcribed with Superscript reverse transcriptase (Gibco BRL, Rockville, MD, USA) and oligo dT at 42°C for 2 h. The reverse-transcribed samples were then subjected to PCR using Taq polymerase. For IL-12 p40 and hypoxanthine phosphoribosyl transferase (HPRT) mRNA detection, specific oligonucleotide primers were designed as follows: IL-12 p40 forward primer, 5′-AGCTTGGATCCTGAGAACTTC-3′, IL-12 p40 backward primer, 5′-GGTTCACTGTTTCTCCAGGG-3′, HPRT forward primer, 5′-CTCGAAGTGTTGGATACAGG-3′, HPRT backward primer, 5′-TGGCCTATAGGCTCATAGTG-3′. These primers were designed to encompass the intronic sequences, so that any PCR products amplified from genomic DNA contaminating the RNA preparation could be distinguished. The PCR reaction conditions were 95°C for 1 min, 60°C for 2 min, 72°C for 2 min, and 22–26 cycles at 94°C for 1 min, 60°C for 1 min, 72°C for 2 min followed by an extension for 8 min at 72°C. PCR products were separated by electrophoresis on 2% agarose gels.

2.5. Immunohistochemistry

Samples were fixed in periodate–lysine–4% paraformaldehyde (4% PLP) overnight at 4°C and frozen in OCT compound (Tissue-TEK, Miles, Elkhart, IN, USA). Serial sections (6 μm thick) were cut with a cryostat and placed on APS (3-amino-propyltriethoxysilane)-coated slides. Blocking was performed in phosphate-buffered saline (PBS, pH 7.4) containing 1% bovine serum albumin (BSA) for 10 min at room temperature. Samples were washed three times in PBS and then incubated overnight at 4°C with a rat anti-mouse IL-12 (p40/p70) monoclonal antibody, C15.6 (PharMingen, San Diego, CA, USA), at a concentration of 2.5 μg/ml in PBS containing 1% BSA. The slides were washed three times in PBS. Endogenous peroxidase was quenched by incubating the samples in 0.3% H_2O_2/methanol for 10 min at room temperature. The slides were incubated overnight at 4°C with the secondary antibody, F(ab′)2 fragments of anti-rat immunoglobulin coupled to horseradish peroxidase (Amersham Pharmacia Biotech, Bucks, UK). Following subsequent washes in PBS, the color reaction was developed with diaminobenzidine. Slides were counterstained with hematoxylin.

3. Results

3.1. T3^b-IL-12 p40 transgenic mice

Transgenic mice were generated using a transgene that contained the 2.8-kb T3^b promoter, the mouse IL-12 p40 cDNA, and rabbit β-globin genomic sequences from the second exon to the polyA signal (Fig. 1). Six founders carrying this transgene were identified, and their phenotypes were apparently normal. These transgenic mice were backcrossed with C57BL/6 mice, and the transgene was transmitted to the next generation in four founder lines: #9, #13, #20, and #24.

The copy number of the transgene in the mouse genome was estimated by Southern blot analysis. As judged from the hybridizing intensity of the bands corresponding to the transgene, the four transgenic founder lines carried 5–10 copies of the T3^b-IL-12 p40 transgene (data not shown).

3.2. Transgene expression in T3^b-IL-12 p40 transgenic mice

To examine whether the T3^b-IL-12 p40 transgene was expressed in the intestine of the transgenic mice, Northern blot analysis was performed using an IL-12 p40 cDNA probe (Fig. 2). Expression levels of IL-12 p40 mRNA in the large intestine were compared (Fig. 2A). IL-12 p40 mRNA was highly expressed in all four lines, and was especially high in line #13, but was not detectable in their non-transgenic littermates.

Expression of the T3^b-IL-12 p40 transgene in other major

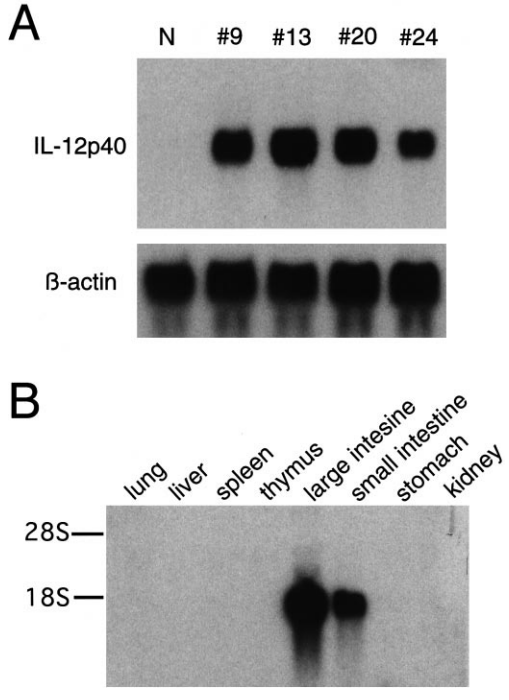

Fig. 2. Expression of IL-12 p40 mRNA in the T3^b-IL-12 p40 transgenic mouse. Total RNAs (10 μg per lane) obtained from tissues of IL-12 p40 transgenic mice and non-transgenic littermates were subjected to Northern blot analysis and probed with IL-12 p40 cDNA or β-actin DNA. A: Expression of IL-12 p40 mRNA in the large intestine of T3^b-IL-12 p40 transgenic mice and non-transgenic littermates from lines #9, #13, #20, and #24. B: Expression of IL-12 p40 mRNA in major organs of a line #20 transgenic mouse.

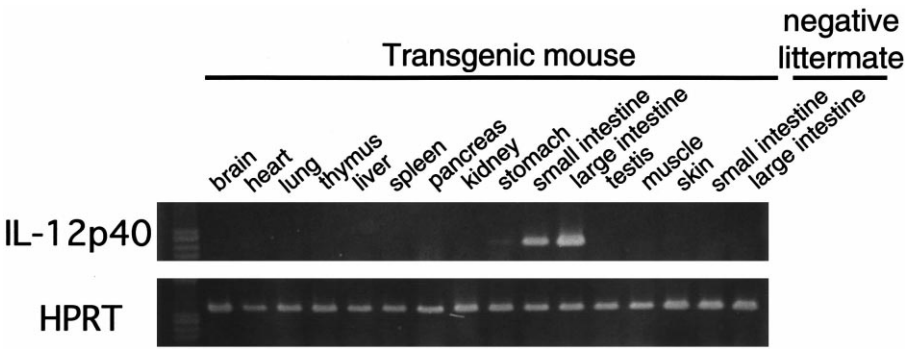

Fig. 3. RT-PCR analysis of IL-12 p40 mRNA in the T3^{b}-IL-12 p40 transgenic mouse. RNA was reverse-transcribed using Superscript reverse transcriptase and an oligo dT primer. PCR was performed to detect IL-12 p40 mRNA (upper panel) and HPRT mRNA (lower panel) using specific primers. PCR products were separated by electrophoresis on 2% agarose gels. The lengths of the expected products were 224 bp for IL-12 p40 and 350 bp for HPRT.

organs was examined in detail using a transgenic mouse from line #20. IL-12 p40 mRNA was detected in both the large and small intestines of the transgenic mouse. Low levels of the transgene transcript were also detected in the stomach, but none was seen in any other organs, including the thymus (Fig. 2B).

For a more detailed and sensitive analysis of the tissue specificity of transgene expression, total RNA was isolated from more organs and tissues of a line #20 transgenic mouse and its non-transgenic littermate and subjected to RT-PCR (Fig. 3). Transgenic IL-12 p40 mRNA was expressed exclusively in the gastrointestinal tract, following this order: large intestine > small intestine > stomach, consistent with the results from the Northern blot analysis. Analysis of a line #13 transgenic mouse by Northern blot and RT-PCR gave almost identical results (data not shown).

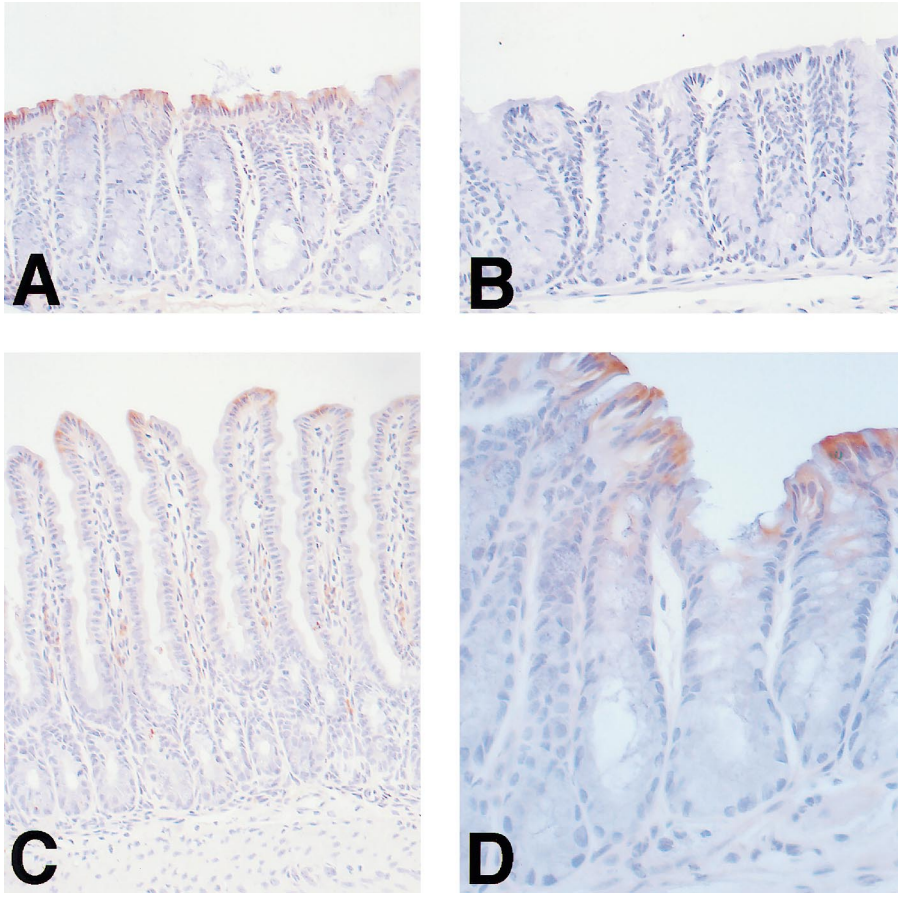

Fig. 4. Immunohistochemical analysis. Sections of organs taken from a transgenic mouse and its non-transgenic littermate (6 μm thick) were incubated with rat anti-mouse IL-12 monoclonal antibody, C15.6, and then incubated with F(ab′)2 fragments of anti-rat immunoglobulin coupled with horseradish peroxidase. Color reactions were developed by diaminobenzidine. Samples were counterstained with hematoxylin. A: Large intestine of the transgenic mouse. B: Large intestine of its non-transgenic littermate. C: Small intestine of the transgenic mouse. Magnification: ×78. D: Large intestine of the transgenic mouse. Magnification: ×156.

3.3. Detection of IL-12 p40 molecule in transgenic mice

To investigate the cellular localization of transgene expression, immunohistochemical analysis of several tissues derived from a #20 transgenic mouse and its non-transgenic littermate were conducted using a monoclonal antibody against IL-12, C15.6 (Fig. 4). The epithelial cells of the large intestine in the transgenic mouse were strongly stained by C15.6 (Fig. 4A). The colonic epithelial cells consist of two types of cells, i.e. absorptive cells and mucus-secreting goblet cells. Close examination of the sections revealed that transgene expression was restricted to mature absorptive cells facing the lumen, and that immature absorptive cells in the crypt and goblet cells failed to express the transgene (Fig. 4D). No significant staining was observed in the epithelial cells of the non-transgenic littermate (Fig. 4B). No staining of the large intestine of the transgenic mouse was seen in controls using the secondary antibody alone (not shown).

Villous epithelial cells of the small intestine in the transgenic mouse were also stained by C15.6 (Fig. 4C). The staining pattern was similar to that of the colonic epithelial cells and significant staining was restricted to mature epithelial cells facing the lumen. Epithelial cells of the stomach were not stained above background (not shown).

4. Discussion

A number of tissue-specific promoters have been used to produce transgenic mice for various purposes; e.g. the preproinsulin promoter [21], α-myosin heavy chain promoter [22], and Clara cell 10-kDa protein (CC10) promoter [23] have been shown to direct transgene expression restricted to pancreatic β cells, heart muscle cells, and lung Clara cells, respectively. Until now, a few promoters that direct intestinal epithelial cell-specific expression in transgenic animals have been reported [24–28]. However, they are not necessarily appropriate for transgenic studies on IBD in terms of activity and tissue specificity; e.g. the fatty acid binding protein promoter drives expression only in the small intestine, but not in the large intestine [24–26]. Here we show that transgene expression under the $T3^b$ promoter was stronger in the epithelium of the large intestine than in the small intestine in our transgenic mice. The transgene expression under the $T3^b$ promoter was largely restricted to the intestinal epithelium. Thus, this promoter should provide a useful tool to study the molecular mechanisms of the pathogenesis of chronic inflammatory enterocolitis and also colorectal cancers. Furthermore, this promoter may be used to express genes exclusively in intestinal epithelial cells when they are introduced via viral vectors for therapeutic purposes.

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