Peroxisome proliferator-activated receptor gamma is induced during differentiation of colon epithelium cells

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Abstract

Peroxisome proliferator-activated receptor γ (PPARγ), a fatty acid-activated nuclear receptor, is implicated in adipocyte differentiation and insulin sensitisation. In view of the association of dietary fat intake and bowel disease, the expression of PPARγ in rodent and human intestine was studied. Expression of PPARγ mRNA was examined by Northern blot hybridisation, RNase protection, and/or competitive RT-PCR assays, whereas PPARγ protein levels were evaluated by immunoblotting and immunohistochemistry. PPARγ mRNA and protein were abundantly expressed in colon relative to the small intestine both in rodents and in man. Interestingly, expression of PPARγ was primarily localised in the more differentiated epithelial cells in the colon. The level of expression of PPARγ in colon was similar to the levels seen in adipose tissue. Expression of PPARγ increased from proximal to distal segments of the colon in man. In Caco-2 and HT-29 human adenocarcinoma cells, PPARγ expression increased upon differentiation, consistent with PPARγ being associated with a differentiated epithelial phenotype. High-level expression of PPARγ was observed in the colon, but not in the small intestine, suggesting a potential role of this nuclear receptor in the colon.

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Introduction

Bowel diseases, including inflammatory and neoplastic bowel disease, are prevalent disorders in Western populations. In fact, colorectal cancer is the second leading cause of death from cancer (Wingo et al. 1995). A remarkable difference in incidence worldwide has led to the attractive hypothesis of an association between dietary fat intake and the incidence of colorectal cancer (Potter & McMichael 1986, De Vries & Van Noorden 1992, Potter 1992). Moreover, recent data demonstrated effects of dietary fat content on intestinal polyp formation and survival in a mouse model of multiple intestinal neoplasia (Wasan et al. 1997). Non-steroidal anti-inflammatory drugs, such as aspirin or indomethacin, have been shown to reduce the frequency of colorectal cancer (Thun et al. 1991, Giovannucci et al. 1995), an effect linked to their ability to inhibit cyclooxygenase activity and to block the production of arachidonic acid-derived prostaglandins. Inflammatory bowel disease also seems to be associated with perturbations in lipid metabolism. In fact, increased levels of prostaglandin E₂ and leukotriene B₄ are found in intestinal mucosa from patients with inflammatory bowel disease and have been suggested to play an important role in its pathogenesis (Sharon & Stenson 1984, Lauritsen et al. 1986). Furthermore, changing the quality of dietary fat has been shown to beneficially influence inflammatory bowel disorders (Lee & Austen 1985, Stenson et al. 1992, Belluzzi et al. 1996).

The link between perturbations in fatty acid and arachidonic acid metabolism with both colorectal cancer and inflammatory bowel disease led us to investigate the expression of the fatty acid-activated transcription factor, peroxisome proliferator-activated receptor γ (PPARγ) in the intestine. PPARγ, a nuclear hormone receptor, has been shown to trigger adipocyte differentiation (Tontonoz et al. 1994) and improve insulin sensitivity (reviewed in Spiegelman & Flier 1996, Schoonjans et al. 1997). A metabolite of arachidonic acid, 15-deoxy-Δ¹₂,1₄-prostaglandin J₂, has been identified as a natural ligand of PPARγ, whereas anti-diabetic thiazolidinediones are synthetic ligands (Forman et al. 1995, Kliwer et al. 1995).
Fatty acids and prostaglandins, present in the lumen of the colon or provided to the intestinal cells through the circulation, could be potential activators for PPARγ (Göttlicher et al. 1993). In this study, we investigated the expression of PPARγ in the intestinal tract of rodents and humans. PPARγ was highly expressed in the colon at levels similar to those found in adipose tissue. These in vivo observations were corroborated by in vitro studies, which demonstrated high-level expression of PPARγ in the human colon cell line, Caco-2. Interestingly, PPARγ levels increased in this cell line with differentiation.

Materials and Methods

Tissues samples

Human ileum and colon biopsies were obtained from untreated patients (n=4; females, mean age 57 ± 10 years) having had a colonoscopy for irritable bowel disease as part of their diagnostic evaluation. Moreover, six endoscopic biopsies per patient were taken systematically from macroscopically normal mucosa at different locations of the colon between caecum and rectum. Mesenteric and subcutaneous white adipose tissues were obtained from patients undergoing elective gallbladder surgery (n=3; females, mean age 49 ± 7). These biopsies were immediately frozen and later used to evaluate expression of PPARγ mRNA by competitive PCR, and PPARγ protein by immunoblot analysis. Subjects gave informed consent and the protocol of the study was approved by the ethics committees of the University Hospital in Lille and the Landeskanken Anstalt of Salzburg. Duodenal, ileal and different parts of colon were systematically obtained from C57BL6/J mice (IFFA-Credo, Arbesle, France). Different parts of colon and epididymal adipose tissues were also obtained from 7-week-old normal Sprague–Dawley rats (IFFA-Credo). Biopsies of the animals were processed for Northern blot, immunoblot, and immuno-histochemical analysis.

Cell culture

The human colon adenocarcinoma cell lines Caco-2 and HT-29 were obtained from ATCC, Rockville, MD, USA. Caco-2 cells were grown in Dulbecco’s modified Eagle medium containing 15% fetal calf serum (v/v), 50 µg/ml streptomycin, 50 µg/ml penicillin and 0·1 mM non-essential amino acids. The cells were subcultured when they were 80% confluent by using 0·5% trypsin and seeded at a density of 5 × 10^4 cells/cm². Differentiation of the Caco-2 cells into enterocytes can be spontaneously obtained in the absence of any inducers by maintaining them at confluence for approximately 20 days (Pinto et al. 1983). HT-29 cells were grown in McCoy’s medium supplemented with 10% fetal calf serum (v/v), 50 µg/ml streptomycin, and 50 µg/ml penicillin. After confluency, HT-29 cells growing in the absence of glucose exhibit an enterocytic differentiation (Zweibaum et al. 1985).

RNA analysis by Northern blot, competitive reverse transcription (RT)-PCR and RNase protection assay

RNA was isolated by the acid guanidinium thiocyanate/phenol/chloroform method (Chomczynski & Sacchi 1987). Northern blot hybridisation with a random primed-labelled cgPPARγ probe (Aperlo et al. 1995) and 36B4 (encoding the human acidic ribosomal phosphoprotein, PO) as a control probe (Masiakowski et al. 1982), was performed exactly as described (Saladin et al. 1995). In human tissue samples, the absolute mRNA concentration of PPARγ was measured from 0·1 µg of total RNA by reverse transcription (RT) reaction followed by competitive PCR (RT–competitive PCR) exactly as described previously (Fajas et al. 1997). After 40 cycles of amplification, results were expressed as amol/µg total RNA. The RNase protection experiments, using an anti-sense riboprobe containing the untranslated exons A1 and A2 of the human PPARγ gene, were performed as described previously (Fajas et al. 1998). Labelled synthetic anti-sense RNA probe (5 × 10^5 c.p.m.) was hybridised with 10 µg of different RNA samples in a total volume of 25 µl for 16 h at 30 °C. Samples were incubated for 1·5 h at the same temperature with a mixture of RNase T1 (2 mg/ml, Boehringer Mannheim, Meylan, France) and RNase A (40 mg/ml, Sigma, St Louis, MO, USA). Nucleases were
then inactivated by adding a mixture of 20% SDS and 20 mg/ml proteinase K during 15 min at 37 °C followed by phenol/chloroform extraction. After precipitation with ethanol, the samples were resuspended in 5 µl formamide loading buffer and resolved on a denaturing 6% polyacrylamide/urea sequencing gel, which was dried after the electrophoresis and exposed to X-ray film.

**SDS-PAGE and immunoblotting analysis**

Cells were homogenised in a cell lysis buffer consisting of PBS with 1% Triton X-100 (Sigma) to which a freshly prepared protease inhibitor cocktail was added consisting of 40 µg/ml 4-(2-aminoethyl)-benzene sulfonylfluoride (AEBSF), 5 mg/ml EDTA-Na2, 1 µg/ml leupeptin, 1 µg/ml pepstatin (ICN, Orsay, France). Tissues were homogenised with a polytron in extraction buffer consisting of PBS with 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS (Sigma) and the fresh protease inhibitor cocktail described above. Protein extracts were obtained after centrifugation of the lysate at 13 000 g at 4 °C and protein concentrations were determined at 595 nm with the Biorad protein colorimetric assay system (Biorad, Ivry-sur-Seine, France). Ten micrograms of each total protein lysate were separated on a 12% polyacrylamide gel according to Laemmli (1970), and proteins were transferred to nitrocellulose filters as described by Towbin et al. (1979). The membrane was blocked for 2 h at 21 °C in blocking buffer (20 mM Tris, 100 mM NaCl, 1% Tween-20, 5% skimmed milk). Next, the filter was incubated overnight at 4 °C with a 1:500 dilution of rabbit IgG anti-human PPARγ (0.5 mg/ml), developed against an N-terminal human PPARγ peptide (amino acids 20–104). The secondary antibody, a horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (Pasteur Diagnostic Sanofi, Marnes la Coquette, France) diluted at 1:5000, was incubated with the filter for 1 h at 21 °C. The detection of the reaction was done by enhanced chemiluminescence (ECL) according to the manufacturer’s protocol (Amersham, Les Ulis, France).

**Immunohistochemistry and immunocytochemistry**

Colon tissue from normal Sprague–Dawley rats was prepared as follows. The vasculature of a deeply anaesthetised animal was cleared by perfusion with PBS followed by periodate-lysine–paraformaldehyde (PLP) fixative (McLean & Nakane 1974). The colon was removed, a section from the middle third was harvested and fixed a further 6 h at 4 °C in fresh PLP fixative. The fixed tissue was then cryoprotected by incubation for 4 h in 10% sucrose/0.1 M phosphate buffer pH 7.3 and frozen in OTC media in cryomoulds prior to storage at −70 °C. Cryosections (6 µm) were cut on a Reichert–Jung

**Figure 2** Immunolocalisation of PPARγ in normal rat colon. (A) Immunohistochemical analysis of PPARγ in rat cryosections. Note the reaction product seen within the nuclei of the epithelial cells (arrowheads) and in the monocytic cells of the lamina propria (arrows). (B) Control rat section in which the primary antibody was incubated with the section in the presence of competing free peptide of the same sequence as that used to immunise the original rabbit. In this example the specific labelling is entirely eliminated and only a faint outline of the tissue is visible. Original magnification × 630.
Cryocut 1800 cryostat. Immunohistochemical labelling was performed using the Vector ABC labelling protocol as follows (Vector Laboratories Inc., Burlingame, CA, USA). Sections were pre-treated with 3% hydrogen peroxide in methanol for 20 min to inactivate endogenous peroxidases, followed by permeabilisation in 0·1% Triton X-100 in PBS at 4°C for 5 min. Putative biotin and avidin binding sites within the sections were blocked with biotin and avidin solutions as specified in the Vector blocking kit. To further reduce the possibility of non-specific antibody binding, the sections were then incubated with 1·5% normal donkey serum followed by incubation with a solution containing 5% non-fat dry milk and 0·1% BSA. Specific labelling was then carried out by incubation with 5 µg/ml IgG isolated from a rabbit immunised with an 18 amino acid fragment of PPARγ (amino acids 116–134; J Auwerx, unpublished observations). In order to demonstrate specificity, sequential sections were incubated with immune IgG in the presence of 20 µg/ml of immunising peptide or in the presence of non-immune IgG. Bound antibodies were visualised by incubation first with biotinylated donkey F(ab')2 anti-rabbit IgG, followed by incubation with avidin–biotin–HRP complexes prepared from the Vector Elite immunoperoxidase kit. Peroxidase reaction product was developed using the glucose oxidase/diaminobenzidine (DAB)/nickel method (Shu & Fan 1988). Digital micrographs were taken using a Kodak Megaplex camera mounted on a Zeiss Axiophot microscope using a ×40 objective.

Mouse colon tissues were fixed in 4% paraformaldehyde acid (PFA) at 4°C overnight, dehydrated in alcohol and embedded in paraffin. Five-micron sections were then deparaffined with xylene and rehydrated by ethanol treatment. Caco-2 cells were fixed in 3% PFA/2% sucrose for 15 min at 21°C and permeabilised in cold methanol/acetone (v/v) for 5 min at 21°C. Immunohistochemistry was performed with the peroxidase-anti-peroxidase universal DAKO Stain Kit according to the manufacturer’s protocol (DAKO, Copenhagen, Denmark) and using a rabbit IgG anti-human PPARγ (Fajas et al. 1997) diluted 1:50. Negative controls were incubated with preimmune serum.

Figure 3 Immunolocalisation of PPARγ in normal mouse colon. (A and B) Immunohistochemical analysis of PPARγ in paraffin sections of mouse colon. Note the intense brown coloration in nucleus and cytoplasm of the epithelial cells (arrows) and in smooth muscle cells (arrowhead). Nuclei were counterstained with haematoxylin. Original magnification × 630.
Results

Expression and immunohistochemical localisation of PPARγ in rodent intestine

To study the expression of PPARγ in mouse intestine, we analysed the presence of the mRNA in various parts of the mouse gastrointestinal tract by Northern blot. A single 1.8 kb mRNA species corresponding to PPARγ mRNA was present in the entire intestine, but the colon showed at least a 10-fold higher expression level relative to other parts of the intestine (Fig. 1A). Next, we asked whether the PPARγ mRNA expression correlated with PPARγ protein expression across the gastrointestinal tract. Therefore, a rabbit anti-PPARγ polyclonal antibody (Fajas et al. 1997) was used in a Western blot experiment to analyse PPARγ protein expression in the same intestinal fragments as used for RNA extraction (Fig. 1B). Immunoblotting experiments showed the presence of a 50 kDa band corresponding to PPARγ only in the colon. To localise PPARγ expression in the colon, immunohistochemistry was performed on serial cryosections of the rat colon. PPARγ was expressed in epithelial cells facing the colonic lumen (Fig. 2A). Immunolocalisation of PPARγ in normal rat colon seen within the nuclei of the epithelial cells (arrowheads, Fig. 2A) and in the monocytic cells of the lamina propria (arrows, Fig. 2A). In the control section the specific labelling was entirely eliminated and only a faint outline of the tissue was visible (Fig. 2B). Lower levels of PPARγ were observed in the muscularis mucosae, submucosal and muscular layers (data not shown). PPARγ expression was also studied in paraffin sections of normal mouse colon (Fig. 3A and B). Similar to in the rat, PPARγ was abundantly expressed in epithelial cells (arrows) and to a lower level in the muscular layer of the mouse colon (arrowhead). The expression of PPARγ could be observed in both the nucleus and cytoplasm of epithelial cells.

Quantification of PPARγ mRNA and protein in human colon

To verify whether PPARγ was also present in the human gastrointestinal tract, ileum and colon biopsies were collected from four normal subjects. PPARγ protein expression was analysed in human ileum and colon by Western blotting. Similar to findings in rodents, PPARγ expression was more pronounced in colon (Fig. 4A, lanes 5–8) in comparison with ileum (lanes 1–4). Multiple biopsies were taken at different locations throughout the colon. Total RNA was extracted and used to quantify PPARγ mRNA by competitive RT-PCR. High-level expression of PPARγ was demonstrated in the entire colon, but the expression was consistently higher in the distal colon (up
to 30 amol/µg total RNA) relative to the proximal colon and caecum (10 amol/µg total RNA) (Fig. 4B). In addition, proteins were extracted from the same biopsies for immunodetection of PPARγ by Western blotting. PPARγ protein is present throughout the entire colon, from caecum to rectum, but its expression appears somewhat less pronounced in the caecum (Fig. 4B, inset).

Comparison of PPARγ expression in colon and adipose tissue in rat and human

The relative levels of PPARγ mRNA and protein expression were then analysed in adipose tissue and colon. We compared the level of expression of PPARγ mRNA in colon and adipose tissue of normal rats by Northern blot hybridisation (Fig. 5A). Expression in rat colon epithelium (lanes 1 and 2) was at the same level as the expression of PPARγ mRNA in rat epididymal adipose tissue (lanes 3 and 4). To extend these results to humans, PPARγ protein expression was analysed by immunoblotting in human colon and adipose tissue (Fig. 5B). PPARγ protein was present at comparable levels in human colon (lane 1), human subcutaneous (SC. AT) (lane 2) and human mesenteric (VISC. AT) (lane 3) adipose tissue biopsies were separated on 10% SDS-PAGE and blotted onto a nitrocellulose filter. Immunodetection of PPARγ protein was performed with a rabbit anti-PPARγ antibody and visualised with the ECL system.

Expression and cytolocalisation of PPARγ in human colon adenocarcinoma cells

In order to establish an in vitro model for the study of the role of PPARγ in colonic function and metabolism, we investigated the expression of PPARγ in the human Caco-2 cell line, which was derived from an adenocarcinoma of human colon. This cell line was shown to be an appropriate model of enterocytic differentiation, since it can undergo spontaneous differentiation when maintained confluent for extended periods (Pinto et al. 1983). Caco-2 cells were differentiated during 20 days. Total RNA was isolated at different times of the differentiation process (Fig. 6A, lanes 2–7) and was analysed by RNase protection assay, using an anti-sense riboprobe containing the untranslated exons A1 and A2 of the human PPARγ gene, allowing us to measure the mRNA expression level of PPARγ1 and a newly described PPARγ isoform, PPARγ3 (Fig. 6A) (Fajas et al. 1998). Both PPARγ1 and γ3 give rise to an identical PPARγ1 protein product (Fajas et al. 1998). Whereas a slight induction of the PPARγ1 mRNA levels could be observed, PPARγ3 mRNA was induced approximately 2·5-fold upon differentiation of the Caco-2 cells (Fig. 6B). Western blot analysis showed that PPARγ was poorly expressed in undifferentiated cells (lanes 1–3) compared with differentiated Caco-2 cells (lanes 4–6), where PPARγ protein was expressed at a high level (Fig. 6C). Along the same lines, the differentiation of another human adenocarcinoma cell line, HT-29, is associated with an induction of PPARγ protein level (Fig. 6C, lanes 4–6). This result suggests that the up-regulation of PPARγ protein expression is at least in part a consequence of the increased transcription of the PPARγ gene, although a post-transcriptional effect on mRNA stability cannot be excluded. Cytolocalisation of PPARγ
was performed both in undifferentiated and differentiated Caco-2 cells (Fig. 7). PPARγ protein seems highly enriched in the differentiated cell nucleus. The special morphology of the differentiated cells, with numerous tightly packed cells in which the cytoplasm is not easily distinguishable and which have a tendency to pile up in domes, does not allow, however, a clear demonstration of whether PPARγ protein is also present in the cytoplasm.

Discussion

PPARs are fatty acid-activated transcription factors belonging to the nuclear receptor superfamily. PPARγ, a subtype of the PPAR family, is predominantly expressed in adipose tissue, where it controls critical steps of lipid homeostasis and functions as a key trigger of adipocyte differentiation (reviewed in Auwerx et al. 1996). Two others subtypes of PPAR, PPARα and δ have been identified. PPARα, the predominant subtype expressed in liver, plays an important role in hepatic fatty acid β-oxidation and its expression has been detected in rodent small intestine (Mansen et al. 1996). Also, PPARγ was reported to be expressed in the gastrointestinal tract in rodents (Braissant et al. 1995). From these studies it is unclear, however, whether it is preferentially expressed in small intestine (Braissant et al. 1995) or colon (Mansen et al. 1996). We recently described the predominant expression of PPARγ in the human adenocarcinoma cell line Caco-2. (A) Total RNA from Caco-2 cells at different times of the differentiation process were analysed by RNase protection assay (lanes 2–7). The RNase protection experiments were done using an anti-sense riboprobe containing the untranslated exons A1 and A2 of the human PPARγ gene (lane 1: control probe). (B) The autoradiograph shown in panel (A) was scanned densitometrically and the RNA values were expressed in relative absorbance units (RAU) taking the undifferentiated control values as 100%. (C) Proteins from Caco-2 and HT-29 cell lysates were separated by SDS-PAGE and transferred onto nitrocellulose filters. Immunodetection was performed with rabbit anti-PPARγ antibodies and visualised with the ECL system. The first three lanes (1–3) were loaded with 10 μg protein obtained from the non-differentiated Caco-2 or HT-29 cells, the last three lanes (4–6) were loaded with 10 μg protein from differentiated-Caco-2 or HT-29 cells.
Figure 7  Immunocytochemical analysis of PPARγ expression in Caco-2 cells. Immunolabelling was performed in non-differentiated Caco-2 cells (A) (NDi; non-differentiated) and in differentiated Caco-2 cells (B and C). Negative controls were performed for differentiated Caco-2 cells by incubation with preimmune serum (B) (PI; preimmune serum). Positive cells were visualised with brown DAB (C) (Di). Original magnification × 400.
expression of PPARγ in human colon by competitive RT-PCR (Auboeuf et al. 1997, Fajas et al. 1997). Although PPARα and δ are also expressed in the human gastrointestinal tract, their level of expression is much lower than that of PPARγ (Auboeuf et al. 1997).

In this more detailed study on PPARγ expression, high levels of PPARγ mRNA and protein were detected in rodent and human colon. Both in rodents and in man, PPARγ protein is more expressed in colon relative to the other parts of the intestinal tract. The expression of PPARγ in human colon had a tendency to increase in the distal segments of the colon relative to the proximal colon. By immunohistochemistry of the mouse and rat colon, PPARγ expression was confined to differentiated epithelial cells, mononuclear cells, and some cells in the muscular layer. We also detected PPARγ in human adenocarcinoma colon cell lines Caco-2 and HT-29. Expression of PPARγ protein increased with differentiation of these cells, consistent with its expression in differentiated epithelial cells in rat and mouse colon. The results in the present study confirm and extend previous reports (Mansen et al. 1996, Auboeuf et al. 1997) showing a predominant expression of PPARγ in colon, with PPARα preferentially expressed in the small intestine. These observations, as well as our current results, are, however, not consistent with in situ hybridisation results reported by Braissant et al. (1995) who describe a decrease of PPARγ expression from jejunum to colon in the rat intestinal tract.

Our data show high levels of PPARγ expression in colon, comparable to the expression in the adipose tissue, which was previously considered to be the main site of PPARγ expression. Contamination of colon samples with adipose tissue cannot account for the high levels of PPARγ observed in the colon, since colon samples obtained in mice were carefully dissected and all fat was carefully removed. In addition, rehybridisation of the Northern blot with a leptin cDNA (Zhang et al. 1996) did not reveal any signal, excluding adipose tissue contamination (data not shown). Furthermore, human biopsies cannot be contaminated with visceral adipose tissue because only mucosa was harvested.

Several clinical reports have noted the importance of dietary fat content and fatty acid-derived products, such as prostaglandins, in relation to diseases of the colon (Lee & Austen 1985, Potter & McMichael 1986, de Vries & van Noorden 1992, Potter 1992, Stenson et al. 1992, Belluzzi et al. 1996, Wasan et al. 1997). Recent studies have shown that the arachidonic acid metabolite 15-deoxy-Δ12,14-PGJ2 is a natural ligand of PPARγ (Forman et al. 1995, Kliever et al. 1995), suggesting that some effects of dietary fat in the colon might be mediated by changing the quantity or quality of fatty acids, which are precursors of PPARγ ligands. The relevance of dietary fat content to diseases of the colon, as well as the results of the present study, which demonstrates high-level expression of PPARγ in differentiated epithelial cells of the colon, warrants a careful evaluation of the involvement of PPARγ in colon pathology. The conservation of PPARγ expression in the colon in several species (man and rodents), as well as differentiation-dependent expression in Caco-2 and HT-29 cells, provides both in vivo and in vitro models for such further exploration.

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References


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