Developmental regulation of the catalytic subunit of the apolipoprotein B mRNA editing enzyme (APOBEC-1) in human small intestine

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Abstract Apolipoprotein (apo) B mRNA editing is a site-specific cytidine deamination reaction responsible for the production of apoB-48 in mammalian small intestine. This process is mediated by an enzyme complex that includes the catalytic subunit, APOBEC-1. In the present study, it is shown that the developmental regulation of apoB mRNA editing in fetal human small intestine is closely mirrored by accumulation of APOBEC-1 mRNA. Similar results were obtained using Caco-2 cells, the data further suggesting that culture of these cells under conditions previously shown to promote differentiation produce an earlier and more marked induction of APOBEC-1 mRNA abundance. Complementary analysis of APOBEC-1 protein accumulation using immunocytochemical localization reveals its appearance to be temporally coordinated with the accumulation of APOBEC-1 mRNA and its distribution to be confined to villus-associated enterocytes. Previous studies demonstrated a close temporal association between the development of triglyceride synthesis and apoB mRNA editing in the rat liver and small intestine. Analysis of fatty acid CoA ligase, monoacylglycerol acyltransferase, and diacylglycerol acyltransferase activity in preparations of human liver and small intestine demonstrates activity of all three enzymes in the late first and early second trimester, suggesting that certain aspects of complex lipid biosynthesis in the human fetal small intestine and liver are regulated developmentally. The cues that modulate the post-transcriptional regulation of fetal human small intestinal apoB gene expression may thus include both temporal programming and events related to the emergence of lipid transport capability.—**Giannoni, F., S-C. Chou, S. F. Skarosi, M. S. Verp, F. J. Field, R. A. Coleman, and N. O. Davidson.** Developmental regulation of the catalytic subunit of the apolipoprotein B mRNA editing enzyme (APOBEC-1) in human small intestine. *J. Lipid Res.* 1995. **36:** 1664–1675.

Supplementary key words triglyceride synthesis · lipoprotein assembly

Apolipoprotein B (apoB) is an essential structural component of triglyceride-rich lipoproteins secreted by the liver and small intestine that circulates in two distinct forms, apoB-100 and B-48 (1). Human liver synthesizes and secretes apoB-100, a protein containing 4536 residues that circulates in association with very low (VLDL), intermediate (IDL), and low density lipoproteins (LDL). Mammalian small intestine secretes apoB-48, a truncated form of the protein that is colinear with the amino terminal 2152 residues of apoB-100 (2, 3). Tissue-specific production of apoB-48 is the result of posttranscriptional editing of the nuclear apoB mRNA whereby a CAA codon, specifying glutamine in apoB-100 mRNA, undergoes site-specific cytidine deamination to produce a UAA stop codon (2, 3). This modification has a significant impact upon the catabolism of apoB-containing lipoproteins because apoB-48 lacks the domains, present in apoB-100, that are responsible for binding of LDL to the LDL receptor (4). ApoB mRNA editing thus imposes an additional level of regulation of gene expression with profound consequences for both the assembly and catabolism of this protein.

ApoB mRNA editing is mediated by an enzyme or enzyme complex (reviewed in ref. 5) of which the catalytic subunit, referred to as APOBEC-1 (apolipoprotein B mRNA editing enzyme, catalytic polypeptide #1), was

Abbreviations: apoB, apolipoprotein B; APOBEC-1, apolipoprotein B mRNA editing enzyme, catalytic polypeptide #1; LDL, low density lipoprotein; FA CoA ligase, fatty acid CoA ligase; MGAT, monoacylglycerol acyltransferase; DGAT, diacylglycerol acyltransferase; RT-PCR, reverse transcription–polymerase chain reaction.

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originally cloned from rat small intestine (6). APOBEC-1 mRNA appears to be ubiquitously expressed in the rat and demonstrates both tissue-specific and developmental regulation (7). Additionally, APOBEC-1 mRNA abundance is regulated in the rat liver in response to nutritional modulation, for example, in the fasting-refeeding model (7). These findings, considered in the context of previous work showing that apoB mRNA editing is regulated in the rat liver in response to dietary, nutritional, and hormonal manipulations, suggest that APOBEC-1 gene expression may be a critical determinant in the regulation of apoB mRNA editing (7, 8). Homologous gene products to rat APOBEC-1 have recently been cloned from human and rabbit small intestine and, in contrast to the distribution pattern found in the rat, demonstrate a restricted pattern of expression with confinement essentially to the small intestine and colon (9–11). There is no information, however, concerning the regulation of APOBEC-1 gene expression in the human small intestine and its potential role in the regulation of intestinal apoB mRNA editing.

Earlier studies demonstrated that apoB mRNA editing is developmentally regulated in the human small intestine with a progressive increase between 11 and 20 weeks of fetal gestation (12, 13). Additionally, a variable increase in the extent of apoB mRNA editing was noted in Caco-2 cells studied over the course of differentiation, suggesting that this colon carcinoma-derived cell line may be a useful model in which to examine aspects of the developmental regulation of apoB mRNA editing (14–16). By contrast, more recent studies, using adult human small intestinal RNA, demonstrated that apoB mRNA editing is not responsive to alterations in lipid flux (17). These findings are consistent with results from studies in postconfluent, differentiated Caco-2 cells exposed to various preparations of micellar lipid in which apoB mRNA editing was unchanged from samples incubated in control media (16). Taken together, these results imply that apoB mRNA editing is regulated developmentally but, once expressed at its maximal level, is not regulated in response to exogenous lipid flux.

The present study reports the results of an examination of the developmental regulation of APOBEC-1 and apoB mRNA editing in both human fetal small intestine and in the Caco-2 cell line. The studies used a quantitative reverse transcription-polymerase chain reaction (RT-PCR) approach for mRNA quantitation coupled with immunocytochemical detection to demonstrate the developmental appearance of this gene product in the human small intestine. In addition, assays were performed to determine the relative specific activity of enzymes controlling several key steps in triglyceride synthesis in both the developing liver and small intestine.

MATERIALS AND METHODS

Tissue procurement and processing

Fetal tissue was obtained from normal elective or spontaneous pregnancy terminations. Fetal heart sounds were confirmed by ultrasound and no tissue was harvested from cases associated with intrauterine death or known fetal abnormality. Fetal age was determined by ultrasound measurements of biparietal dimeter and femur length and confirmed where possible by independent reference to the last menstrual cycle. Adult tissue was obtained either from the National Disease Research Interchange tissue procurement services or during the course of surgical intestinal resection. The use of such tissues was approved by the Institutional Review Board of the University of Chicago Hospitals. Tissue samples were snap-frozen within 30 min and stored at −80°C prior to RNA extraction (12). For determination of enzyme activity relating to triglyceride synthesis, tissues were placed in ice-cold medium I (0.25 M sucrose, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4) and homogenized with 10 up and down strokes of a motor-driven Teflon-glass homogenizer. The homogenate was centrifuged at 100,000 *g*, protein concentration was determined and aliquots of the total particulate preparation were stored at −70°C until use.

Cell culture

Caco-2 cells, passage number 34 to 57, were grown on either plastic tissue culture flasks or polycarbonate filters (Transwell, Costar) that, when indicated in the figure legends, were coated with Matrigel as recommended by the manufacturer (Collaborative Research). Total RNA was extracted by lysing the cells directly in 4 M guanidinium thiocyanate or after 2 h incubation with 50 U/ml dispase (Collaborative Research) in the case of cells plated on Matrigel.

Quantitation of APOBEC-1 mRNA abundance by reverse transcription–polymerase chain reaction

This was conducted as previously validated (16), with minor modifications. Aliquots of total RNA (5–20 μg) from the indicated source were treated with 0.5 units of DNase RQ1 (Promega, WI) at 37°C for 45 min in 50 μl 40 mM Tris-HCl pH 7.5, 6 mM $MgCl_2$, 10 mM NaCl, 20 U RNAse inhibitor (RNAsin, Promega). The RNA was then sequentially extracted with phenol–chloroform and chloroform, precipitated with ethanol, washed once in 70% ethanol and resuspended in water just prior to use. RNA concentration was then redetermined by absorbance at 260 nm. Five hundred ng total RNA from human intestine or Caco-2 cells was used for RT-PCR. Reverse transcription was performed at 65°C for 15 min in a final volume of 20 μl RT buffer, containing 10 mM

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Tris-HCl, pH 8.3, 90 mM KCl, 1 mM $MnCl_2$, 200 mM each dNTP, 60 pmol reverse primer, 5 U rTth (# N808-0097, Perkin-Elmer-Cetus, CT), and the reaction was covered with 80 µl mineral oil. After reverse transcription, 80 µl of PCR buffer was added to each tube, containing 8 µl chelating buffer (50% v/v glycerol, 100 mM Tris-HCl, pH 8.3, 1 M KCl, 7.5 mM EGTA, 0.5% Tween 20), 6 µl 25 mM $MgCl_2$ (1.5 mM final concentration) 60 pmol forward primer in 66 µl of water together with 0.3 µl [α-^{32}P]dCTP (3000 Ci/mmol, 10 mCi/ml, DuPont). After 3 min at 95°C, PCR was performed for the established number of cycles in a Perkin-Elmer thermocycler as follows: 30 sec at 95°C, 1 min at 55°C, 1.5 min at 72°C. A final 10-min extension was added after the last cycle. For each RNA sample, an internal mRNA was examined as a housekeeping control, either GAPDH for human tissue samples or β_2-microglobulin (β_2m) for Caco-2 cells. These internal standards were reverse transcribed and amplified in identical but separate tubes. Additionally, independent negative controls for all mRNAs were included to exclude DNA contamination, by using rTth and leaving the tubes on ice during reverse transcription. Fifteen µl of the reaction material was analyzed by 2% agarose–1% NuSieve (FMC Corporation) gel electrophoresis and bands of the expected size, corresponding to APOBEC-1, GAPDH, or β_2m PCR products, excised, and subjected to liquid scintillation counting (Packard 1500 LS, Downers Grove, IL). Negative controls were run in parallel and an equivalent sized band was excised and counted to check for DNA contamination. Primers for APOBEC-1, GAPDH, and β_2m were chosen to flank introns, giving products of 373, 453, and 201 base pairs, respectively. Nevertheless, due to the presence of pseudogenes in GAPDH (18), DNase digestion was necessary to avoid the presence of comigrating bands. Preliminary studies demonstrated that β_2m mRNA abundance is developmentally regulated in the human small intestine, necessitating the alternate choice of GAPDH for use as an invariant housekeeping control (data not shown). Under the conditions detailed above for RT-PCR, 22 and 28 cycles, respectively, represent the mid-point of the linear portion of the exponential phase of GAPDH and APOBEC-1 cDNA amplification from human small intestinal RNA (**Fig. 1A**). Similarly, 30 cycles represents the mid-point of linearity for APOBEC-1 and 20 cycles for β_2m cDNA amplification from Caco-2 RNA (Fig. 1B).

Immunocytochemical detection of APOBEC-1

An antipeptide antibody raised against residues 17–36 (RIEPwEFnVFyDPRELRKEa) of the rat apoB mRNA editing protein (Research Genetics, AL) containing four differences from the human sequence, shown in lower case, was used to localize APOBEC-1 in human small intestine. Five µM sections were prepared on Vectabond-coated slides and treated with 1% hydrogen peroxide–methanol at room temperature for 15 min (7). The slides were blocked in 5% normal goat serum/3%

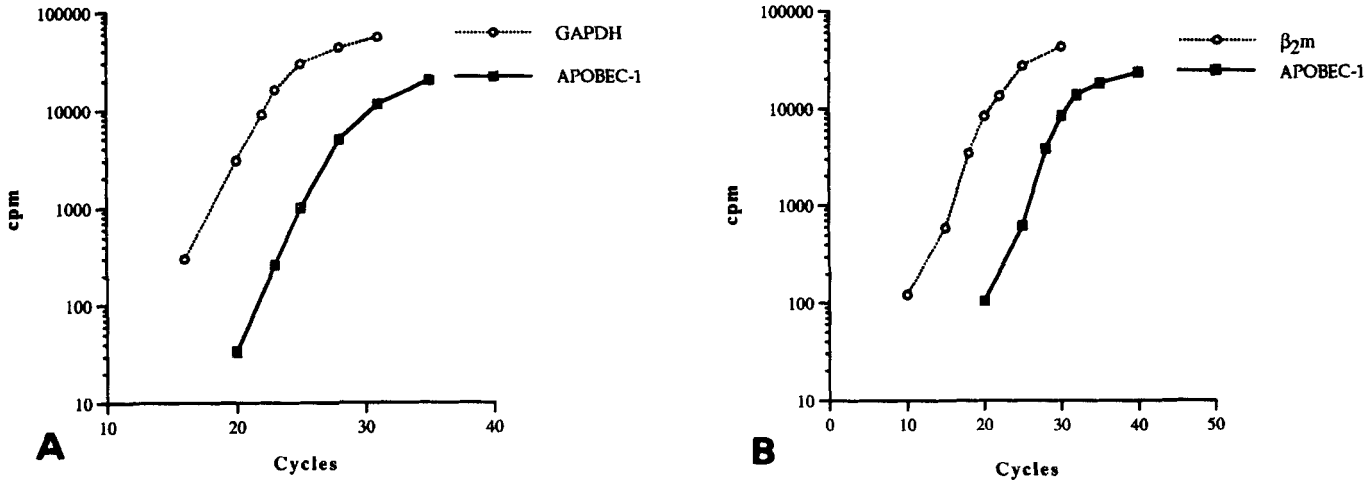

Fig. 1. Quantitation of APOBEC-1 mRNA levels in human fetal small intestine and in Caco-2 cells. A: 500 ng total RNA from a 23 weeks gestational age human fetal small intestine was subjected to RT-PCR in the presence of [^{32}P]dCTP. APOBEC-1 and GAPDH were quantitated in separate tubes over an increasing number of cycles. Aliquots of the amplification reactions were run on a 2% agarose –1% NuSieve gel and bands of the expected size were excised and counted in scintillation fluid. Each value represents the mean of triplicate observations. Parallel and exponential amplification is demonstrated over a wide range of cycle number. B: 500 ng total RNA from 21 day postconfluent Caco-2 cells (38% UAA) was used to reverse transcribe and PCR amplify APOBEC-1 and β_2 microglobulin in separate tubes with increasing number of cycles. Bands of the expected size were excised from 2% agarose–1% NuSieve gels and counted in scintillation fluid. The amplification of a representative experiment is shown together with the graph of the mean values from triplicate experiments. The amplification is exponential and parallel over a wide range of cycle number.

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bovine serum albumin (BSA) and reacted with anti-APOBEC-1 IgG (38 μg/ml final concentration) in PBS–0.3% Triton–1% BSA (30 min each), both at room temperature. Control incubations contained peptide-absorbed anti-IgG. After washing, the slides were incubated with biotinylated goat anti-rabbit IgG and avidin-conjugated horseradish peroxidase (Vectastain Elite, Vector Labs, Burlingame, CA), prior to color development and light counterstaining with hematoxylin as previously described (7).

Enzyme assays

sn-2-Monooleylglycerol, *sn*-1,2-dioleylglycerol, and phospholipids were purchased from Serdary Research Labs, Inc.; other reagents were purchased from Sigma. Monoacylglycerol acyltransferase (MGAT) activity was assayed with 2–8 μg of total particulate protein, 25 μM [^{3}H]palmitoyl-CoA and 50 μM *sn*-2-monooleylglycerol (19–22). An aliquot of the reaction product was chromatographed on silica gel G plates with heptane–isopropyl ether–acetic acid 60:40:4 (v:v:v). MGAT specific activity was calculated as previously detailed (20–22). DGAT activity was measured as previously described using 2–8 μg total particulate protein, 30 μM [^{3}H]palmitoyl-CoA and 200 μM *sn*-1,2-dioleoylglycerol (20, 21). Fatty acid CoA ligase activity was assayed using 10–20 μg of total particulate protein, 50 μM [^{3}H]palmitate (New England Nuclear), 1 mM ATP, and 100 μM CoA (20). All assays were performed at 23°C under optimal substrate concentrations and measured the initial rates of reaction in each tissue.

Oligonucleotides

APOBEC-1 forward primer, HE1-F (5′-GAGAACTTCGTAAAGAGGCC-3′, 5′ at 89*) APOBEC-1 reverse primer HE2-R (5′-CTCCAGCAGTGATAATACTC-3′, 5′ at 461*) *considered from the first codon. β_2m/F (5′-GTGGAGCATTCAGACTTGTCTTTCAGCA-3′, 5′ at 1477) β_2m/R (5′-TTCACTCAATCCAAATGCGGCATCTTC-3′, 5′ at 3537) GAPDH-F2 (5′-ACCACAGTCCATGCCATCAC-3′, 5′ at 520) GAPDH-R2 (5′-GTCCACCACCCTGTTGCTGTA-3′, 5′ at 972).

RESULTS

Developmental regulation of APOBEC-1 gene expression in human small intestine and in Caco-2 cells

Figure 2A shows a representative RT-PCR amplification from total RNA in fetal and adult small intestine. APOBEC-1 mRNA is detectable as early as 11 weeks of gestation, increasing in relative abundance during the second trimester. Maximal abundance of APOBEC-1 mRNA, however, is not achieved until postnatal life (Fig. 2A, B). Owing to the limited availability of tissue from neonatal and early childhood donors, the precise timing of this postnatal increase remains unknown. A more extensive analysis of timed fetal intestinal samples, analyzed in triplicate, reveals a trend of increased APOBEC-1 mRNA abundance up to 17 weeks gestation, followed by a variable but generally reduced abundance in the later gestational aged samples studied. The source of this variability is unknown but is unlikely to represent an artefact of processing full thickness intestinal samples (as opposed to mucosal isolates) as all the adult intestinal RNA samples were prepared similarly and included muscularis and serosal layers. Furthermore, mRNA abundance for GAPDH was comparable between the fetal and adult small intestinal samples (Fig. 2A). ApoB mRNA editing was determined in the same samples by RT-PCR and primer extension assay (16). Endogenous apoB mRNA editing was detectable at 11 weeks of gestation, in parallel with the appearance of APOBEC-1 mRNA and gradually increased with time as previously demonstrated (12, 13). A significant correlation (Fig. 2C) was found between APOBEC-1 mRNA relative abundance and apoB mRNA editing in fetal and adult small intestinal samples, suggesting its importance in this process.

Previous studies demonstrated that the extent of apoB mRNA editing increases in Caco-2 cells in association with cell differentiation (14–16). APOBEC-1 mRNA was quantitated in cells grown on plastic and isolated at different time points. APOBEC-1 mRNA was barely detectable in preconfluent, undifferentiated cells (up to day 4 post plating) with maximal expression achieved soon after confluence (day 8) and little change thereafter, up to 21 days postconfluence (**Fig. 3A**). By contrast, cells grown on either uncoated semipermeable filters or filters coated with matrigel, revealed detectable APOBEC-1 mRNA at day 2, prior to the onset of endogenous apoB mRNA editing. APOBEC-1 mRNA abundance further increased with cell differentiation in cells maintained on semipermeable filters, in parallel with increases in apoB mRNA editing. These data imply that factors associated with the state of cell confluence and differentiation act in concert to regulate apoB mRNA editing in Caco-2 cells and that this regulation is at least partially accounted for by an increase in APOBEC-1 mRNA abundance. Examination of APOBEC-1 mRNA abundance in relation to the extent of apoB mRNA editing (%UAA) in 29 separate preparations from pre- and postconfluent Caco-2 cells demonstrates a significant, positive correlation (0.588, $P < 0.0003$). However, the substantial variability in apoB mRNA editing noted previously, coupled with the observation above concerning the continued increase in apoB mRNA editing in

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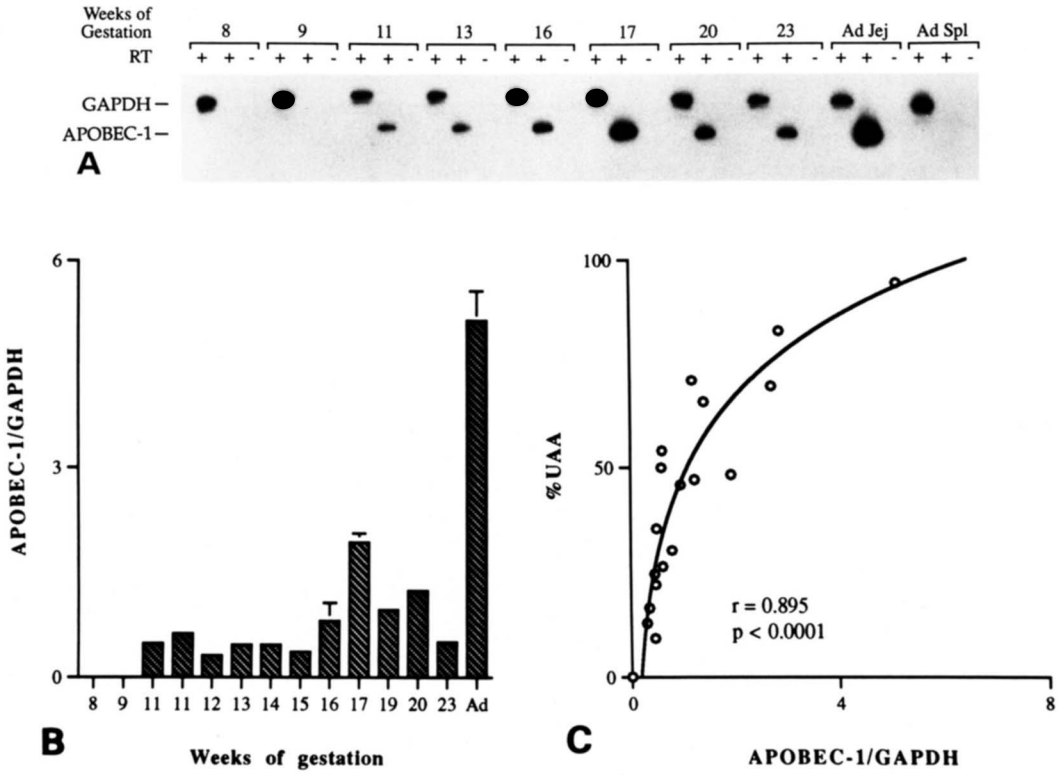

Fig. 2. APOBEC-1 mRNA quantitation in human fetal small intestine. A: 500 ng total fetal small intestinal RNA from 8, 9, 11, 13, 16, 17, 20, 23 weeks of gestation, adult jejunum (Ad. Jej) and spleen (Ad. Spl) was reverse transcribed (RT+) and PCR amplified for APOBEC-1 (28 cycles) and GAPDH (22 cycles) in separate tubes. Approximately 3 μl of the reaction mix was run on a 6% nondenaturing polyacrylamide gel and then subjected to autoradiography. Independent negative controls without reverse transcription (RT–) were combined and run in a single lane to demonstrate the absence of DNA contamination. The corresponding products of APOBEC-1 and GAPDH are shown. B: 500 ng fetal intestinal RNA from 8 to 23 weeks of gestation and adult jejunum (Ad) was reverse transcribed and PCR amplified in the presence of [^{32}P]dCTP for APOBEC-1 and GAPDH mRNA, each sample being assayed in triplicate. Aliquots of the reaction mix were run on a 2% agarose–1% NuSieve gel and the corresponding bands were excised and counted in scintillation fluid. Background counts for each sample were subtracted as detailed in methods. APOBEC-1 mRNA levels are expressed as a ratio APOBEC-1/GAPDH. The values are presented as mean and standard deviation of triplicate experiments. Error bars, where not shown, are contained within the mean. C: Correlation between APOBEC-1 mRNA levels and apoB mRNA editing in human fetal (n = 15) and adult small intestinal RNA samples (two duodenum, two jejunum and one ileum). The equation for the regression is y = 64.035 LOG(x) +48.033.

postconfluent Caco-2 cells grown on plastic despite comparable APOBEC-1 mRNA, suggests that additional factors are likely to be involved in the regulation of this process.

Immunocytochemical localization of APOBEC-1

No immunoreactive material was detectable in fetal small intestine at 10.5 weeks gestational age (Fig. 4A), a representative sample demonstrating an undifferentiated structure with virtually no villus folds (Fig. 4A). Rudimentary villus folds were present in an 11-week gestational age fetal small intestine that demonstrated patchy, intense nuclear staining for APOBEC-1 (Fig. 4B, arrows). This image is shown at higher resolution in **Fig. 5A.** By 13 weeks of gestation, a more established villus architecture was apparent and the cells were uniformly reactive for APOBEC-1, with intense nuclear staining (Fig. 4C). Adult small intestinal samples demonstrated a diffuse pattern of immunoreactivity in all cells along the villus-crypt axis with intense nuclear staining noted in the intervillus regions (Fig. 4D, arrows). These findings are demonstrated in an additional subject, revealing a comparable pattern of intense nuclear staining, particularly in the intervillus region and diffuse immunoreactivity along the villus (Fig. 5B).

Activity of fatty acid CoA ligase (FA CoA ligase), monoacylglycerol acyltransferase (MGAT), and diacylglycerol acyltransferase (DGAT) in fetal small intestine and liver

The temporal pattern of expression of three enzymes involved in fatty acid incorporation into triglycerides was determined in fetal and adult tissue samples as an initial attempt to correlate the emergence of complex lipid assembly with the developmental regulation of apoB mRNA editing and APOBEC-1 gene expression.

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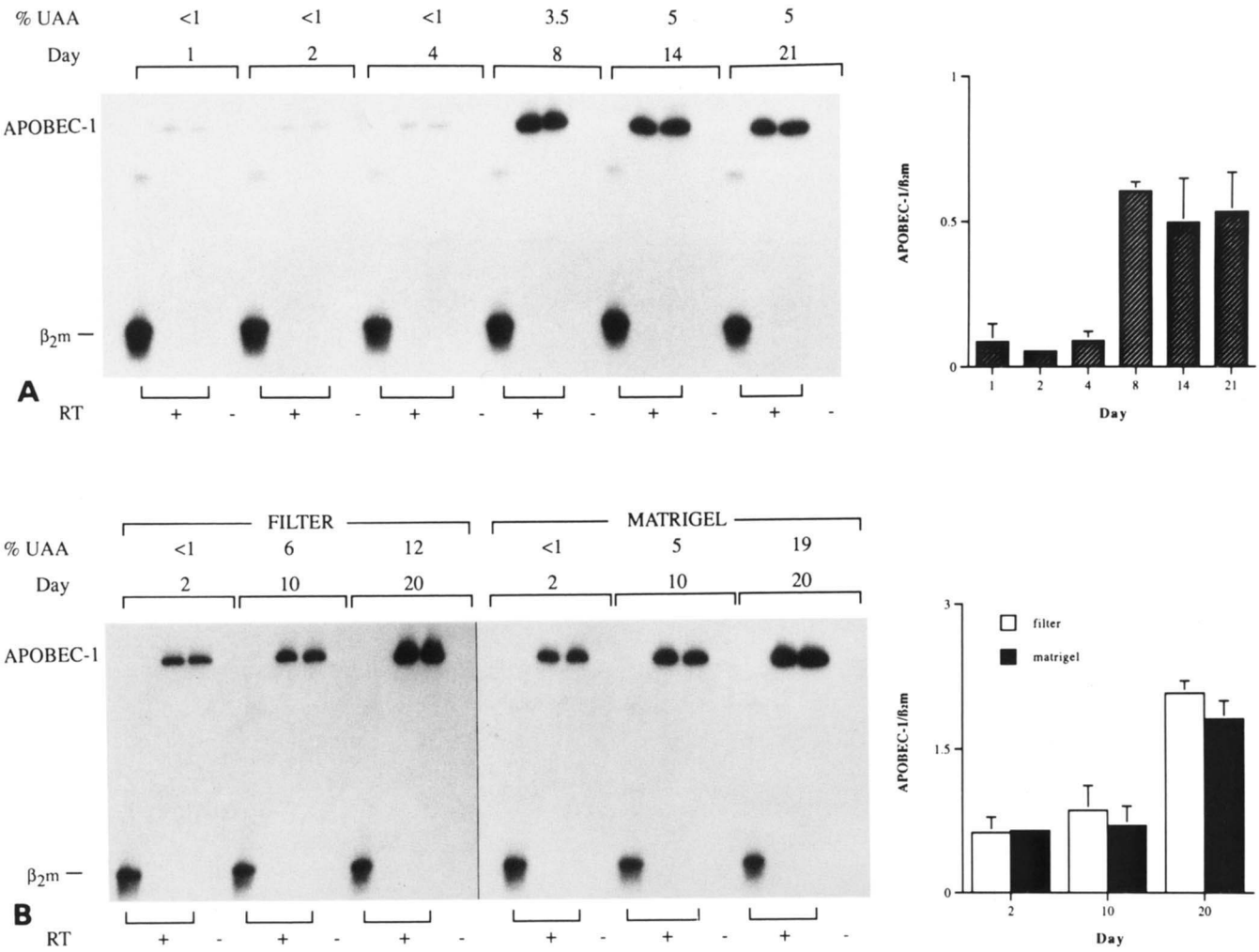

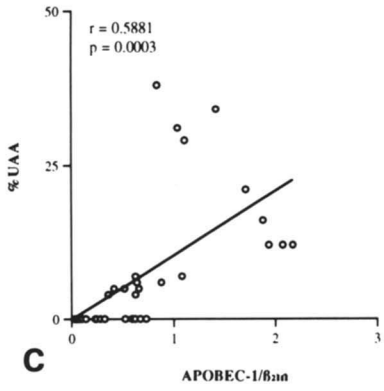

Fig. 3. APOBEC-1 mRNA quantitation in pre- and postconfluent Caco-2 cells. A: 500 ng total RNA from Caco-2 cells grown on plastic for 1, 2, 4, 8, 14, and 21 days (after plating) was reverse transcribed (RT+) and PCR amplified in the presence of [^{32}P]dCTP for APOBEC-1 (30 cycles) and β_2-microglobulin (20 cycles) in separate tubes. Approximately 3 µl of the reaction mix was run on a 6% nondenaturing polyacrylamide gel and the PCR products corresponding to APOBEC-1 (in duplicate) and β_2 microglobulin are indicated (left panel). Independent negative controls for the two amplifications were pooled and run in a single lane (RT–). The graph (right panel) shows the increase in APOBEC-1 mRNA levels in duplicate experiments in which each sample was assayed in triplicate. For these reactions, RT-PCR was performed in the presence of [^{32}P]dCTP and equal volumes of the reaction mix were run on a 2% agarose–1% NuSieve gel. The expected bands were excised and counted as detailed in methods. APOBEC-1 mRNA levels increased significantly between day 4 and day 8, ($P < 0.0001$). B: 500 ng total RNA from Caco-2 cells grown on either uncoated semipermeable filters or on identical filters coated with Matrigel was used to reverse transcribe (RT+) and PCR amplify APOBEC-1 and β_2 microglobulin mRNA in the presence of [^{32}P]dCTP. The right panel shows a representative assay of a time course in which cells were harvested for RNA extraction at 2, 10, and 20 days after plating. Approximately 3 µl of the reaction mixture was loaded on a 6% nondenaturing polyacrylamide gel. For each sample APOBEC-1 (in duplicate) and β_2 microglobulin products are indicated. Separate negative controls were pooled and run in a single lane (RT–). The graph (right panel) shows the results of duplicate (days 2 and 10) or triplicate (day 20) experiments. RNA samples from each experiment were assayed in triplicate. APOBEC-1 mRNA levels increase significantly between day 10 and day 20 in both uncoated and Matrigel-coated filter grown cells ($P < 0.0001$). C: Correlation between APOBEC-1 mRNA abundance and apoB mRNA editing in pre- and postconfluent Caco-2 cells grown on plastic. The equation for the regression is y = 10.394x – 0.057.

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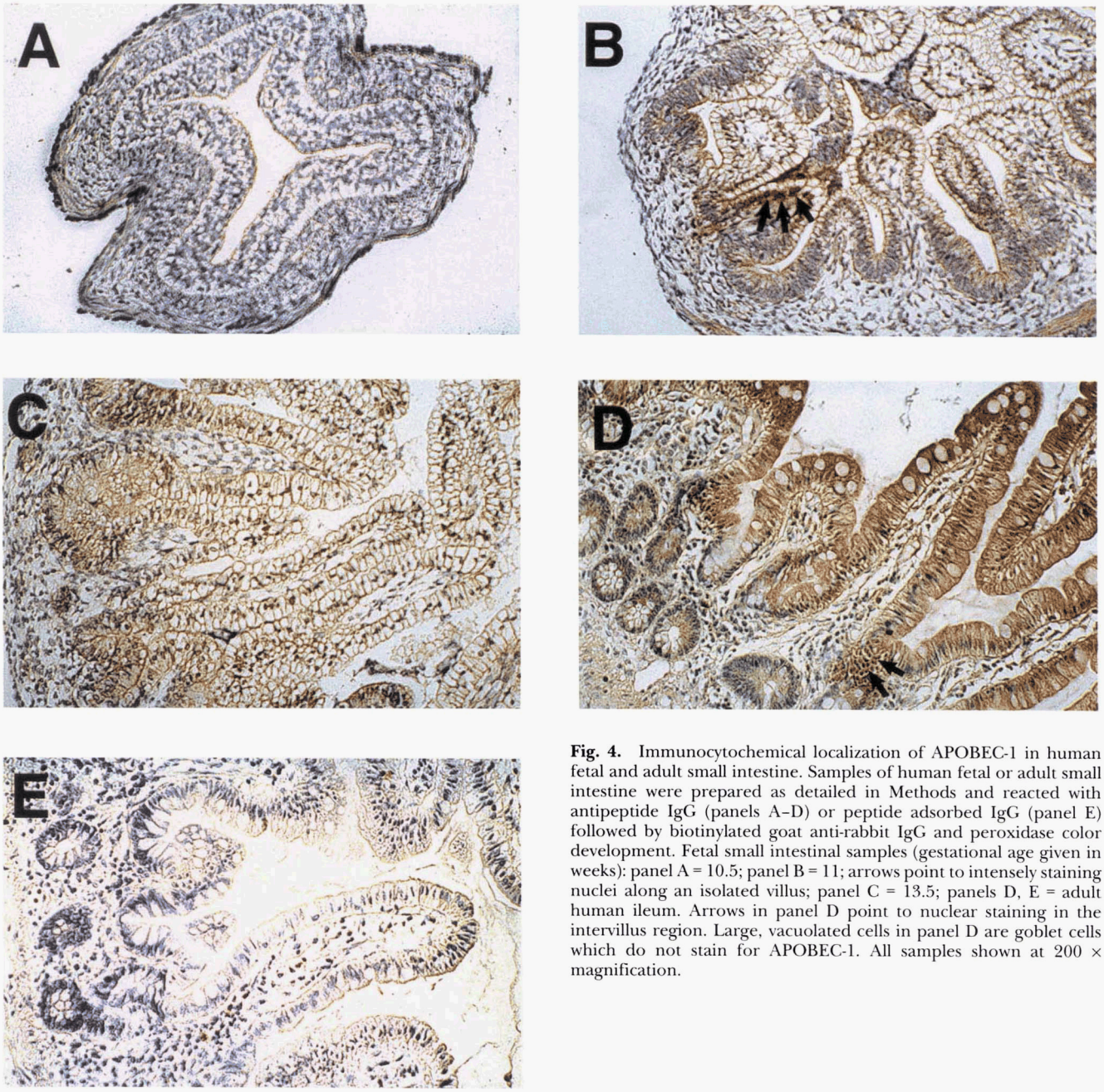

Fig. 4. Immunocytochemical localization of APOBEC-1 in human fetal and adult small intestine. Samples of human fetal or adult small intestine were prepared as detailed in Methods and reacted with antipeptide IgG (panels A–D) or peptide adsorbed IgG (panel E) followed by biotinylated goat anti-rabbit IgG and peroxidase color development. Fetal small intestinal samples (gestational age given in weeks): panel A = 10.5; panel B = 11; arrows point to intensely staining nuclei along an isolated villus; panel C = 13.5; panels D, E = adult human ileum. Arrows in panel D point to nuclear staining in the intervillus region. Large, vacuolated cells in panel D are goblet cells which do not stain for APOBEC-1. All samples shown at 200 × magnification.

The activity of all three enzymes was low in a single 10-week gestational age fetal small intestine, but all samples studied from 12 weeks gestational age onwards yielded values comparable to adult levels (**Fig. 6A**). These data suggest that the late first trimester fetal small intestine may be fully competent to synthesize triglyceride, findings that extend the conclusions of earlier reports using organ explant culture (23). The specific activity of MGAT in the adult sample examined was considerably lower than previously noted in the adult rat small intestinal mucosa, where values in excess of 100 nmol/min per mg protein, are usually observed (21). In order to determine whether this result was influenced by the use of extracts prepared from full thickness adult small intestine, two additional subjects were studied in whom mucosal samples were prepared by scraping the resected tissue. These results are shown in the panel in Fig. 6A, demonstrating approximately three- to fivefold higher specific activity of all three enzymes, but with values for MGAT still an order of magnitude below those found in the adult rat small intestine. For the purposes of comparison with the data from fetal small intestine, extracts were prepared from liver tissue from these same donors and assayed as

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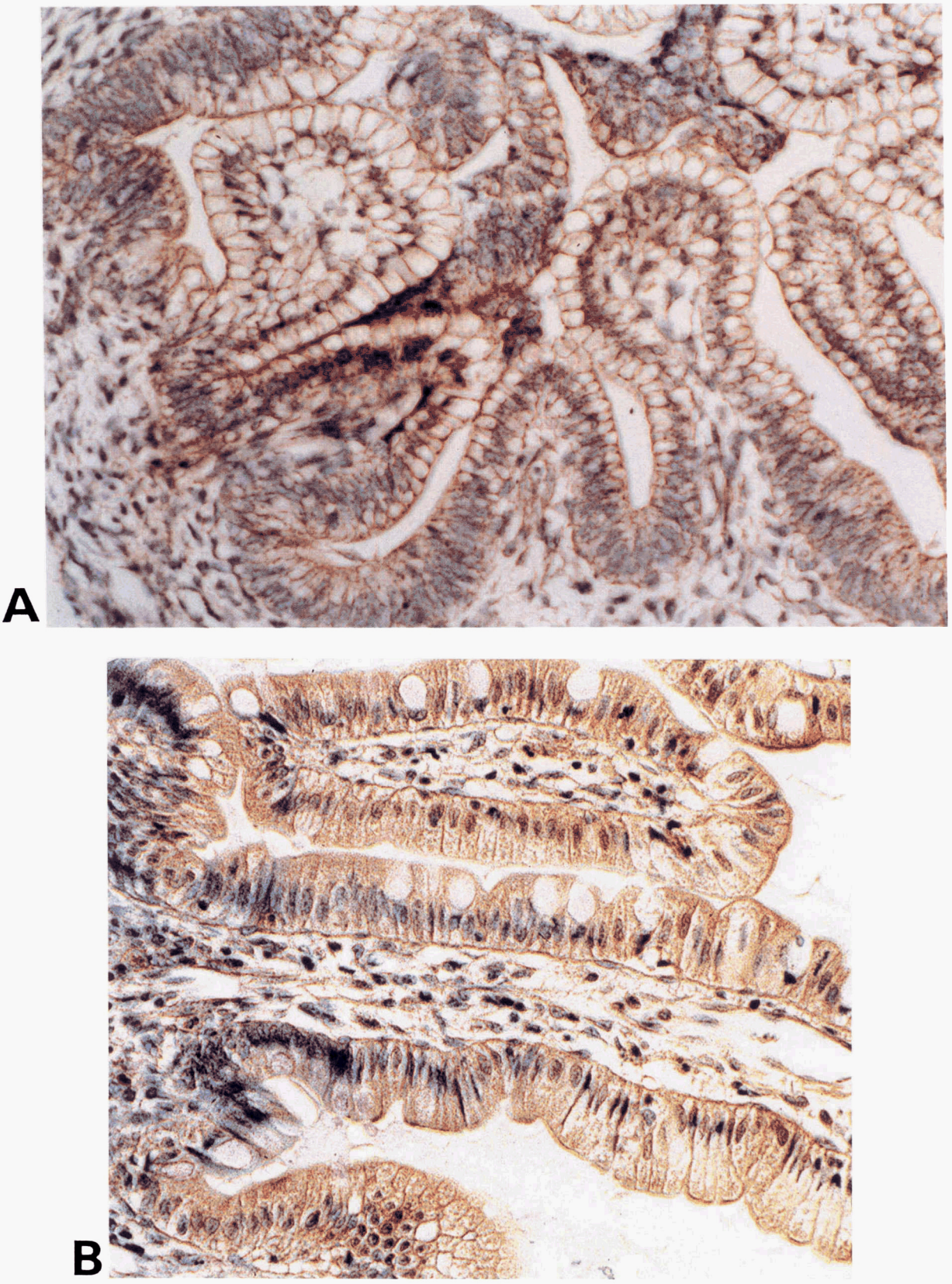

Fig. 5. Immunocytochemical staining of APOBEC-1 in human small intestine. Panel A: higher resolution photograph of the 11 week gestational age sample demonstrated above in Fig. 4B. Note the intense nuclear staining demonstrated in a single villus. Panel B: an additional adult human ileal sample showing diffuse immunoreactivity along the villus with occasionally intense nuclear staining.

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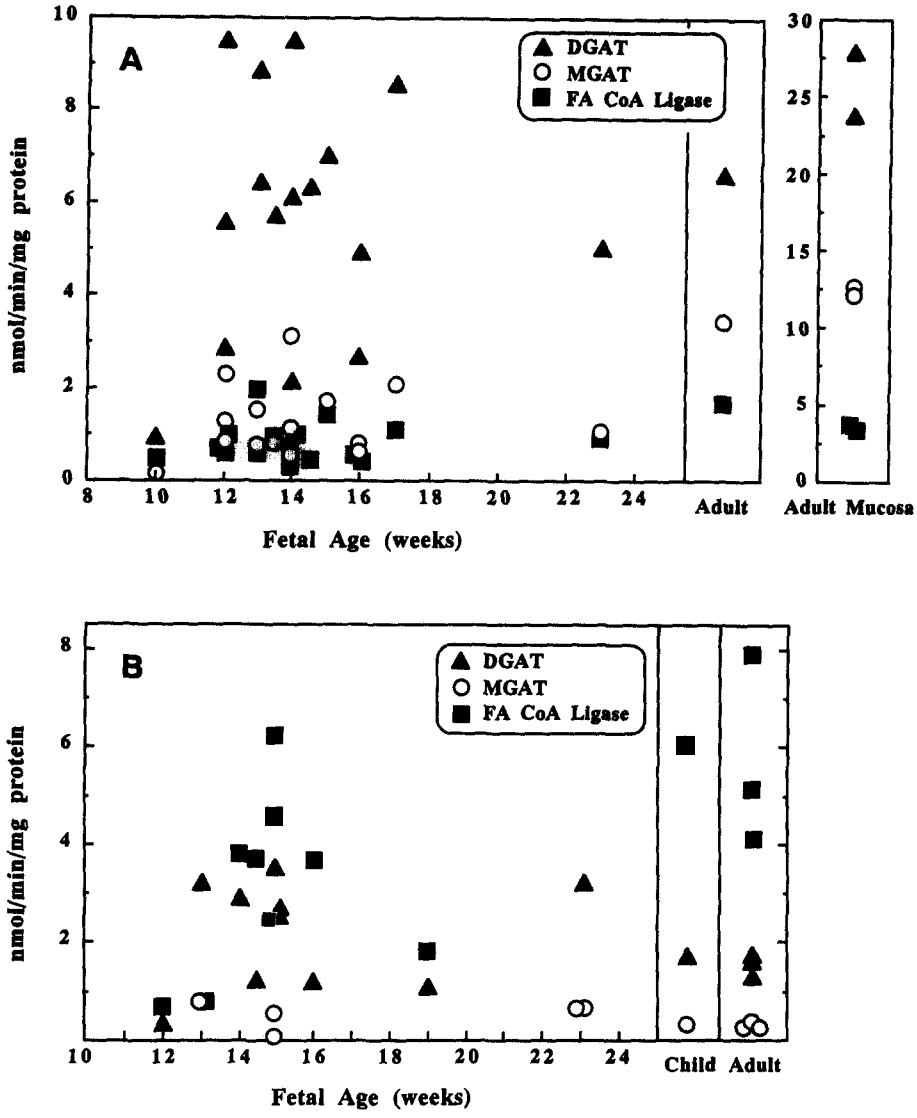

Fig. 6. Specific activity of monoacylglycerol acyltransferase (MGAT), diacylglycerol acyltransferase (DGAT), and fatty acid CoA-ligase (FA CoA Ligase) in samples of human fetal and adult small intestine (panel A) and liver (panel B). Samples (either whole tissue or mucosal extracts) were prepared as detailed above and determinations of enzyme activity were made according to previously validated methods. Each point represents a single sample. The identity of the symbols is given within the inset of each figure.

detailed above. The results show a comparable pattern of developmental regulation to that noted in the small intestine, with adult levels of activity achieved for all three enzymes by 12–13 weeks of gestation (Fig. 6B). Considered as a whole, these data suggest that the endogenous triglyceride synthetic pathway is active very early in human fetal hepatic and intestinal development.

DISCUSSION

The central observation of this study is that the developmental regulation of APOBEC-1 provides a plausible explanation for earlier findings that apoB mRNA editing is developmentally regulated both in the human fetal small intestine and in Caco-2 cells (12–16). The second conclusion to be drawn from these studies is that the early gestational age fetal small intestine and liver manifest enzyme activity, comparable to that found in adult samples for several key steps in triglyceride synthesis. Taken together, these data provide a framework for further understanding the developmental regulation of lipoprotein assembly in the fetal human small intestine. Several aspects of these findings merit further discussion.

Previous studies from our own and other laboratories have investigated the developmental regulation of intestinal apoB mRNA editing in animal species as well as in

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humans (12, 13, 24–26). Along these lines, several studies have demonstrated that rat and mouse small intestinal apoB mRNA editing undergoes a dramatic prenatal induction with adult proportions of apoB-48 mRNA achieved at or around the time of birth (7, 13, 25, 26). More recent results indicate that this increase in endogenous intestinal apoB mRNA editing precedes the developmentally modulated accumulation of APOBEC-1 mRNA in the fetal rat small intestine (7). These latter findings suggest a model in which APOBEC-1 expression in the 19–20 day embryonic rat small intestine is at threshold levels and that the increase in apoB mRNA editing is achieved through the induction of one or more complementation factor(s). More convincing proof of this possibility, however, will await further identification of these factor(s). The current studies undertook to establish the temporal pattern of APOBEC-1 gene expression in the human fetal small intestine, using a combination of RT-PCR and immunocytochemical detection. Our recent analysis of APOBEC-1 mRNA distribution demonstrated that the transcript was undetectable in Northern blots of 14-week gestational age fetal human small intestine (9). Additionally, detection of the mRNA species in adult human small intestine required prolonged exposure of the autoradiogram, suggesting that APOBEC-1 is a low abundance species whose accurate detection requires the application of more sensitive techniques. Accordingly, RT-PCR was chosen as the method of choice in view of the limited quantities of RNA available from human fetal tissues. The results indicate a general trend of increased APOBEC-1 mRNA abundance towards the end of the second trimester, in association with increased endogenous apoB mRNA editing.

The timing of these alterations in fetal human small intestinal apoB mRNA editing, spanning the late first and second trimester, contrasts with the pattern demonstrated in both the rat and mouse small intestine, where apoB mRNA editing undergoes a dramatic increase over the 2–3 days preceding birth. Among the possibilities to consider in understanding these inter-species differences are the relative patterns of intestinal morphogenesis. Previous studies of human fetal intestinal morphogenesis have demonstrated that villus formation begins at 9–10 weeks, followed by the emergence of crypts lined by undifferentiated cells at 10–11 weeks (27, 28). This information is accurately recapitulated in the present study where the gross morphology of a 10.5-week fetal small intestinal sample shows primitive villus folds while an 11-week sample demonstrates the emergence of a more established architecture (Fig. 4). The findings contrast with the pattern of intestinal morphogenesis demonstrated in the rat small intestine, which does not develop a well-established villus architecture until day 19 of gestation, i.e., 2–3 days prior to birth (27, 28). Thus, an important conclusion of this study is that the appearance or activation of genes involved with apoB mRNA editing appears to be temporally coincident with the onset of intestinal ontogenesis in both the human and rat. Although the precise distribution of the transcript among the different cellular elements of the developing gut was not determined in this study, immunocytochemical analysis indicates that essentially all villus-associated enterocytes express APOBEC-1 protein. Additionally, in situ hybridization studies of the rat APOBEC-1 demonstrated a restricted pattern of mRNA distribution, predominantly within enterocytes, with little expression in submucosal and adventitial cells (7). Although these findings have yet to be confirmed in the human small intestine, the protein distribution data presented above suggests that mature villus cells are likely to be the major source of APOBEC-1 gene expression in the human gut.

Important aspects of the developmental regulation of small intestinal apoB mRNA editing have been recapitulated in several analyses of Caco-2 cells (14–16). The current studies demonstrate the appearance of APOBEC-1 mRNA in post-confluent cells grown on plastic, coincident with the onset of apoB mRNA editing. Cells grown on semipermeable filters, which have been previously shown to demonstrate an earlier onset of differentiation-associated characteristics, were found to express APOBEC-1 mRNA at an earlier stage (day 2 post-plating), suggesting that the expression of this mRNA is temporally coincident with other aspects of the differentiation process. It is interesting to note that, despite the appearance of APOBEC-1 mRNA in Caco-2 cells analyzed after 2 days growth on filter supports, endogenous apoB mRNA editing was below detectable limits (Fig. 3). These findings reinforce the concept (8) that the regulation of apoB mRNA editing is dependent upon both the catalytic subunit of the apoB mRNA editing enzyme and the activity of complementation factors.

One further aspect of the developmental regulation of apoB mRNA editing explored in these studies was its temporal relationship to the appearance of enzyme activity involved in triglyceride synthesis. Previous studies in the rat liver have demonstrated modulation of apoB mRNA editing in response to nutritional and hormonal influences, in particular, in response to fasting and refeeding a high carbohydrate diet, a potent stimulus to hepatic lipogenesis and triglyceride accumulation (29). A further feature of this model is that changes observed in hepatic apoB mRNA editing were accompanied by a greater than 5-fold increase in APOBEC-1 mRNA abundance (7). Other studies have demonstrated a close temporal association of both the

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developmental regulation of hepatic apoB mRNA editing and triglyceride synthesis in the neonatal rat, suggesting the possibility that a common underlying mechanism may modulate both elements of lipoprotein assembly (30). Developmental regulation of fatty acid utilization has also been demonstrated in the late fetal and neonatal rat small intestine (31). In regard to the fetal human small intestine, there is only a limited amount of information concerning the regulation of lipoprotein biogenesis. Previous work, using intestinal organ explant cultures, demonstrated complex lipid synthesis and lipoprotein secretion from 17–20 week gestational age samples (23). In regard to apoB gene expression, previous studies demonstrated de novo apoB-100 and B-48 synthesis and secretion from explants prepared from an 11.5-week gestational age fetal small intestine (12). The current results extend these findings by demonstrating that tissue extracts prepared from the late first and second trimester fetal small intestine manifest activity comparable to that found in adult samples for three key enzymes involved in triglyceride biosynthesis. These data, therefore, suggest that many elements of lipoprotein assembly are fully developed at the time of onset of intestinal ontogenesis. This suggestion is consistent with earlier studies demonstrating little change in the accumulation of mRNAs encoding cholesterogenic enzymes, apolipoproteins B, A-I, A-II, or liver fatty acid binding protein in the developing human fetal liver (32). The general conclusion to be drawn from these analyses of ontogenic regulation of lipid metabolism in the developing human fetus is that the capacity for complex lipid assembly and mobilization is established very early.

A major question left unanswered remains: the nature of the developmental cues that act to modulate apoB mRNA editing in the human fetal small intestine. Among the possibilities to consider would be the role of circulating hormones such as insulin, thyroid hormone, and glucocorticoids, all of which have been demonstrated to promote cytodifferentiation when added to intestinal organ explant cultures in vitro (see ref. 33 for review). Additionally, insulin and thyroid hormone have been demonstrated to modulate both complex lipid assembly and apoB mRNA editing, either in isolated rat hepatocytes (34) or in the intact rat liver (35). However, although these factors may be potentially important in the regulation of small intestinal differentiation in vitro, circulating levels of all these hormones in the human fetus rise most dramatically in the third trimester, after the onset of intestinal differentiation (33). Other factors to consider are the trophic actions of luminal lipids, including biliary and amniotic fluid lipid, although it is unlikely that these would be abundant in the 10–12 week fetal small intestine (27, 28). Epidermal growth factor has been suggested to be of importance in the regulation of intestinal lipoprotein secretion (23) and the possibility that this or other factors such as placentally derived peptides (36) may be involved in the developmental regulation of apoB mRNA editing merits further attention (23). These and other issues will be the focus of future reports.

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