Intact Intestinal mRNAs and Intestinal Epithelial Cell Esterase, But Not *Cryptosporidium parvum*, Reach Mesenteric Lymph Nodes of Infected Mice

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Intact Intestinal mRNAs and Intestinal Epithelial Cell Esterase, But Not Cryptosporidium parvum, Reach Mesenteric Lymph Nodes of Infected Mice

Esther M. Ponnuraj*2*‡ and Anthony R. Hayward*†‡

Dendritic cells from the mesenteric lymph nodes (MLN) contain dense esterase-positive inclusions that may originate in effete intestinal epithelial cells and reach MLN without degradation. The MLN esterases have the electrophoretic mobilities of both intestinal and mononuclear cells. Cryptosporidium parvum (CP)-infected mice have CP Ag-positive cells in MLN and also increased numbers of dense esterase-positive cells, but the CP Ag-positive cells do not stain for esterase. To characterize the handling of epithelial cell products by dendritic cells, we analyzed mRNAs in the MLN of control and CP-infected recombination-activating gene−/−DO11.10 mice by oligoarrays. mRNAs for 115 proteins were increased in MLN after CP infection, of which the principal increases in trypsin and chymotrypsin approximated to 250-fold. Colipase, reg-1, C-reactive protein-ductin, and amyloid were also up-regulated >10-fold and all returned to baseline by 28 days after infection. mRNAs for the same proteins were detected in intestinal epithelial cells of infected mice by oligoarrays and RT-PCR after infection. mRNA for CP β-tubulin was detectable in intestinal epithelial cells between 5 and 18 days after infection but was not detected in the MLN throughout the observation period. It appears that host response to CP infection includes expression of mRNA for some pancreatic enzymes by intestinal epithelial cells and their subsequent transport to the MLN. The esterase and trypsin, and mRNAs for chymotrypsin, colipase, and others that may derive from uninfected epithelial cells, appear to be transported to the MLN intact, while mRNA for CP β-tubulin that is derived from infected cells is degraded. The Journal of Immunology, 2001, 167: 5321–5328.

Intestinal epithelial cells are subject to infection by a range of pathogens, so it seems likely that the immune system needs to discriminate between self and microbial proteins before a specific immune response is made. Little is known of the basis for this discrimination and whether it is made in the draining lymph node or by the dendritic cells (DCs)3 that transport Ags to the node. Ags ingested by DCs are generally thought to be digested to polyptides for presentation to T cells (1), so the recent finding that intestinal nonspecific esterase reaches the mesenteric lymph node (MLN) with intact enzymatic activity (2) was unexpected. The intestinal epithelial cells from which this nonspecific esterase is derived are engulfed at the end of their life span by DCs in the lamina propria (3, 4). DCs that have ingested intestinal epithelial cells are therefore a likely means for the transport of esterase to the draining lymph node. Whether DCs could discriminate between infected and uninfected intestinal epithelial cells by digesting the former to peptides while leaving the cytoplasmic proteins of the latter intact is not known. The issue is important in the context of immunity to gut pathogens, including apicomplexan parasites such as Cryptosporidium parvum (CP). This organism has attracted interest recently because of widespread outbreaks (5) and causes severe disease or fatalities in immunodeficient subjects (6, 7).

CP occupies a parasitophorous vacuole in infected epithelial cells, separated from the host cell cytoplasm by an intact cell membrane (8). SCID mice may be infected by CP for months with little or no weight loss (9, 10). A CP infection can be cleared by mice that successfully activate their CD4 T cells to express CD154 and provide a signal to a CD40-positive marrow-derived cell (11–13). Whether the intestinal immune system handles epithelial cells infected by CP differently from uninfected cells is not known. During studies of mice infected by CP, we saw increased numbers of esterase-positive cells and cells that stained for CP Ag in the MLN. To explore the development of an intestinal immune response to CP we first determined whether intestinal esterase would be transported to the MLN of mice, as has been shown previously for rats. DO11.10-transgenic BALB/c mice on a recombination-activating gene (RAG) knockout background (14) were studied because they are known to recover promptly from CP infection despite having T cells of only a single specificity (13). The single TCR expressed by these animals reduces the diversity of the T cell response. Oligoarray and RT-PCR analysis of mRNAs in the MLN were used to identify additional epithelial cell products in MLN and to examine the handling of CP products. The esterase and mRNA approaches both support a model in which the contents of intestinal epithelial cells can be transported intact to the MLN.

MacPherson and colleagues (2) showed that rat DCs with a CD4+OX41− phenotype contain dense inclusions of nonspecific esterase derived from intestinal epithelial cells. The MLN of mice are known to contain esterase-positive cells with macrophage or DC morphology (15), but whether any of this esterase is of intestinal origin is not known. The persistence of the intestinal esterase enzyme activity within the DCs of rats, and possibly mice, would...
argue for a pathway that delivers intact endogenous proteins to MLN. Ags from infectious agents, in contrast, appear to be degraded within DCs and their peptides presented to T cells (1). The integrity of epithelial cell mRNA compared with the degradation of CP mRNA has implications for the maintenance of tolerance during an immune response to an enteric pathogen.

Materials and Methods
Mice and sample collection
Animal conditions and experimentation were approved by the Institutional Animal Care and Use Committee. BALB/c DO11.10RAG"-/-transgenic mice were bred and housed in microisolator cages with sterile food, water, and bedding as before (11, 12). They were infected with “GCH1” CP oocysts (catalog no. 1372; McKesson BioServices, AIDS Research and Reference Reagent Program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health). Three animals each were euthanized by CO2 inhalation on days 5, 8, 12, 15, 18, and 28 following infection, and MLNs were dissected using a microscope. They were frozen on liquid N2 for RNA extraction. For the isolation of intestinal epithelial cells, gastrointestinal tract extending from the duodenum to the terminal ileum was removed. Small pieces of tissue were frozen in OCT compound (Sakura-Finetek, Torrance, CA) for cryosections and the remaining epithelial surface was exposed and rinsed free of debris in Ca2+ (Sakura-Finetek, Torrance, CA) for cryosections and the remaining epithelial surface was exposed and rinsed free of debris in Ca2+ and Mg2+-free HBSS with 5% FCS (HBSS-FCS). The gut was transferred to HBSS-FCS with 1 mM DTT, 0.5 mM EDTA (pH 8.0), and antibiotics (100 U/ml penicillin, 100 gg/ml streptomycin, and 250 ng/ml amphotericin B) for orbital shaking at 37°C for 40 min. The dislodged epithelial cells (16) were centrifuged at 400 x g for 15 min at 4°C on a two-step gradient of 20 and 60% Percoll (Sigma-Aldrich, St. Louis, MO). Epithelial cells were harvested from the 60% interface, washed in HBSS, and spun down at 400 x g for 10 min.

RNA extraction
RNA was extracted from pelleted epithelial cells by sonication and frozen MLN by homogenizing in 750 ul of TRIzol (Life Technologies, Rockville, MD). The homogenate was extracted in chloroform twice and then precipitated by isopropanol. The RNA was quantitated, cleaned on an RNeasy mini spin column (catalog no. 74104; Qiagen, Valencia, CA), and quantitated by spectrophotometry.

cDNA synthesis and microarray analysis
T7-d(T34) primer (100 pM) (Genset, Paris, France) was annealed to 5–10 µg of RNA at 70°C for 10 min. First-strand synthesis was conducted at 42°C for 1 h in a 20-µl reaction using 200 U of Superscript II RNAS H reverse transcriptase in the presence of 50 mM Tris-HCl (pH 8.3), 75 mM KC1, 15 mM MgCl2, 10 mM DTT, and 500 µM each dATP, dTTP, dGTP, and dCTP. First-strand cDNA was used for PCR. For oligoarray analyses, a second strand was synthesized in a 150-µl volume at 16°C for 2 h using 40 U of DNA polymerase I, 10 U of DNA ligase in the presence of 20 mM Tris-HCl (pH 6.9), 4.6 mM MgCl2, 90 mM KCl, 0.15 mM dNTPs, 10 mM (NH4)2SO4, 2 U of RNase H, and 200 µM each dATP, dCTP, dGTP, and dTTP. The second strand was further incubated with 10 U of T4 DNA polymerase for 5 min, the reaction was stopped with EDTA, and the cDNA was cleaned by Phase Lock gel (Brinkman Instruments, Westbury, NY). cDNAs were in vitro transcribed to yield biotin-labeled cRNA using BioArray HighYield Transcript Labeling kit (Enzo Diagnostics, Farmingdale, NY) according to the manufacturer’s instructions. The cRNAs were cleaned on RNeasy mini columns and quantitated. cRNA (20 µg) was fragmented by heating at 94°C. Fragmented cRNA (15 µg) was added to a mixture of 50 pM of control oligonucleotide B2, control cRNA mixture (consisting of 1.5 pM BioS, 5 pM BioC, 25 pM BioD, and 100 pM pGIKS-CRE), 0.1 mg/ml herring sperm DNA, and 0.5 mg/ml acetylated BSA, in MES buffer. The hybridization mixture was heated to 99°C for 5 min and 45°C for 5 min, was centrifuged at 12,000 x g for 5 min, and was applied to a buffer-equilibrated Test 2 probe array (Affymetrix, Santa Clara, CA) for 16 h of incubation at 45°C in a rotisserie incubator. Provided that this array confirmed the integrity of β-actin and GAPDH mRNAs (selected as housekeeping genes) across the 5°, mid, and 3° regions, the fragmented cRNA was applied to the Multi1KsubA array. Following hybridization the arrays were stained with PE-streptavidin (Vector Laboratories, Burlingame, CA) and biotinylated anti-streptavidin Ab and scanned using an HP GeneArray Scanner (Hewlett-Packard, Palo Alto, CA). Comparisons between an uninfected animal and an animal infected by CP for 5–28 days were by MicroArray Suite 4.0 software (Affymetrix, Santa Clara, CA). To analyze infected gastrointestinal epithelial cells, the products of multiple tubes of reverse transcription were transcribed in vitro. The amount of starting RNA was sub- tracted from the in vitro transcribed product to calculate an adjusted yield.

Detection of CP mRNA
β-Tubulin was selected for amplification because it is present in viable but not dead Cryptosporidium and has an 87-ppm intron that distinguishes the DNA from the spliced mRNA products (17). RNA from intestinal epithelial cells and MLN were reverse transcribed with 100 U of Superscript1 reverse transcriptase (Life Technologies) in the presence of 2 µM b-tub2 reverse primer. The products of a nested PCR amplification (17) were analyzed on 2% agarose gels.

RT-PCR analysis of genes selected on the basis of up-regulation data from oligoarray analysis of the MLN
cDNAs were made by T7-d(T34) primer following which PCR was conducted using the primers described in Fig. 1. Genes proven to be up-regulated using the oligoarray analysis in one animal at each time point were further confirmed in three animals’ MLN at each time point by the RT-PCR. To find out whether 300 mRNAs that were up-regulated in the MLN were from the gastrointestinal epithelial cells RT-PCR was done on three to five animals at each time point.

Immunofluorescence and immunohistochemical staining
Sections of gut and MLN (5 µm) were fixed in 80% cold ethanol for 10 min and preincubated in blocking solution (1% BSA with 10% normal goat serum in PBS) for 10 min to reduce nonspecific binding of fluorescence conjugates. FITC-conjugated rat polyclonal Ab to CP (A600FL.Sporo-Glo; Waterborne, New Orleans, LA) was diluted 1/40, applied to sections, and incubated overnight at room temperature. Sections were washed in PBS and mounted in 90% glycerol in PBS for viewing.

Cytokeratin was stained by a rabbit wide-spectrum anti-keratin Ab (cat-alog no. Z0622; DAKO, Carpinteria, CA) followed by FITC anti-rabbit IgG (catalog no. 110-095-144; Jackson Immunoresearch Laboratories, West Grove, PA). α-Naphthyl-butyrate esterase test (kagol no. 181-B; Sigma-Aldrich) was stained on 5-µm sections of gut that were fixed with the recommended acetone-formaldehyde-citrate (ACF) fixative for 10 s at room temperature and rinsed with de-ionized water for 45 s. Subsequent incubations with freshly prepared substrate at 37°C for 15 min were followed with tap water rinses and methylene blue counterstain. When esterase and immunofluorescence methods were combined, the sections were fixed in 80% ethanol instead of ACF. The esterase stain was performed first and the methylene blue counterstain was omitted. Sections were examined on a Leitz microscope with incident UV light and transmitted phase contrast or conventional optics. Images were captured with a Spot camera (model 1.3.0; Diagnostic Instruments, Sterling Heights, MI) and processed with Adobe Photoshop software (Adobe Systems, San Jose, CA).

Characterization of esterase-positive cells
MLN from DOTg mice were digested by collagenase (catalog no. C9263; Sigma-Aldrich) and DNase (catalog no. D5025; Sigma-Aldrich). The cells were centrifuged on a 14.5% metrizamide (catalog no. M3383; Sigma-Aldrich) gradient and stained with an isotype control (catalog no. 340041; BD Biosciences, San Jose, CA) or for CD11c (catalog no. 550261; BD PharMingen, San Diego, CA) and CD8a (catalog no. RM2204-3; Caltag Laboratories, Burlingame, CA). CD11c+ cells were identified among cells with the light scatter properties of DCs and sorted on a MoFlo cell sorter (Cytomation, Fort Collins, CO) into CD8a+ and CD8a− subsets. The sorted cells were cytotoxic for 7 min at 700 rpm onto SuperFrost+ slides (Fisher Scientific, Pittsburgh, PA), fixed with ACF, and stained for esterase according to the manufacturer’s instructions (catalog no. 181-B; Sigma-Aldrich). At least 300 cells on each slide were scored as esterase-positive or -negative by two observers and the results were averaged and expressed as a percentage.

Zymograms for esterase isoforms
Cells tested were adhered splenocytes, metrizamide-purified DCs from MLN, and intestinal epithelial cells. In each case 10° or more cells were lysed in Tris buffer containing 0.1% Triton X-100 and 15 mM EDTA. Lysates were stored frozen at −70°C and were sonicated before loading.
onto a 7.5% polyacrylamide gel. They were run at 15 mA for 5 h and then fixed in ACF for 1 min. The gels were rinsed and developed for esterase activity using the Sigma-Aldrich kit.

Measurement of tryptic activity

Suspensions of MLN cells obtained from mice, three uninfected and three obtained 12 days after CP infection, were prepared with a Dounce homogenizer and adjusted to 10^6 cells/ml. One-milliliter aliquots were centrifuged and the supernatant was decanted. The cells were rapidly frozen over liquid N2, then thawed, resuspended in 400 μl of PBS, and sonicated for 5 s. One hundred-microliter aliquots of cell sonicates were mixed with 1 U of purified enterokinase (catalog no. E0885; Sigma-Aldrich) in 50 μl of PBS for 5 min before addition of 50 μl of Bzipar (catalog no. R-6505; Molecular Probes, Eugene, OR) to a final concentration of 10 μM (18). Substrate cleavage was followed as changes in relative fluorescence units under conditions in the manufacturer’s product sheet at room temperature on a Spectramax Gemini fluorometer (Molecular Devices, Sunnyvale, CA) using 498-nm excitation and 521-nm emission.

Results

CP infection increased the number of esterase-containing cells in the MLN

DCs that have endocytosed intestinal epithelial cells can be identified by their dense esterase-positive granules in the lamina propria of rats (2), and comparable cells in the lamina propria of mice may transport effete intestinal epithelial cells to the MLN. The frequency of esterase-positive cells in the MLN of DOTg mice increased from an average of 0.8 (SD 0.2) per 100 field (20 fields

FIGURE 1. Schematic diagram of genes of interest selected for RT-PCR. Target nucleotides on the Mu11kSubA Affymetrix oligoarray are shown together with the region amplified by PCR. PCR primer sequences, predicted PCR product sizes, and sample RT-PCR products are also shown. a, Trypsin gene located in chromosome 6 within the TCR-β locus (GenBank accession no. AE000664) comprises 741 nucleotides of which nucleotides 273–689 are the chip target sequences. The sense primer spanning nucleotides 67–91 and the antisense primer complementary to nucleotides 700–719 amplify a 649-bp fragment. The insert shows the ethidium bromide-stained 1% agarose gel with a 1-kb marker in lane 1, a reverse transcription-negative control without product in lane 2, and a 649-bp positive band in lane 3. b, The chip targets nucleotides 1614–1824 of chymotrypsin like protease precursor (GenBank accession no. AA244542). The chymotrypsin gene consists of seven exons (filled) and six introns (open). The sense primer spanning nucleotides 1655–1674 and the antisense primer complementary to nucleotides 1792–1811 amplify a 157-bp fragment. The insert shows the ethidium bromide-stained 1% agarose gel with a 1-kb marker in lane 1, a negative (uninfected gut) sample in lane 2, and a 157-bp positive band in lane 3. c, A sequence 87 bases long from GenBank accession no. AA611440 was used as target for colipase precursor. Colipase RNA contains 446 bases. RT-PCR amplifies a 232-bp fragment between nucleotides 88 and 320. The insert shows the ethidium bromide-stained 2% agarose gel with a 50-bp marker in lane 1, a negative sample in lane 2, and a 232-bp positive band in lane 3. d, Reg-1 gene (GenBank accession no. D14010) contains 3433 nucleotides with six exons and five introns. The chip targets nucleotides 3251–3397 in exon six. RT-PCR amplifies 295 bases spanning exons three through five. The insert shows the ethidium bromide-stained 2% agarose gel with a 50-bp marker in lane 1, a 295-bp positive band in lane 2, and a negative control sample in lane 3.
FIGURE 2. Cryostat sections of MLN stained for esterase 5 (a), 12 (b), 18 (c), and 28 (d) days after CP infection. CD11c/CD8α staining profile of MLN cells (e) that were sorted into CD11c+CD8α+ (f) and CD11c+CD8α− (g) subsets. The cells in f and g were cytocentrifuged before esterase staining and the arrowhead indicates diffuse esterase staining while the arrow shows esterase granules. h, Zymogram of adhered splenic macrophages showing principal mononuclear cell esterase isoform (1), metrizamide gradient-purified DCs from MLN (2), and intestinal epithelial cells showing gut esterase isoforms (3). Cells staining for CP Ag are present in the MLN (i) 12 days after infection but are not seen in cervical node (j). At ×100, the esterase staining of MLN (k) and CP immunofluorescence (l) views are seen to stain different populations of cells when applied in combination. The arrow indicates a CP-positive cell in the two views. Original magnifications: a–d, i, and j = ×25; k and l = ×100. m, RT-PCR for CP β-tubulin in 2% agarose gel. Lane 1, Bright band shows 350-bp band of 50-bp m.w. marker. Lane 2, RT-PCR product of CP oocysts. Lane 3, negative control. Lanes 4–10, A representation of PCR products.
per animal, \( n = 3 \) in uninfected controls to 4.8 (SD 0.5) per \( \times 100 \) field (20 fields per animal, \( n = 3 \)) 12 days after infection by CP. There was no difference in frequency of esterase-positive cells between uninfected controls and animals infected for 5 days. Fig. 2, a–d, show sections for animals 5, 12, 18, and 28 days after infection.

Esterase-positive cells were of a CD11c+CD8α+ phenotype

To determine whether the esterase-positive cells seen in MLN could be DCs, we collagenase-digested MLN and centrifuged the suspension on a metrizamide gradient. Between 76 and 84% of the cells layering over the 14.5% metrizamide had the CD11c+ phenotype of DCs (Fig. 2c), and these cells were further sorted into CD8α-positive and -negative subsets. The sorted cells were cyto-centrifuged and then stained for esterase. Fig. 2f shows two different kinds of esterase staining in the CD11c+CD8α+ subset. One is diffuse (shown by arrowhead) and the other is small distinct granules (shown by arrow). The CD8α− subsets also had esterase-positive cells but were fewer (Fig. 2g). The percentage of cells with any type of esterase staining in cytospin preparations of the CD8α− subset was 34 (\( n = 3 \), range 27–37%), compared with 15% in the CD8α+ (\( n = 3 \), range 8–18%). The CD11c+CD8α− phenotype has previously been associated with DCs that elicit Th-1-type responses by CD4+ T cells (19). To find out whether CP-containing cells were esterase-positive, the slides were dual-stained for CP and esterase. CP-containing cells were seen in both the CD8α− and CD8α+ subsets. However, cells that stained for CP were negative for esterase as was in the sections of MLN.

Intestinal isoforms of esterase were detected in the DCs from MLN

Different isoforms of esterase are known to occur in cells from intestinal epithelium and bone marrow (2). To determine whether one or both isoforms would occur in the MLN, we electrophoresed Triton X-100 tissue lysates of MLN cells that had been enriched for the DC subset by centrifugation on a 14.5% metrizamide gradient (20). The gels were then developed for esterase activity with an α-naphthyl butyrate substrate. The results (Fig. 2h, lane 1) show predominance of a higher m.w. double band in adhered splenic mononuclear cells while gut (Fig. 2h, lane 3) has two lower m.w. bands but lacks the mononuclear isofom. MLN (Fig. 2h, lane 2) has both the higher and lower m.w. bands that were seen in mononuclear and intestinal cells. Insufficient esterase activity was detected in lysates of whole MLN (without metrizamide gradient centrifugation) for analysis.

MLN cells that stained for CP by immunofluorescence did not stain for esterase

A CP infection is followed by the appearance in the MLN of cells stained by FITC-Ab for CP Ag (Fig. 2i). The mean frequency for CP Ag-positive cells at 12 days was 0.9 (SD 0.2) per \( \times 100 \) field for two mice examined, while CP Ag-positive cells were not seen in three uninfected mice (Fig. 2j). When the esterase stain is combined with the immunofluorescent CP stain, separate populations staining for one or other marker are distinguished (Fig. 2k, lane l). So, while a CP infection increased the number of DCs with esterase in the MLN, the DCs that contain CP Ag did not have intact esterase.

CP mRNA was present in intestinal epithelium but not MLN following infection

Signal for the 282-bp β-tubulin mRNA of CP was detected by nested RT-PCR in the intestinal epithelium of CP-infected DOTg mice between 5 and 18 days after infection (Fig. 2m, lanes 5–9). In Fig. 2m, lane 10 shows the 369-bp DNA signal for the β-tubulin gene of CP with a single intron seen in epithelial extracts 18 days after infection, while samples at 28 days were negative for β-tubulin mRNA and DNA (not shown). mRNA extracted from the MLN of CP-infected DOTg mice was consistently negative for a CP signal in the 5- to 28-day period after infection that was studied (not shown). The absence of a β-tubulin signal from these nodes indicates that the cells that were stained by FITC-Ab for CP Ag in the MLN of infected mice in the weeks following infection had not retained intact CP nucleic acids.

mRNAs for pancreatic proteins increased in MLN following CP infection

Twelve days following a CP infection the levels of mRNA for trypsin, chymotrypsin, colipase, and reg-1 in the MLN increased by 100-fold or greater (Fig. 2n). Data in this figure are limited to the 11 mRNA species showing the greatest relative increases, adjusted for the expression of the same mRNA in uninfected MLN. Microarray image intensities for the 20 probe sets for the four principal mRNAs are shown in Fig. 2o. The increases seen on day 12 occur in the upper (perfect match) and not in the lower (mismatch) probe cells. These four mRNAs were also detected by RT-PCR in MLN of infected animals (Table I). These results are novel in that they point either to the transport of intestinal mRNA species to the lymph node by migrating cells or, conceivably, to the synthesis of gut epithelial-associated mRNAs by cells that normally reside in the MLN. To determine whether intestinal epithelial cells reached the MLN intact, frozen sections of nodes were stained for the epithelial cell marker, cytokeratin. Positive staining was observed only for fragments of cells, within mononuclear cells, but no intact epithelial cells were seen in the MLN (not shown). This cytokeratin staining result argues against the passage of intact epithelial cells from the intestine to the MLN.

CP infection increased intestinal expression of pancreatic proteins

Oligoarray analysis of mRNA species from intestinal epithelial cells at two time points after CP infection showed a 3- to 7-fold increase of expression of C-reactive protein-ductin, but there was little if any increase in expression of reg-1 protein, trypsin, and chymotrypsin mRNA (Table II). mRNAs for trypsin, colipase, and reg-1 were found in intestinal epithelial cells of one of four uninfected mice by RT-PCR. The frequency with which these mRNAs were found increased to peak between 12 and 15 days after CP infection (Table II). The difference in the combined frequency of positive results by RT-PCR between uninfected animals (3 of 15) of gut epithelial cell samples of mice autopised on days 5 (lanes 4–6), 8 (lanes 7 and 8), 12 (lane 9), and 18 (lane 10). Bands of β-tubulin message (282 bp) (lanes 5–9) and a 369-bp band of β-tubulin DNA in a sample of day 18. n, Expression of eight mRNA species in MLN of CP-infected mice expressed as fold increase over uninfected mice (defined as 1) as measured by Affymetrix Mu11k oligoarray. o, Fluorescent image of oligoarray hybridized with cRNA from MLN of animals uninfected or infected for 5, 12, 18, and 28 days. The 20 perfect matches on day 12 with bright signals for trypsin, chymotrypsin, colipase, and reg-1 (GenBank accession nos. AE000664, AA244542, AA611440, and D14010) are shown above the 20 mismatches in the lower 20 squares. Probe sets outside the white lines are for genes that did not vary in expression according to infection status.

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Table I. Number of MLN and gastrointestinal epithelial samples positive for pancreatic protein mRNA by RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession No.</th>
<th>Uninfected (n = 5)</th>
<th>5–8 (n = 8)</th>
<th>12–15 (n = 6)</th>
<th>18 (n = 5)</th>
<th>28 (n = 2)</th>
<th>Uninfected (n = 4)</th>
<th>5–8 (n = 9)</th>
<th>12–15 (n = 6)</th>
<th>18 (n = 5)</th>
<th>28 (n = 3)</th>
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<td>Trypsin</td>
<td></td>
<td>0</td>
<td>7</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td></td>
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<td>3</td>
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<td>0</td>
<td>0</td>
<td>6</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Colipase</td>
<td></td>
<td>0</td>
<td>8</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>9</td>
<td>6</td>
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<tr>
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<td></td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>Absent</td>
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<td>1</td>
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<td>3/12</td>
<td>17/36</td>
<td>22/24</td>
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Table II. Expression of pancreatic protein mRNA in gut epithelial cells after CP infection

<table>
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<tr>
<th>Gene Name</th>
<th>Accession No.</th>
<th>Detection in Uninfected Animals</th>
<th>Fold Change 5 Days after Infection Over Uninfected Control</th>
<th>Fold Change 12 Days After Infection Over Uninfected Control</th>
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<td>C-reactive protein-ductin</td>
<td>U37438</td>
<td>Present</td>
<td>3.21</td>
<td>7.01</td>
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<td>Reg-1</td>
<td>D14010</td>
<td>Present</td>
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<td>Trypsin</td>
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<td>−1.3</td>
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<td>aa244542</td>
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<td>Colipase</td>
<td>a611440</td>
<td>Present</td>
<td>−0.97</td>
<td>−1.6</td>
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</table>

* Fold change calculated as described in MicroArray Suite software from infected and uninfected animal data.

b mRNA expression in control was higher than in experimental animal.

c mRNA expression was absent in control.
intestinal esterase in the MLN argues for some DC transport occurring without digestion. In the case of trypsin, increased proteolytic activity in lysates of mesenteric node cells was revealed by enterokinase. This finding would be consistent with the possibility that the trypsinogen was translated either in epithelial cells or in the DCs themselves. An epithelial cell origin would imply that trypsinogen was not entirely degraded during transport to the MLN. Because cytokeratin staining did not show intact epithelial cells in MLN, it is their products (mRNAs and trypsinogen) that can escape degradation. The failure of DCs to degrade Ags fully has precedent (22).

The mRNAs for trypsin, chymotrypsin, colipase, and reg-1 were detected in most infected intestinal epithelial cells by RT-PCR. Oligoarray analysis did not detect trypsin or chymotrypsin in intestinal epithelial cells of uninfected animals, and RT-PCR was positive for trypsin, colipase, and reg-1 in one of four uninfected mice. Infection did not increase trypsin, chymotrypsin, colipase, or reg-1 mRNAs detected by oligoarray analysis, although the frequency of positive signals by RT-PCR was increased in the infected animals. Because the RT-PCR appeared to be a more sensitive indicator of mRNA expression, the use of oligoarray analysis on intestinal epithelial cells was limited to two time points.

mRNA for β-tubulin of CP was present in intestinal epithelial cell preparations from infected mice, as was expected. Nevertheless, we did not find mRNA for CP β-tubulin in the MLN. The cells in MLN that stained for CP Ag were negative for esterase inclusions, while those that contained esterase inclusions did not stain for CP. The simplest explanation for this difference is that DCs that engulf a CP-parasitized epithelial cell respond by degrading the contents of their phagocytic vacuole, while DCs that engulf uninfected epithelial cells allow the contents of their phagocytic vacuoles to remain intact. The absence of β-tubulin message from the MLN of infected mice may therefore result from fusion of lysosome with the parasite-containing phagosome of a DC. If this view is correct, it could be peptides derived from CP that are detected by immunofluorescent staining within MLN. A polyclonal Ab was used for this staining and molecules as small as haptenated amines, within the size range of products of phagolysosomal digestion, are known to suffice as Ab epitopes (23).

CP infections interfere with stimulated apoptosis of infected cells in vitro (24) while apoptosis of neighboring, uninfected, epithelial cells is increased (25). If a CP infection increases the apoptosis of uninfected epithelial cells in vivo, these cells could be engulfed by DCs in the lamina propria and some of their mRNA and proteins could be transported intact to the MLN. Twelve days after CP infection, the frequency of esterase-positive cells in the MLN increased to outnumber cells staining by immunofluorescence for CP Ag. Enzyme from uninfected epithelial cells that have apoptosed in response to CP infection of a neighboring cell is a possible source for this esterase. If this is the case, the apoptotic cells would most likely be transported to the MLN by DCs. This transport could account for the increased mRNAs for trypsin and other intestinal products that we detected in MLN of infected mice by the oligoarray analysis. The absence of CP β-tubulin mRNA from MLN obtained after CP infection, despite its presence in gut epithelial cells, most likely results from the active degradation of the contents of phagosomes by DCs that have taken up infected epithelial cells. The lack of DCs that stain for both esterase granules and CP from the MLN of infected mice is significant because it suggests that DCs that engulf infected cells respond by degrading endocytosed proteins. CP-derived CpG dinucleotides (26) might trigger the degradation of endocytosed intestinal epithelial cells.

Our results suggest that some components of uninfected but apoptotic intestinal epithelial cells may remain intact on their arrival in the MLN. Perhaps this allows tolerance to be maintained to normal cell constituents during an infection. The degradation of the products of infected epithelial cells should, in contrast, allow for their loading onto histocompatibility Ags and the generation of a specific immune response.

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References


