Innate BALB/c Enteric Epithelial Responses to *Trichinella spiralis*: Inducible Expression of a Novel Goblet Cell Lectin, Intelectin-2, and Its Natural Deletion in C57BL/10 Mice


*J Immunol* 2004; 173:1894-1901; doi: 10.4049/jimmunol.173.3.1894

http://www.jimmunol.org/content/173/3/1894

---

**References**
This article cites 26 articles, 9 of which you can access for free at:
http://www.jimmunol.org/content/173/3/1894.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Innate BALB/c Enteric Epithelial Responses to Trichinella spiralis: Inducible Expression of a Novel Goblet Cell Lectin, Intelectin-2, and Its Natural Deletion in C57BL/10 Mice


Infection of mice with the nematode parasite Trichinella spiralis induces changes in the proteome of the jejunal epithelium, including substantial up-regulation of a novel variant of interlectin. In this study we sequence this novel lectin, termed intelectin-2, and compare expression levels during T. spiralis infection of resistant (BALB/c) with susceptible (C57BL/10) mouse strains.

Intelectin-2 was cloned and sequenced from BALB/c mRNA extracted on day 14 of infection, and was found to have 91% amino acid identity with interlectin (within our study termed interlectin-1). Intelectin-2 transcripts were up-regulated early (day 3) during infection with T. spiralis in BALB/c mice, suggesting an innate response, and levels remained high through to day 14 (time of parasite rejection). Immunohistochemistry of jejunal sections with a rabbit polyclonal Ab to Xenopus laevis 35-kDa cortical granule lectin (XL35; 68% identity with interlectin-2) followed a similar pattern, with intense labeling of goblet and Paneth cells at day 14. However, interlectin-2 transcripts and protein were absent, and immunohistochemistry negative when C57BL/10 mice were infected with T. spiralis. Genomic PCR and Southern blotting confirmed that the intelectin-2 gene is absent from the C57BL/10 genome. The presence of intelectin-2 in resistant BALB/c mice, its absence from the susceptible C57BL/10 strain and the kinetics of its up-regulation during T. spiralis infection suggest that this novel lectin may serve a protective role in the innate immune response to parasite infection. The Journal of Immunology, 2004, 173: 1894–1901.

The mucus layer that coats and protects the simple epithelia of the gut and lung is composed of mucin glycoproteins and additional components that help to protect the epithelium against invasion by pathogens. In the gastrointestinal tract, such components include antibacterial defensins (1), lysozyme (2), trefoil factors (3), and members of the galactose-binding lectin family (galectins) (4). The mucus layer plays a significant role in the response to intestinal nematodes in which the worms become physically entrapped in mucus and are expelled (reviewed in Ref. 5). Mucus trapping is associated with changes in the physical properties of mucus, and worm expulsion in primary infection is associated with goblet cell hyperplasia and augmented mucus synthesis (5). Certain lectins, such as the sheep galectin, ovgal11, also appear to be up-regulated in response to parasite infection (6). Proposed roles for such lectins in the luminal environment include opsonization of bacteria and binding to mucins, to alter the viscoelastic properties of mucus itself (4, 6).

We recently described (7) the appearance of a novel lectin in extracts of exfoliated jejunal epithelium from BALB/c mice infected with the nematode parasite Trichinella spiralis. This protein, which was absent in uninfected mice, appeared to be a closely related variant of mouse interlectin, which is expressed by small intestinal Paneth cells (8). Mouse interlectin belongs to a small family of related lectins, with members also described in Xenopus laevis (9–11) and in human (12, 13). Various roles have been postulated for the Xenopus lectins in fertility and development (10, 11). Human interlectin (12) (also known as HL-1 (13) and lactoferrin receptor (14)) was expressed as a recombinant protein, and found to bind oligosaccharides, including galactofuranose moieties and galactofuranose-containing bacterial cell wall preparations (12). Hence, a role in recognition of bacteria was proposed. This lectin is expressed in the small intestine and colon, but is also highly expressed in heart tissue (12, 13), and has been immunolocalized to endothelium (13). The second human interlectin variant, HL-2, was found to be expressed specifically in the small intestine (13).

The aim of this study was to define the site of expression of the novel mouse interlectin (7) and its relationship to the development of resistance against the intestinal nematode T. spiralis. We have sequenced this interlectin variant and compared its expression with that of the previously described mouse interlectin (8). Our results show that the expression of the interlectin variant is confined to the ileum in normal BALB/c mice, but that during infection there is strong jejunal expression in goblet and Paneth cells that peaks at the time of worm expulsion. In contrast, the genome of the C57BL/10 strain, in which expulsion of T. spiralis is delayed (15), lacks this gene and there is no detectable expression in the gut.
Breedling Laboratories (Margate, U.K.); C57BL/6j and C57BL/10 mice by Harlan Olac (Biocenter, U.K.); and 129S6/SvEv mice were a gift from R. Thresher (University of Cambridge, Cambridge, U.K.).

**Parasite infections and tissue preparation**

Maintenance, infection, and recovery of T. spiralis larvae was based on standard methods (16). Sex-matched 8- to 15-wk-old BALB/c or C57BL/10 mice were infected by gavage with 200–300 muscle larvae per mouse in 0.2 ml of PBS/1% agar. Larvae were freshly isolated from muscle cysts from 30 to 90 day infected BALB/c mice. To check infections were successful, adult worms were isolated from groups of four to five of the mice at 6–7 days after infection as previously described (17). For RT-PCR analysis, mice were killed on day 14 following infection (worm expulsion phase) and epithelium exfoliated by EDTA perfusion as previously described (18, 19). Samples were also prepared from age-matched uninfected controls. Additional infections were set up in age- and sex-matched BALB/c mice, which were killed on days 1, 3, 7, 14, 28, and 56 postinfection (p.i.) for epithelium isolation. Stripped epithelium was transferred directly into TriReagent (Sigma-Aldrich, Poole, U.K.) for RNA isolation as previously described (19) or processed for protein extraction as described below. Subsequent purification and removal of contaminating DNA using DNA-free DNase (Ambion, Austin, TX) has been previously described (17). To check the purity of stripped epithelium preparations, samples were simultaneously treated with proteinase K, trypsin, and perchloric acid, and the isolated RNA was reverse transcribed using 2.5 μM (dT)18 as previously described (20). One-twentieth the volume was amplified by PCR using gene-specific primers for intelectin-1 (ILN1P6F/ILN1P7R) and intelectin-2 (ILN2P6F/ILN2P7R), or for the housekeeping gene GAPDH as previously described (17) with equivalent quantities of nonreverse transcribed RNA as negative controls. Reaction conditions were optimized to ensure the number of cycles used correlated with the amplification stage of the PCR. Amplifications for intelectin-1 and intelectin-2 were conducted for 40 s at 94°C, 40 s at 60°C, and 120 s at 72°C for 35 cycles (32 cycles for epithelial RNA preparations). Amplifications for GAPDH were conducted for 40 s at 94°C, 40 s at 60°C, and 60 s at 72°C for 35 cycles, with a final magnesium concentration of 1.5 mM, pH 8.3. To confirm specificity of the intelectin-1 and intelectin-2 PCR products, restriction digests of 4 μl of each 50-μl PCR product were conducted with EcoRI, which only cuts the intelectin-2 PCR product to give digests of 200 bp and 660 bp, and SacI, which only cuts the intelectin-1 PCR product to give digests of 472 bp and 388 bp (see Fig. 1). In addition, single PCR products from two day 14 epithelium samples amplified using ILN1P6F/ILN1P7R or ILN2P6F/ILN2P7R were purified and sequenced as described to verify the specificity of the primers. PCR products were visualized as earlier described and hybridized with a common probe for both intelectins, ILNR2, to detect any weak bands.

**Detection of transcripts by semiquantitative RT-PCR**

Total RNA (1 μg) from isolated epithelium, or other tissues as already described, was reverse transcribed using 2.5 μM (dT)18 as previously described (20). One-twentieth the volume was amplified by PCR using gene-specific primers for intelectin-1 (ILN1P6F/ILN1P7R) and intelectin-2 (ILN2P6F/ILN2P7R), or for the housekeeping gene GAPDH as previously described (17) with equivalent quantities of nonreverse transcribed RNA as negative controls. Reaction conditions were optimized to ensure the number of cycles used correlated with the amplification stage of the PCR. Amplifications for intelectin-1 and intelectin-2 were conducted for 40 s at 94°C, 40 s at 60°C, and 120 s at 72°C for 35 cycles (32 cycles for epithelial RNA preparations). Amplifications for GAPDH were conducted for 40 s at 94°C, 40 s at 60°C, and 60 s at 72°C for 35 cycles, with a final magnesium concentration of 1.5 mM, pH 8.3. To confirm specificity of the intelectin-1 and intelectin-2 PCR products, restriction digests of 4 μl of each 50-μl PCR product were conducted with EcoRI, which only cuts the intelectin-2 PCR product to give digests of 200 bp and 660 bp, and SacI, which only cuts the intelectin-1 PCR product to give digests of 472 bp and 388 bp (see Fig. 1). In addition, single PCR products from two day 14 epithelium samples amplified using ILN1P6F/ILN1P7R or ILN2P6F/ILN2P7R were purified and sequenced as described to verify the specificity of the primers. PCR products were visualized as earlier described and hybridized with a common probe for both intelectins, ILNR2, to detect any weak bands.

**Long-template (LT)-PCR**

To confirm the presence or absence of intelectin-1 and intelectin-2 sequences in the genome of BALB/c, C57BL/10, and 129/SvEv strains of mice, genomic DNA was prepared using a Qiagen DNeasy Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. LT-PCR was conducted using methods and PCR conditions previously described, but using an annealing temperature of 60°C (20). The primers used were ILNP6F/ILNR1 for intelectin-1, and ILNP6F/ILNP25 for intelectin-2. These primers were predicted from the intelectin-1 genomic sequence to amplify LT-PCR products of 3185 and 3146 bp from the intelectin-1 and intelectin-2 genes, respectively. PCR products were hybridized with ILNF1, a common probe for both genes.

**Primers and probes**

The sequences of the primers were the following: ILNF1 (sense) TCTC TATTCTCTGGCGCAG; ILNR1 (antisense) GCTGACTGGACCATC GATC; ILNR2 (antisense) GGAAGATCCTCCCTCACCAGCA; ILNP25 (sense) TGGTCTCT ACCGAAAGATTCGGG; ILNP23 (antisense) CTTTCTCATATTTTCAC CGGATA; ILNP24 (antisense) CTTGCTGTGATGCGGCGAGT GLN25 (antisense) CATGTTGTTCTGGCCACACTA; ILNP16 (sense) TTAT CATGTTGTTGTACCAGAGG; ILNP17R (antisense) GAGTTCACAT CATCCCAATC; ILNP26F (sense) TTATCATGATTGGCCAGAGATG; ILNP27R (antisense) GAGTTGCTATCATCGCCATGTC; INTLF1 (sense) TGGGCGAATTTCTTCTTCTTCTC; INTLR1 (antisense) CTGGTGTAC CCATTGCTATCCATCT; Primers and internal complementary probes were designed with the help of the “Raw Primer” program available at http://ales.med.unm.edu/rawprimer.html.

**Southern blot analysis**

Genomic DNA was prepared from mouse spleens, digested with HindIII, and transferred to Hybond-N+ membranes (Amersham Biosciences, Piscataway, NJ) according to standard protocols. The blot was then hybridized to an intelectin-1 probe, random-prime labeled (Amersham Biosciences) with [α-32P]dCTP. This probe consists of 812 bp of the intelectin-1 coding region (NM_010584; bases 1895–1975 inclusive) and was generated by PCR using the primers INTLF1 and INTLR1. For hybridization, membranes were

Abbreviations used in this paper: p.i., postinfection; 2D-GE, two-dimensional gel electrophoresis; LT-PCR, long-template PCR.
prehybridized at 65°C in a buffer containing 5× SSC, 5× Denhardt’s solution, 0.5% SDS, and 50 μg/ml sheared salmon sperm DNA (Stratagene, La Jolla, CA), labeled probe added, and hybridization continued overnight. The membranes were sequentially washed at 65°C in low (2× SSC, 0.1% SDS), medium (1× SSC, 0.1% SDS), and high (0.1× SSC, 0.1% SDS) stringency buffers as required.

Two-dimensional gel electrophoresis

Jejunal epithelium was obtained from BALB/c and C57BL/10 mice at day 14 of *T. spiralis* infection, as previously described. To inactivate rapidly any residual proteases, samples were extracted with 1 ml of 60 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, preheated to 95°C. The samples were then heated on a boiling water bath for 5 min, followed by cooling and centrifugation at 20,000 × g for 30 min at 15°C. Aliquots of extracts (typically 5–15 μl) were diluted in rehydration buffer (8 M urea, 2% CHAPS, 0.4% DTT, 0.2% Bio-Lytes 3-10 (Bio-Rad, Hercules, CA)) to give a final SDS concentration of <0.1% and CHAPS:SDS ratio of >2:1. Samples were then analyzed by 2D-GE as previously described (7).

Briefly, first dimensional separation was with immobilized 7-cm or 17-cm pH gradient 3-10 strips (Bio-Rad) with focusing for 20,000 Vh or 45,000 Vh, respectively, using a Bio-Rad Isoelectric Focusing IEF cell. Strips were then equilibrated and run on 12% acrylamide SDS gels as previously described (7). Gels were then either stained with silver nitrate (PlusOne method; Amersham Biosciences) followed by imaging with a flatbed scanner (ImageScanner; Amersham Biosciences) or transferred to membrane (Immobilon P; Millipore, Bedford, MA) for Western blotting.

Western blotting

Following transblotting to Immobilon P membrane, blots were blocked with 50 mM Tris-HCl, pH 7.5, 0.15M NaCl, 1% milk powder, 0.5% Tween 80. Blots were then incubated for 1.5 h with either 1 μg/ml rabbit anti-XL35 Ig (13) or 1 μg/ml control rabbit IgG in blocking solution. Following washing, blots were then treated for 1 h with mouse monoclonal anti-rabbit IgG alkaline phosphatase conjugate (Sigma-Aldrich; 1/20,000), then washed and developed with 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/NBT.

Immunostaining with anti-XL35 Ab

Samples of small intestine were collected from BALB/c, 129/SvEv, and C57BL/10 mice at days 0 and 14 of *T. spiralis* infection (see above), and further samples from BALB/c mice at day 7 and 28 of infection. Tissues were fixed in 4% paraformaldehyde, processed into paraffin blocks and sections (4 μm) were made onto coated slides (Snowcoat X-tra; Surgipath, Wilmington, MA, Canada). Sections were dewaxed in xylene and endogenous peroxidase activity blocked by treatment with hydrogen peroxide. After rehydration, sections were treated with 0.1% trypsin in 1 mM Tris-HCl, pH 7.5, 0.1% CaCl2 at 37°C for 20 min. Slides were then washed, blocked with PBS containing 0.5 M NaCl and 0.5% Tween 80, and incubated with anti-XL35 Ig (13)(1 μg/ml in blocking buffer) or control rabbit IgG (1 μg/ml) for 1 h at 21°C. After treatment with biotinylated donkey anti-rabbit IgG (BA4001, 1/400; Vector Laboratories, Burlingame, CA), followed by avidin-HRP conjugate (ABC kit; Vector Laboratories) sections were stained with diaminobenzidine, counterstained with hematoxylin, then dehydrated and mounted.

Results

Cloning and sequencing

Direct sequencing of the PCR product from primers ILNF1 and ILNR2 fortuitously gave only the intelectin-2 sequence, rather than the human analogues HL-1 and HL-2, and the frog analogue XL35, with which it has 91%, 79%, 77%, and 68% amino acid identity, respectively. Regions of the intelectin-2 sequence are indicated that correspond to the tryptic mass fingerprinting of the corresponding 2D-GE spot.

Differential PCR for intelectin-1 and intelectin-2

To investigate the expression of intelectin-1 and intelectin-2 in uninfected and *T. spiralis*-infected jejunal epithelium, specific primers were designed. Amplifications between forward primers ILN1P6F and ILN2P6F, specific for intelectin-1 and intelectin-2, respectively, and common reverse primer ILNR2, gave products of 860 bp (Fig. 3A). A single PCR product from both sets of primers was partially sequenced to confirm their specificity for intelectin-1 and intelectin-2. It was noted that although intelectin-1 was expressed in both uninfected and infected epithelium, transcripts for intelectin-2 were only detected in infected samples.

To confirm the identity of all PCR products generated by the ILN1P6F and ILN2P6F primers, restriction digests were conducted with SacI or EcoRI, which have unique cleavage sites in the intelectin-1 and intelectin-2 sequences, respectively (Fig. 1). Restriction enzyme digests of the PCR products are shown in Fig. 3A. SacI digestion produced restriction fragments of the expected size (472 bp and 388 bp) only from intelectin-1 PCR products, whereas EcoRI produced the expected restriction fragments only from intelectin-2 PCR products.

Anatomical distribution of intelectins in the mouse

RNA samples from various anatomical sites were analyzed by RT-PCR for intelectin-1 and intelectin-2 (Fig. 3B). The distribution of intelectin-1 transcripts appeared to be largely restricted to the intestine, and in particular, no transcripts were detected in heart tissue, in contrast to the distribution of human intelectin (12). Intelectin-2 was even more restricted in its distribution, only being detected in the ileum of uninfected mice. The effect of infection was negligible on intelectin-1 distribution, apart from a possible up-regulation in gastric tissues. However, intelectin-2 expression was markedly increased on infection, with a strong RT-PCR signal being detected in small intestine and colon but not in stomach (Fig. 3B).

Intelectin-1 transcripts were abundant in uninfected jejunal epithelium as detected by RT-PCR, and remained at high levels throughout infection with *T. spiralis* (Fig. 3C). Intelectin-2 transcripts were detected neither in uninfected jejunum nor at day 1 of infection, but were up-regulated by day 3 p.i. and strongly expressed at days 7 and 14. Intelectin-2 transcripts were markedly reduced at day 28, and were undetectable in samples collected from day 56 p.i.

2D-GE and Western blotting

2D-GE resolved intelectin-2 in extracts of *T. spiralis*-infected (day 14) BALB/c epithelium as a complex of 4 or 5 spots (Fig. 4A) as previously described (7). Western blotting of such separations with mouse intelectin-2 transcripts were marked and were undetected in samples collected from day 56 p.i.
The response of C57BL/10 mice, which reject T. spiralis from the gut much slower than the BALB/c strain, was compared. Extracts from day 14 of T. spiralis infection did not exhibit intelectin-2 spots by 2D-GE (Fig. 4B), and Western blotting with anti-XL35 Ig was negative (data not shown). This suggested that intelectin-2 expression was much reduced or absent in the C57BL/10 strain on T. spiralis infection.

Immunostaining with anti-XL35 Abs

Jejunum from day 14 of T. spiralis infection in BALB/c, 129/SvEv, and C57BL/10 mice was immunostained with polyclonal rabbit anti-XL35 Ig. Immunostaining of goblet and Paneth cells was observed in infected BALB/c and 129/SvEv mice (Figs. 5, A and B, respectively), but not in C57BL/10 mice. Normal rabbit IgG controls were negative (data not shown). Anti-XL35 immunostaining of BALB/c jejunum from days 0, 7, 14, and 28 of T. spiralis infection (Fig. 5, D, E, F, and G, respectively) showed that staining was absent in uninfected jejunum (Fig. 5D), despite the presence of high levels of transcripts for intelectin-1. However, staining was evident at days 7 and 14 of infection in goblet cells (Fig. 5, E and F, respectively) and Paneth cells (data not shown). Significantly, Fig. 5, E and F showed immunoreactive material being released apically from goblet cells into the lumen. At day 28 (Fig. 5G), following resolution of infection, there was little remaining evidence.

FIGURE 1. cDNA sequence encoding mouse intelectin-2. The cDNA sequence obtained for mouse intelectin-2 (mITLN2) is aligned with the published sequence for mouse intelectin-1 (mITLN1) (8). Locations of sequencing primers (see Materials and Methods) are underlined. The SacI and EcoRI restriction sites are indicated, which selectively cut intelectin-1 and intelectin-2, respectively. Start and stop codons are shown in upper case.
of anti-XL35 immunoreactivity in goblet cells. Normal rabbit IgG controls from day 0 and day 14 of infection were also negative (Fig. 5, H and I, respectively). The staining pattern with anti-XL35 is therefore consistent with the expression of intelectin-2, as detected by RT-PCR and 2D-GE, whereas intelectin-1 could not be detected when using this Ab to immunostain sections that nonetheless express high levels of intelectin-1 mRNA transcripts.

Comparison of intelectin-1 and intelectin-2 expression by BALB/c, C57BL/10, and 129 strains of mice

Using primers specific for intelectin-1 and intelectin-2 (see previous), RT-PCR was conducted on RNA from the jejunal epithelium of uninfected and T. spiralis-infected (day 14) C57BL/10 mice (Fig. 6A). As with BALB/c mice, intelectin-1 was strongly expressed by C57BL/10 mice at both day 0 and day 14. However, no intelectin-2 transcripts were noted for C57BL/10 mice at any stage of infection, in agreement with the lack of intelectin-2 protein as previously shown.

The full genomic sequence of the closely related C57BL/6J strain (provided by the Mouse Genome Sequencing Consortium at http://www.ensembl.org/Mus_musculus with version 16.30.0, release date 06/05/03) indicated a complete absence of the intelectin-2 gene in this strain. The intelectin-1 gene was present, and 20 kb of genomic DNA sequence encompassing the intelectin-1 gene was downloaded for sequence analysis. This predicted that a single band of ~5.5 kb would be detected in a Southern blot of HindIII-digested DNA, using an 812-bp intelectin-1 cDNA probe.

Therefore the corresponding Southern blot was performed, comparing the genomic organization of intelectin gene(s) in BALB/c and 129/SvEv strains, which respond rapidly to T. spiralis (22), with C57BL/6J and C57BL/10 strains, in which worm expulsion is delayed (23). Southern blot analysis indicated the presence of the expected 5.5-kb restriction fragment in DNA from C57BL/10 and C57BL/6J mice (Fig. 6B), confirming the presence of a single intelectin-1 gene in these strains. However, an additional band of ~6 kb was detected in DNA from BALB/c and C57BL/10 mice (Fig. 6B), suggesting that a second intelectin gene is present in these mice. This observation is consistent with our 2D-GE (Fig. 4), immunostaining (Fig. 5), and PCR (Fig. 6A) data, which demonstrates that a second form of intelectin is expressed in BALB/c mice but not in C57BL/10 mice. As the 5.5-kb band is common to all tracks in the figure, it is reasonable to suggest that it represents the intelectin-1 gene.

A further investigation using LT-PCR was conducted on genomic DNA from C57BL/10, BALB/c, and 129/SvEv strains. The PCR primers were designed to span two exons, based on the genomic sequence of intelectin-1. As expected, all three strains gave the appropriate PCR product for intelectin-1 (Fig. 6C), but was not detected for intelectin-2.
the intelectin-2 product was only observed for BALB/c and 129/SvEv mice. Therefore, we conclude that the intelectin-2 gene is absent from C57BL/10 mice, and that it is present in both BALB/c and 129/SvEv strains.

Discussion
We describe in this report a novel epithelially derived lectin, intelectin-2, that is exclusively expressed in the intestine in BALB/c and 129/SvEv mice. It is located in the goblet-Paneth cell population, is substantially up-regulated at the site of infection with *T. spiralis*, and levels of expression of intelectin-2 are maximal at the time of worm expulsion. In our previous study (7), intelectin-2 was found to be the second most abundant protein, after β-actin, in 2D-GE of exfoliated jejunal epithelium on day 14 or day 15 of infection. The fact that the protein is located in the two cell types that secrete apically into the gut lumen indicates that intelectin-2 is likely to be released into the gut lumen. The presence of luminal secretion was confirmed in this study by immunohistochemistry, and considering the abundance of intelectin-2, it is likely that substantial quantities of intelectin-2 are secreted into the lumen by goblet cells during the expulsion phase of *T. spiralis* infection.

Mass spectrometric analysis of tryptic digests of intelectin-2 showed that it was similar to the previously described lectin, intelectin (8). Using sequence similarities, and regions of consensus sequence between other species variants of intelectin, we determined the cDNA sequence for intelectin-2. This cDNA encodes a 313 amino acid glycoprotein with 91% identity with mouse intelectin-1.

In uninfected BALB/c mice, mRNA for intelectin-1 was expressed at high levels throughout the gut, but was not detected in significant amounts in other tissues, including the heart, in which human intelectin has been shown to be highly expressed (12). Nematode infection did not cause any apparent change in intelectin-1 expression in the gut. By contrast, intelectin-2 expression in uninfected mice was confined to the ileum, and was highly up-regulated during nematode infection, occurring throughout the gut. Increased message for intelectin-2 was noted in the jejunum as early as day 3 of infection and was high at days 7 and 14, but returned to undetectable levels at day 56, following resolution of infection.

The intelectin response to infection was also studied using an affinity-purified rabbit antiserum to the frog intelectin analogue, XL35. Western blotting 2D-GE separations of exfoliated epithelial extracts of nematode-infected jejunum with the Abs detected a 313 amino acid glycoprotein with 91% identity with mouse intelectin-1. Western blotting 2D-GE separations of exfoliated jejunal epithelium from infected mice would suggest that intelectin-1 is not present in the extracts.

The up-regulation of intelectin-2 expression as early as day 3 of *T. spiralis* infection, as well as during the expulsion phase, suggests that this lectin may be a component of the early innate response to nematode infection. It was of interest therefore to compare the expression of intelectins in the low responder C57BL/10 strain, which fail to expel *T. spiralis* efficiently (15). Comparison of 2D-GE profiles of exfoliated jejunal epithelium from *T. spiralis*-infected BALB/c and C57BL/10 mice indicated an apparent lack of up-regulation of intelectin-2 in the latter strain. This was further confirmed by the immunostaining of goblet cells and Paneth cells in sections from *T. spiralis*-infected BALB/c but not C57BL/10 mice. There was virtually no staining in uninfected mice, which is noteworthy because mRNA for intelectin-1 is highly expressed by...
Paneth cells in these mice (8). The lack of detection of intelectin-1 in uninfected mice by 2D-GE and by immunohistochemistry suggests that the protein is not present in significant amounts. This might reflect effects such as translational inhibition or a high turnover rate, or alternatively, intelectin-1 may be rapidly transformed to a form that is refractory to the analyses attempted thus far.

Therefore, it seems likely that the immunostaining of sections from infected BALB/c mice is due to the induced expression of intelectin-2.

Using RT-PCR, the pattern of expression of intelectin-1 by the C57BL/10 strain was similar to that of BALB/c mice, but we failed to detect intelectin-2 in the jejunum of both normal and T. spiralis-infected (day 14) C57BL/10 mice.

Inspection of the current release of the mouse genome (Mouse Genome Sequencing Consortium, Ensembl Mouse release 16.30.1), which is based on the C57BL/6J strain, shows only one intelectin gene, intelectin-1, located on chromosome 1H2. The intelectin-2 gene is not present in the C57BL/6J genome. By comparison, in the human genome, both intelectins (HL-1 and HL-2) are situated on chromosome 1q23.3 \( \sim 60,000 \) bp apart. Southern blotting and genomic PCR, comparing the low responder C57BL/10 strain with the high responder BALB/c and 129/SvEv mouse strains, supports the presence of the intelectin-1 gene in all three strains, and the absence of intelectin-2 in C57BL/10 mice, whereas this gene is present in the BALB/c and 129/SvEv strains. This suggests that a deletion of the intelectin-2 gene probably existed in the progenitors of the C57BL/6 and C57BL/10 strains, which both originated from the same parents.

The early expression of intelectin-2 on day 3 of infection, which is relatively soon after the infective larvae enter their intraepithelial niche in the jejunum, suggests that this is an innate epithelial response that may be independent of the adaptive immune response. It is highly unlikely that the lack of intelectin-2 in C57BL/6 and C57BL/10 mice is solely responsible for their impaired ability to reject T. spiralis because these strains differ from the BALB/c strain at many other loci (24), and exhibit important
differences in their ability to produce regulatory cytokines, such as IL-10, in response to infections (25). However, the fact that intelectin-2 is so markedly up-regulated in response to infection, becoming one of the most abundantly expressed proteins in infected epithelium (7), suggests that it may play an important role in the innate immune response to parasite infection. The putative role has yet to be determined, and it may indeed be multifunctional, including recognition of foreign carbohydrate moieties, as described for human intelectin (12), and the alteration of mucin properties, by similarity to the aggregation of the mucin-like egg jelly coat protein by the Xenopus intelectin analogue, XL35 (26).

In the few immunohistochemical sections in which worms were evident, there was no sign of anti-XL35 staining on the worm surface (data not shown). In view of the widespread up-regulation of intelectin-2 throughout the gut, rather than just at the site of infection, a role for this lectin in preserving the mucosal barrier might be more likely.

Future studies should investigate the signals responsible for up-regulating intelectin-2 expression, and whether they can induce intelectin-2 expression at mucosal surfaces other than the intestinal epithelium. Importantly, the viscoelastic properties of purified mucins in the presence or absence of intelectin-2 should be explored. Furthermore, because there is a natural knockout of the gene in C57BL/10 mice, the effect of making these mice transgenic for goblet cell-specific expression of intelectin-2, on the expulsion kinetics of T. spiralis, would demonstrate its potential role in the innate protective response against infection. It would also be important to determine whether HL-1 or HL-2, the human analogues to mouse intelectins, are regulated in a similar manner to their murine counterparts.

Acknowledgments

We thank Dr. Jeremy Brown for assistance in microscopy imaging and Judith Pate for technical assistance.

References