Isolation and Characterization of a Novel Inducible Mammalian Galectin*

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A novel mammalian galectin cDNA (ovgal11) was isolated by representational difference analysis from sheep stomach (abomasal) tissue infected with the nematode parasite, Haemonchus contortus. The mRNA is greatly up-regulated in helminth larval infected gastrointestinal tissue subject to inflammation and eosinophil infiltration. Immunohistological analysis indicates that the protein is localized in the cytoplasm and nucleus of upper epithelial cells of the gastrointestinal tract. The protein is also detected in mucus samples collected from infected abomasum but not from uninfected tissue. The restricted and inducible expression of ovgal11 mRNA and limited secretion of the protein support the hypothesis that OVGAL11 may be involved in gastrointestinal immune/inflammatory responses and possibly protection against infection.

The immune response of mammals to helminth parasite infection, like that of an allergic response, involves recruitment of lymphocytes and eosinophils to the site of infection (1). Although the coordinated events leading to leukocyte migration are being elucidated (primarily through the isolation of CC chemokines), there still remains a great deal to be defined regarding the response of infected tissue during allergergic-type immune reactions and the recruitment of inflammatory cells across epithelial surfaces.

Sheep have been used successfully in many immunological studies to analyze the immune response of large animals, including humans. The advantages of using this animal model are the comparable size and closer physiological and phylogenetic relationship with humans compared with rodent models. Haemonchus contortus is a natural nematode parasite of sheep that inhabits the abomasum (gastric stomach). Challenge infection of immunized sheep can result in massive infiltration of lymphocytes and eosinophils within abomasal tissue (1–2), indicating that this tissue may be a good source of inflammatory molecules associated with allergic-type immune responses.

The aim of the present study was to identify molecules that are specifically up-regulated in tissues subject to intense allergic-type reactions compared with unstimulated control tissues. Hence representational difference analysis (RDA;1 see Refs. 3–4) was chosen to isolate genes with up-regulated expression within H. contortus immunized and challenged abomasal tissue.

A differentially expressed cDNA clone was isolated that is clearly up-regulated in infected abomasum and intestine supporting eosinophil infiltration, compared with uninfected tissue. This clone exhibits most similarity to a family of carbohydrate-binding proteins called galectins, particularly within the putative carbohydrate recognition domain (CRD), but not with sufficient overall homology to be classified as the ovine homologue of one of the ten presently identified galectins. To conform to the current nomenclature regarding galectin family members this clone has been named ovgal11 (5). Given the tissue distribution of OVGAL11 expression, and its homology to other proteins that play an immunomodulatory role, it is anticipated that OVGAL11 will be involved in the immune response of mammals to infection of the gastrointestinal tract.

EXPERIMENTAL PROCEDURES

Abomasal Tissues and Mucus—Nematode-free adult Merino sheep (Ovis aries wethers) were immunized by oral infection with 4,000 L3 larvae of the nematode, H. contortus, once a week for 9 weeks, drenched with levamisole (10 ml/sheep; Nilverm; Cooper’s Animal Health, North Ryde, NSW, Australia), and maintained nematode-free for nine weeks. They were then challenged with 1 × 10⁹ ex-sheathed H. contortus L3 larvae by direct injection into the abomasal lumen after surgically exposing the abomasum and were killed 2 days later (I1). Another group of adult sheep was infected orally with 5,000 L3 larvae each week for 12 weeks, drenched, and rested for 12 weeks before being challenged orally with 5 × 10⁴ L3 larvae and were killed 3 (I2) or 5 (I3) days later. Control sheep were treated equally but received phosphate-buffered saline instead of larvae at the last challenge. Samples of abomasal tissue were collected for histology and RNA preparation.

Tissues from primary H. contortus infections of Merino sheep were obtained by orally infecting 3-month-old worm-free sheep once with 1–5 × 10⁴ H. contortus L3 larvae and sacrificing them 3 or 5 days post-infection. Control sheep were uninfected. Tissue was collected for RNA preparation and histology. In addition, mucus was collected from abomasal tissues of these sheep for Western blot analysis by carefully scraping mucus off the surface of the mucosa. Mucus was diluted 1/3 (w/v) with citrate buffer (10 mM citric acid, pH 5), spun at 12,000 rpm for 30 min, and the supernatant was collected and stored at −20 °C.

Intestinal Tissue Samples—Adult Merino sheep were orally infected with ~1 × 10⁵ L3 larvae of the intestinal parasite, Trichostrongylus vitrinus, and sacrificed 3 days later. Samples were collected from small and large intestine for RNA preparation and histology. Intestinal samples from uninfected sheep were taken as controls. Mesenteric lymph nodes associated with the intestinal tissue were also collected for RNA preparation.

Mammary Tissue Samples—Mature non-lactating Merino ewes were primed by weekly intramammary infusions of ~5 × 10⁹ ex-sheathed H. contortus L3 larvae. BAL, bronchoalveolar lavage; IL, interleukin; RACE, rapid amplification of cDNA ends; UTR, untranslated region; RT, reverse transcriptase; GST, glutathione-S-transferase; PCR, polymerase chain reaction; IPTG, isopropyl-1-thio-β-D-galactopyranoside; bp, base pair.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EBI Data Bank with accession number(s) AF252548.‡ To whom correspondence should be addressed. Tel.: 61-39347-4083; E-mail: e.meeusen@vet.unimelb.edu.au.

† The abbreviations used are: RDA, representational difference analysis; CRD, carbohydrate recognition domain; MAL, mammary lavage;
contortus L3 larvae in 5 ml of sterile saline for 4–5 weeks as described previously (6–7). The ewes were then reared for 3–4 weeks before being challenged by intramammary infusion of ~5 × 10^6 ex-sheathed *H. contortus* L3 larvae and were killed 4 days later, when large numbers of lymphocytes and eosinophils are known to be present in the tissues (6). Ovine monocytes and lymphocytes were isolated by treatment with one intramammary infusion of ~5 × 10^6 ex-sheathed *H. contortus* L3 larvae or were primed but not challenged. Control sheep received one intramammary infusion of sterile saline alone.

Unprimed leukocyte populations were also induced by a single infusion of *H. contortus* larvae in the mammary glands of ewes orally infected with 10^7 metacecariae of the liver fluke, *Fasciola hepatica* (7), and mammary tissues were collected 2 days later.

**Lung Tissue Samples**—Lung tissues with eosinophilic inflammation were obtained from sheep naturally infected with the small lung worm, *Muellerius capillaris*, as diagnosed by the presence of small nodules in the lung tissue and detection of nematodes in tissue sections. Histological examination demonstrated extensive infiltration of eosinophils and eosinophilic granulomas into the lung tissue of infected ewes.

**Mammary Lavage Fluid (MAL) Leukocytes**—MAL of *H. contortus* sensitized and challenged (primed), or liver fluke infected and *H. contortus* challenged (unprimed) ewes, were collected and 4 days post-challenge, respectively, by infusing 7 ml of sterile saline into the teat of the ewe to wash out the MAL within the MAL were collected 2 days later with sterile saline before being resuspended directly into guanidinium thiocyanate solution for RNA preparation. Some of the unprimed leukocytes were cultured for 5 or 24 h with 100 ng/ml of recombinant human interleukin 5 (IL-5; Genzyme Diagnostics, Cambridge, MA) before RNA extraction (7). The proportion of eosinophils in these MAL, as assessed by Giemsas-stained cytospots, varied from 52–92%. Monocytes and lymphocytes constituted the remainder of the cell population (5–32 and 3–16%, respectively).

**Bronchoalveolar Lavage Fluid (BAL) Leukocytes**—Lung worm infected or control sheep were sacrificed, and the lung and heart were removed from the chest cavity. Approximately 500 ml of saline was poured down the trachea, the lung was massaged for 2 min to ensure an even distribution of saline, and the saline/BAL fluid mixture was then drained from the lung. The procedure was repeated once. The BAL fluid mixed was centrifuged, and pelleted cells were washed twice before being used for RNA preparation. Cytospot analysis indicated that eosinophils represented 8% of cells in the BAL in infected sheep, whereas none were present in control sheep.

**RNA Preparation**—Total RNA was purified from tissues using a standard guanidinium thiocyanate, phenol/chloroform extraction (8). 0.1–1 g of tissue, 0.5–1 ml of packed larvae, or ~1 × 10^6 cells were used for RNA preparation. Poly(A)^+ RNA was purified from the total RNA using Dynal beads as instructed by the manufacturer (Dynal, Melbourne, Vic, Australia).

**Representation Difference Analysis**—RDA was performed as described previously (9). RNA prepared from uninfected abomasal tissue was used as the RNA template. RNA preparation, reverse transcription reaction, and PCR were performed as described previously (3–4). RNA prepared from uninfected abomasal tissue was used as a template for the reverse transcriptase (RT) reaction. The purity of the recombinant protein preparation was confirmed by automated sequencing after being extracted from agarose gels (PerkinElmer Life Sciences) and amplified by PCR (3). Three rounds of subtractive hybridization PCR were completed before three individual PCR bars were cloned into the pGEM®-T Easy vector (Promega) and sequenced using the Big Dye sequencing kit (PerkinElmer Life Sciences).

**Rapid Amplification of cDNA 5’-Ends (5’-RACE)**—The 5’-end of the full-length *ogsal11* cDNA was isolated using the 5’/3’-RACE kit from Roche Molecular Biochemicals and two specific primers (SP1, 5’TATCCACGGGTGCTTGGTCCTGCGCCAG and SP2, 5’TATCCACGGGTGCTTGGTCCTGCGCCAG; see Fig. 1). Approximately 2 μg of total RNA prepared from an abomasum sample collected 3 days post-*H. contortus* challenge was used as the template for the reverse transcriptase (RT) reaction. As negative controls, a no-template RT sample and a no-tail cDNA sample were also used as templates in the subsequent PCRs. PCR products generated by the second round (nested) PCRs were subcloned into the pGEM®-T Easy vector (Promega) and sequenced using the Big Dye sequencing kit (PerkinElmer Life Sciences).

**Immunohistochemistry**—Immunohistochemistry was performed on sections of Optimal cutting temperature compound (Tissue Tek; Miles Inc., Elkhart, IN)-embedded, frozen tissue blocks were cut and fixed in 95% ethanol for 10 min at 4°C or 5 μm sections of 100% ethanol fixed and paraffin-embedded tissue blocks were cut. Endogenous peroxidase was quenched by adding DAKO peroxidase blocking reagent. Primary polyclonal serum (diluted 1/500) was incubated with the slides for 1–2 h at room temperature in a humidity chamber. A 1/100 dilution of secondary antibody (horseradish peroxide-conjugated anti-rabbit IgG (DAKO, Carpinteria, CA)) for 30 min, signals were detected by ECL (Amersham Pharmacia Biotech). Fuji XAR film was exposed to the strips for 10–30 s. Rabbit polyclonal anti-IL-7 serum and polyclonal antiserum against an IL-10 GST fusion were detected by Western blot analysis and enzyme-linked immunosorbent assay (not shown).

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TABLE I
Nucleotide and amino acid identities between known galectins and ovgal11

| Species        | Name      | Nucleotide identity | Amino acid identity |
|----------------|-----------|---------------------|---------------------|
| Human chromosome 19 | galectin 10 | 48.08               | 34.30               |
| Human mouse | partial galectin 10 | 55.12\(^*\)        |                     |
| Orangutan   | partial galectin 10 | 52.20\(^*\)        |                     |
| Human rat   | galectin 4 | 42.95               | 29.20               |
| Rabbit      | galectin 3 | 38.03               | 29.20               |
| Human rat   | galectin 9 variant | 38.68               | 27.74               |
| Rat         | galectin 5 | 37.18               | 25.55               |
| Human rat   | galectin 7 | 38.89               | 25.55               |
| Human rat   | galectin 9 | 38.89               | 24.80               |
| Pig         | galectin 4 |                     | 23.36               |
| Mouse       | galectin 6 | 43.16               | 22.63               |
| H. contortus | galectin 1 | 38.25               | 22.63               |
| Rat         | galectin 7 | 36.54               | 22.63               |
| Human rat   | galectin 8 | 40.38               | 21.90               |
| Pig         | galectin 2 | 31.84               | 16.06               |
| Sheep       | galectin 1 |                     | 16.06               |
| Rat         | galectin 1 | 31.84               | 16.06               |
| Cow         | galectin 1 | 15.33               |                     |
| Human rat   | galectin 3 | 28.85               | 15.33               |
| Human rat   | galectin 1 |                     | 14.60               |

\(^*\) These identities are probably over-estimates of the total nucleotide identity given that the partial galectin 10 clones used in the comparison correspond to the highly conserved CRD.

RESULTS

Isolation of a Novel Galectin cDNA—RDA was used to amplify cDNAs expressed at greater levels in H. contortus immunized and infected abomasum compared with uninfected abomasum. This resulted in the amplification of three predominant bands. Each band was subcloned individually, and at least eight clones were sequenced from each. This led to the identification of 12 different cDNAs. A BlastN search of all the nucleotide sequences revealed that one of the cDNA amplified, a 365-bp fragment, was most closely related to galectins (Table I). This cDNA, which represented 12% of the total number of clones sequenced, demonstrated marked differential expression by Northern blot analysis in infected and uninfected abomasum and was studied further. Given that this cDNA was isolated from a whole animal model of helminth infection, the initial characterization of the molecule has been conducted in whole animal infection models. Isolation of murine and human homologues of ovgal11 may make tissue culture-based systems more amenable to the study of this molecule in the future.

From comparison to other galectins, a putative translation of the nucleotide sequence was performed. Using this putative reading frame a stop codon was found at the 3'-end of the clone only 14 bp upstream of the Sau3A restriction enzyme site that was used in the RDA. This stop codon is considered to be a preferred mammalian stop codon. However, the ATG start was considered to be a preferred mammalian stop codon (11). Hence, it was anticipated that the entire 5'-coding region was contained within the RDA product. However the 5'-end of the coding region did not contain an in-frame initiation codon (ATG) and therefore was considered incomplete. 5'-RACE was utilized to isolate the remaining 5'-sequence.

The 5'-RACE resulted in a PCR product of 195 bp that contained 66 bp of overlap with the original RDA sequence (not including the SP2 primer). The 5'-RACE product appears to contain the remaining coding sequence. An in-frame ATG codon is found 42 bp downstream from the first nucleotide (Fig. 1), and it is contained within an almost perfect Kozak sequence (12).

The short (41 bp) 5'-untranslated region of the cDNA contains an E box consensus sequence 30 bp downstream of the first nucleotide (Fig. 1). Comparison to the consensus sequence matrices in Mat inspector v2.22 indicate that this E box may be bound by the Class A basic helix loop helix transcription factor AP4.

Preliminary analysis of the ovgal11 gene has demonstrated that it displays a similar genomic organization to that of other galectins. A partial genomic clone was amplified by PCR and found to contain an intron of 549 bp (not shown). The position of the intron (300 bp downstream from the putative translation start site; see Fig. 1) matches that reported for prototype galectins (13). Given reports of strong transcriptional regulatory elements within the introns of other genes, including galectin 3 (14), we have also searched this intronic sequence for regulatory elements. Mat inspector v2.2 identified many potential regulatory sites. These include a Class B E box that may be bound by upstream stimulating factor, myc/max, or other Class B helix loop helix transcription factors and an AP1 consensus sequence.

The sequence of the entire putative coding region of ovgal11 was confirmed by sequence analysis of RT-PCR products from three sheep (GenBank™ accession number AF252548). One of the three sheep (also used in 5'-RACE) was found to contain the following 6 nucleotide substitutions when compared with the other two clones isolated: G126 to C126, G205 to C205, C212 to T212, T215 to C215, G216 to C216, and T384 to G384 (Fig. 1). These of these six nucleotide changes resulted in amino acid substitutions in the predicted amino acid sequence, Ala29 to Pro29, Trp35 to Ser35, and Gly59 to Arg59. Given that the other two clones are identical, their predicted amino acid sequence is presented (Fig. 1). The presence of natural polymorphisms within ovgal11 is consistent with the fact that, unlike rats and mice, which are greatly inbred species, sheep, even within the same breed, are known to be genetically diverse.

The predicted amino acid sequence (OVGAL11) encoded by the mRNA is 137 amino acids long. Blast searches of both the full nucleotide and predicted amino acid sequences confirm that this clone is related to the family of galectins but not with sufficient identity to be considered the ovine homologue of a known galectin. Hence, the clone was considered to be a novel member of the galectin family and was named ovgal11. Galectins are sugar-binding proteins that all share a CRD. Of the known galectins, the predicted amino acid sequence of ovgal11 shows most homology to a prototype galectin called galectin 10 (overall nucleotide identity ~42% and amino acid identity ~34%; see Table I and Fig. 2). Galectin 10 is also known as the Charcot-Leyden crystal and is an eosinophil lysophospholipase that may also be present in basophils. Unlike the other known galectins, galectin 10 only exhibits weak binding to lactose, and an endogenous ligand for its putative CRD has not yet been identified (15–16).

The nucleotide Blast search also identified a human genomic clone isolated from chromosome 19. Part of this genomic clone corresponds to the human galectin 10 gene (positions 10343–17535); however, a sequence 19,497 bp downstream of this gene also demonstrates similarity to the clone (Table I). This putative gene is not considered to be the human homologue of the sheep clone, because it demonstrates only 48% nucleotide identity, whereas the galectins compared across species are usually well conserved (e.g. sheep and human galectin 1 share 87% amino acid identity, rat and human galectin 7 share 73% nucleotide identity, and human and rat galectin 1 share 62% nucleotide identity).

This program is available via the World Wide Web.
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No signaling peptide to target OVGAL11 to intracellular or extracellular locations has been identified in the putative amino acid sequence. A eukaryotic transmembrane domain prediction program (TM predict) indicates that the protein does not span a membrane. A putative protein kinase C phosphorylation site (position 120; SVK), two putative casein kinase II phosphorylation sites (positions 65 and 133; TFTD and TPTD, respectively), and a cell attachment sequence (position 127; RGD) have been recognized by a PROSITE data base search (see Fig. 1 and Ref. 17).

Northern Blot Analysis of ovgal11 mRNA Levels—Despite the homology of ovgal11 to human galectin 10, expression of this clone within eosinophils was not detected by Northern blot hybridization. No expression was detected in total RNA extracted from any leukocyte population studied regardless of the percentage of eosinophils present, whether they were obtained from BAL (Fig. 3C) or from the mammary lavage of nematode challenged ewes, or were stimulated with IL-5 (Fig. 3A). No expression was detected in any mammary tissue regardless of the level of eosinophil infiltration found in those tissues (Fig. 3B).

Ovgal11 mRNA transcripts were not detected in total RNA prepared from adult *H. contortus* or L3 larvae indicating that the clone was of sheep, and not parasite, origin (Fig. 3A). This was subsequently confirmed by Southern blot analysis (data not shown).

In contrast, ovgal11 expression within 12 of 13 infected abomasal tissue samples of sensitized sheep was easily detected by Northern blot analysis at 2, 3, and 5 days post-challenge (Fig. 3D). In this experiment, none of the 8 controls had detectable levels of ovgal11 mRNA. The one infected abomasal sample in which expression could not be detected had very poor eosinophil infiltration, no detectable tissue larvae, and a large number of intraepithelial mast cells, indicating that rapid rejection of the nematode larvae through degranulation of mast cells may have occurred in this sheep (1). Hence, expression was only detectable in those infected abomasum samples that supported tissue infiltration by large numbers of eosinophils. However, there did not appear to be a direct correlation between eosinophil numbers and the level of ovgal11 expression. There was no detectable expression within lymph nodes collected from the same infected abomasum tissue or control abomasum tissues (not shown).

Northern blot analysis of abomasum samples detected strong ovgal11 expression in the immunized sheep by 2 days post-infection (Fig. 3, A–D). In contrast strong expression in abomasum samples obtained from naive sheep after primary infection with *H. contortus* was not detected until 5 days post-infection (Fig. 3E). Significantly increased lymphocyte and eosinophil infiltration in these primary infected sheep was also not apparent until day 5 post-infection (18). In this group of sheep, very low levels of ovgal11 mRNA in two of three control samples were also detected when longer exposures were used (not shown).

High ovgal11 mRNA levels were detected within the small intestine of sheep infected with *T. vitrinus* larvae (Fig. 3E). Histology confirmed that this tissue was also supporting eosinophil infiltration. This demonstrates that ovgal11 expression is not limited to *H. contortus* larval infection or to the abomasum. Once again, no ovgal11 mRNA was detected in uninfected tissue. As with abomasal lymph nodes, there was no detectable expression in the mesenteric lymph nodes associated with infected or uninfected intestinal tissue (Fig. 3E).

Post-mortem examination of sheep infected with *T. vitrinus* revealed a natural infection of adult *Chabertia ovina* in the large intestine. Despite this infection ovgal11 mRNA could not be detected in the large intestine samples (Fig. 3E). Unlike larval infections, eosinophil infiltration is not a major feature of adult nematode infections (1).

OVGAL11 expression was not detected in lung tissue collected from lung worm-infected ewes, even though eosinophil infiltration was supported by this tissue or from control lung tissue (Fig. 3C).

In Vivo Expression of OVGAL11 Protein—Immunohistochemical staining clearly demonstrates OVGAL11 protein expression within the upper epithelial layer of infected abomasum and
small intestine (Fig. 4, A and D). Those sections known to contain larvae exhibit greater and more widespread expression of OVGAL11 than those that do not (Fig. 4 A). Nuclear, as well as cytoplasmic, staining is apparent in some immunohistology sections (Fig. 4 C).

Although most of the uninfected abomasal and intestinal tissues were clearly negative (Fig. 4 F), some control uninfected tissue showed significant uniform staining, suggesting that low levels of constitutive expression may occur that are not great enough in whole tissue at the mRNA level to be detected by Northern blot analysis.

In addition to cytoplasmic and nuclear staining, immunohistology indicates that OVGAL11 may be secreted into the lumen (Fig. 4 D) where it may interact with carbohydrate motifs such as mucins. Western blot analysis does detect OVGAL11 protein in mucus samples collected from _H. contortus_-infected sheep, but not in mucus of uninfected sheep (Fig. 5). The apparent molecular mass of the protein detected in mucus corresponds to that of OVGAL11 from infected tissue and recombinant OVGAL11 (~14 kDa, Fig. 5). No change in apparent molecular mass was observed when mucus samples were run under reducing or non-reducing conditions (not shown).

**DISCUSSION**

Representational difference analysis was used successfully to isolate a novel cDNA (ovgal11) whose expression is greatly up-regulated in gastrointestinal tissue in response to helminth larval infections. Both nucleotide and predicted amino acid Blast searches have identified this clone as a novel galectin. Of the three possible galectin subtypes (proto, tandem repeat, and chimera), _ovgal11_ can be classified as proto-type (19). As well as fitting the structural criteria of this group, _ovgal11_ exhibits most amino acid identity to galectin-10, which is also a proto-type galectin.

Natural polymorphisms were found between the sheep studied within the putative coding region of _ovgal11_. These nucleic acid substitutions would result in 3 amino acid substitutions (2 within the putative CRD); however it remains to be determined whether these differences affect the function of OVGAL11 and how common these polymorphisms are.

Endogenous OVGAL11 extracted from mucus and run under reducing conditions on SDS polyacrylamide gels was the same size as recombinant protein. This indicates that the endogenous protein is not cleaved post-translation (as expected, because no obvious signal peptide is present to be removed) and is not glycosylated or phosphorylated despite the presence of putative protein kinase C and casein kinase II sites.

Given the amino acid identity between the OVGAL11 predicted amino acid sequence and known galectins, it is assumed that this protein will only exhibit weak affinity for lactose. Like human galectin-10, of the seven residues thought to be important to carbohydrate binding by galectin-1, OVGAL11 shares...
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Figure 3. Northern blot analysis of ovgal11 mRNA levels. Five Northern blots are shown with corresponding 18 S rRNA to show loading. Panel A, Northern blot hybridization of the ovgal11 RDA clone to total RNA prepared from adult or L3 larvae of H. contortus (H.c. A and L3, respectively), abomasum (Abo.) infected with H. contortus collected 2 days post-challenge (I2), or uninfected (U) larvae, eosinophil-rich leukocyte populations extracted from the MAL of H. contortus primed (P) ewes, or F. hepatica primed ewes before (F) and after culture with IL-5 (IL). Panel B, Northern blot hybridization of the ovgal11 RDA clone to total RNA prepared from H. contortus infected abomasum collected 2 days post-challenge (I2) and mammary tissue collected from primed ewes (P), primed and challenged ewes (PC), or challenged but not primed ewes (C). Panel C, Northern blot hybridization of the ovgal11 RDA clone to total RNA prepared from nematode-infected lung tissue (I), uninfected lung tissue (U), leukocytes extracted from the BAL of nematode-infected (I) or uninfected (U) lung, and abomasum (Abo.) infected with H. contortus collected 2 days post-challenge (I2), or uninfected (U). Panel D, Northern blot hybridization of the ovgal11 RDA clone to total RNA prepared from uninfected abomasum (U), or H. contortus infected abomasum collected 2, 3, or 5 days post-infection (I2-5). *, this infected abomasum sample did not show signs of inflammation. Panel E, Northern blot hybridization of the ovgal11 RDA clone to total RNA prepared from various portions of uninfected small intestine (U), small intestine infected with T. circumcisus larvae (I), large intestine (LI) infected with adult C. ovina (U) or uninfected (U), mesenteric lymph nodes (MLN) associated with infected (I) or uninfected (U) small intestine, abomasum (Abo.) infected with H. contortus collected from naive sheep 3, 5, or 36 days after primary infection (I3, I5, and I36, respectively), or uninfected (U).

The detection of high ovgal11 mRNA levels in helminth infected but not control gastrointestinal tissues implies that this galectin plays a role in the immune/inflammatory response. Other galectins have similarly been implicated in immunological interactions including allergic-type responses (22). For example galectin-3 has been reported to increase the production of superoxide by neutrophils (23), bind to IGE (24–25), and induce degranulation of mast cells and basophils (26). Galectin-3 knockout mice have less granulocytes in the peritoneal cavity during acute peritonitis compared with wild-type mice (27). Also a galectin 9 variant called egalectin has recently been isolated from a T-cell line that exhibits potent and specific eosinophil chemoattractant activity (28).

The earlier induction of ovgal11 mRNA expression after infection of immunized sheep compared with naive sheep implies that OVGAL11 may be regulated by, or play a part in, the adaptive (memory) immune response. Other galectins have been implicated in both innate and adaptive immunity (22).

OVGAL11 protein could be detected by immunohistochemistry in some of the control tissues, which did not have detectable levels of ovgal11 mRNA, indicating that low constitutive levels of expression may occur that are not great enough to be detected at the mRNA level in whole tissue by Northern blot analysis. The contrasting expression patterns of ovgal11 mRNA and protein may also be the result of protein storage rather than de novo protein synthesis.

Although both Western blot analysis and immunohistochemistry clearly demonstrate the secretion of OVGAL11 into the gastrointestinal lumen/mucus, no signal peptide to direct its secretion has been identified. Hence, like other galectins it seems likely that OVGAL11 will be secreted by a non-classical pathway independent of the endoplasmic reticulum-Golgi network (29–31). It is likely that this secretion will occur from the apical surface of polarized cells by exocytosis, because this has been reported for other galectins (29–30).

The presence of large quantities of OVGAL11 protein in mucus from larval infected sheep suggests that OVGAL11 may interact with mucus or other carbohydrate-containing molecules present in the mucus. Recombinant galectin-1 has been shown to bind mucins and epithelial cell surface glycoconjugates of the gastrointestinal tract, and it has been postulated that this galectin may act to cross-link mucins into a macromolecular mass and thus protect the epithelial surface against luminal contents including foreign organisms (32). However expression/secretion of galectin-1 in the gastrointestinal tract is constitutive and has not been shown to be up-regulated by helminth infections (32). Galectin-3 on the other hand has a 2-fold increased binding affinity for mucins from highly metastatic colon cancer cells than from low-metastatic colon cancer cells (33), suggesting that not only may some galectins have exposure to mucus only at particular time points, but also that they may bind mucins only under specific conditions. The mucus of the gastrointestinal tract of sheep and mice has been shown to be significantly altered after repeated nematode infection and to contain immune mediators that affect larval motility (33–35). It is likely that the abundant secretion of OVGAL11 into the mucus after parasite infection contributes to the altered properties and activities of immune mucus, and this requires further investigation.

Ovgal11 mRNA was not detected in the mammary glands of sheep subjected to the same conditions that caused high expression in the gastrointestinal tissue, nor in lung tissue. Sheep mammary gland has no mucus-producing epithelial cells, which may explain the lack of ovgal11 expression. High
mucus production is however a feature of the allergic lung. The lung tissue used in the present study, although containing a high number of eosinophils, was different in that it was subject to an established chronic, rather than acute, infection. It remains to be seen whether different stimuli would induce ov-gal11 expression in the lung.

In addition to being secreted, immunohistology has indicated nuclear and cytoplasmic localization of OVGAL11, demonstrating that like other galectins, OVGAL11 may have multiple localizations indicative of multiple functions (36–38). Other galectins have demonstrated simultaneous nuclear and cytoplasmic staining (39), and roles for both galectin-1 and -3 in pre-mRNA splicing have been postulated (38–39). Possibly this staining represents the association of OVGAL11 with the nuclear matrix as demonstrated for galectin-3 (36).

It remains unclear whether helminth parasites induce ov-gal11 expression directly or induction of the gene is due to tissue damage and/or the inflammatory response induced by the parasite. The last possibility is most likely, as adult parasite stages, which also cause tissue damage but not eosinophilic inflammation, do not induce expression. Preliminary studies have identified many potential regulatory elements within the untranslated regions of the ovgal11 gene, including E boxes and AP1 binding sites. E boxes are known to regulate the expression of other galectins, and it has been speculated that

FIG. 4. Immunohistology of stomach and intestinal tissue. Immunohistology of H. contortus infected abomasum tissue collected 5 days post-challenge (A, B, and C) and T. vitrinus infected (D and E) or uninfected (F) small intestine. Sections were stained using indirect immunoperoxidase with polyclonal OVGAL11 antibody (OVGAL11) and anti-IL-7 polyclonal antibody (IL-7). Abomasum sections A and B contain an H. contortus L3 larva (thin arrow). Strong OVGAL11 staining is detected in the upper epithelial layer of both infected abomasum and small intestine (A, C, and D). OVGAL11 staining of the mucosal surface of villi is apparent in D (arrow head), and nuclear staining is apparent in C (thick arrow). No staining was apparent in the sections probed with anti-IL-7 antibodies (B and E) or when using an IL-10-GST fusion protein polyclonal antibody (not shown). All sections are shown at 100× magnification, except section C, which is shown at 200×. L, lumen.

FIG. 5. Western blot analysis of OVGAL11 protein levels in abomasal mucus and tissue samples. OVGAL11 polyclonal antibody was probed against mucus isolated from abomasum of 2 uninfected sheep (uninfected mucus) or from abomasum collected 3 or 5 days after primary infection of naive sheep with H. contortus larvae (infected mucus). The OVGAL11 polyclonal antibody was also probed against infected abomasum tissue collected 5 days post-challenge (L) and 500 ng of recombinant OVGAL11 (rGAL11).
they may regulate gene expression in proliferating and differentiating epithelial cells (40). Similarly a 3′-AP1 site regulates the epithelial cell line specific expression of human keratin 19 (41). The presence of putative AP1 binding sites suggests that IL-1 or other cytokines may be able to regulate the transcription of ovgal11. Currently IL-1 is known to regulate many immediate early genes vital to the inflammatory response through modulation of AP1 activity (42). Regulation of ovgal11 expression by cytokines would be consistent with its accelerated induction after challenge compared with primary infection. However, the role, if any, of these putative regulatory elements remains to be defined by functional studies. The isolation and characterization of regulatory elements upstream of the transcription start site should also greatly enhance the understanding of transcriptional control of this gene.

Despite the uncertainties that remain regarding OVgal11 function, the restricted gene expression and secretion of this novel galectin implies that it plays a role during gastrointestinal inflammation and may be involved in the protection of the gastrointestinal tract from infection.

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