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TOLL-LIKE RECEPTOR 2 AND *MUC2* EXPRESSION ON HUMAN INTESTINAL EPITHELIAL CELLS BY *GYMNOPHALLOIDES SEOI* ADULT ANTIGEN

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ABSTRACT: Goblet cell hyperplasia and mucin hypersecretion are important for the expulsion of the intestinal trematode, *Gymnophalloides seoi*, from mice. However, regulatory mechanisms underlying these processes remain elusive. To better understand the effects of *G. seoi* antigen on the host's intestinal epithelial cells, we determined whether *G. seoi* induces expression of Toll-like receptors (TLRs) and mucin-related (*MUC*) genes on a human intestinal epithelial cell line (HT29 cells). We treated HT29 cells with *G. seoi* or other adult helminth antigens and measured mRNAs of *TLRs* and *MUCs*. We also performed reverse transcriptase-polymerase chain reaction (RT-PCR) and flow cytometry to determine whether *TLR* and *MUC* expression is regulated by interferon (IFN)-γ, interleukin-4, or monoclonal antibodies (mAbs) against *G. seoi* 46 kDa antigen. *Gymnophalloides seoi* antigen significantly induced expression of *TLR2* and *MUC2* in HT29 cells, and IFN-γ was found to upregulate *TLR2* expression on the surface of the cells. The expression of *MUC2* was increased by IFN-γ, but was decreased significantly via the combination of mAbs-to-human TLRs and *G. seoi* antigen. These results demonstrated that *G. seoi* antigen upregulates *TLR2* and *MUC2* expression on human intestinal epithelial cells. These effects reflect a helminth-induced, IFN-γ–dependent, and innate mucosal immune mechanism in this human intestinal cell line.

Gymnophalloides seoi (Digenea: Gymnophallidae) is a human intestinal trematode endemic along the western and southern coasts of the Republic of Korea (Lee et al., 1993; Chai et al., 2001, 2003). Infection occurs after eating contaminated raw oysters (Chai et al., 2003). Previous studies using infected mice showed that mucosal goblet cell (GC) hyperplasia in *G. seoi*–infected mice is regulated by CD4+ T cells, but alteration at the terminal sugar chain of mucin is probably independent of CD4+ T cells (Guk et al., 2009). GC hyperplasia and worm expulsion are mediated by T-helper 2 (Th2) cell–derived factors, such as interleukin (IL)-4 and IL-5 (Seo et al., 2003; Guk et al., 2009). However, the regulatory mechanisms underlying mucosal GC hyperplasia during parasitic infections are unclear. Mucin hypersecretion contributes to expulsion of nematodes and trematodes from the gastrointestinal tract (Nawa et al., 1994; Seo et al., 2003). The expression and regulation of mucin-related (*MUC*) genes were reported in rodents infected with intestinal nematodes, including *Trichinella spiralis* and *Nippostrongylus brasiliensis* (Shekels et al., 2001; Yamauchi et al., 2006).

There are 2 major classes of mucins, i.e., secreted and membrane-tethered. The secreted mucins, MUC2, MUC5AC, MUC5B, and MUC6, are stimulated by tumor necrosis factor (TNF)-α, IL-6, IL-4, or IL-9 in colonic or epithelial cell lines (Cohn et al., 1999; Dabbagh et al., 1999; Enss et al., 2000; Deplancke and Gaskins, 2001; Rose et al., 2001). The membrane-tethered mucins, MUC1, MUC3, MUC4, MUC12, and MUC13, may contribute to airway mucus obstruction (Rose et al., 2001). Most mucin studies have focused on the respiratory epithelial layer in asthma models (Cohn et al., 1999; Kraft et al., 2008). However, intestinal parasitic infections also trigger mucin hypersecretion, which is an important response of the innate immune system (Moncada et al., 2003). The regulatory mechanisms underlying GC activation during parasitic infections remain to be elucidated.

Toll-like receptors (TLRs) contribute to the maintenance of epithelial barrier functions in response to enteric parasites (McGuinness et al., 2003; Moncada et al., 2003). TLR signaling involves the upregulation of pro-inflammatory cytokines and chemokines and the induction of a local immune response (Harris et al., 2006). Although TLR signaling pathways have been studied extensively, very limited information is available on the role of these receptors in intestinal parasitic infections. Intestinal trematodes, such as *G. seoi*, dwell in the intervillous space of the small intestine and are probably trapped by mucins before expulsion (Seo et al., 2003). This suggests that TLRs in intestinal epithelial cells (IECs) of infected hosts should affect parasitic processes that would escape from the first-line host defense, including GC hyperplasia.

Recent reports have detailed the relationship between TLR signaling and expression of MUC proteins in epithelial cells by using an asthma model (Ueno et al., 2008). TLR signaling is suppressed by *MUC1* (human) and *Muc1* (non-human species), and it may play a crucial role in airway infection by various pathogenic bacteria and viruses (Ueno et al., 2008). In rat biliary epithelia, treatment with anti-TLR2 antibodies or anti-TLR4 antibodies downregulated *MUC2* expression that had been induced by lipopolysaccharide (LPS) (Ikeda et al., 2007). Kraft et al. (2008) suggested that the TLR2 signaling pathway is involved in *MUC5AC* expression in airway epithelial cells exposed to *Mycoplasma pneumoniae*.

Gymnophalloides seoi induces strong and rapid GC hyperplasia in the early phase of infection in mice (Seo et al., 2003). Mast cells did not appear to be essential for worm expulsion (Seo et al., 2003). Therefore, in the present study, we investigated mRNA expression of various *TLRs* and *MUCs* on a human IEC line after stimulation with *G. seoi* or other parasite antigens. We also studied whether interferon (IFN)-γ and IL-4 regulate the expression of *TLRs* and *MUCs*. An increase in the level of these cytokines in mesenteric lymph nodes was previously reported in mice infected with *G. seoi* during the period of GC hyperplasia and worm expulsion (Guk et al., 2009).

MATERIALS AND METHODS

Antigen

Naturally infected oysters (*Crassostrea gigas*) were collected from Aphaedo Island, Shinan-gun, a known endemic area of gymnophalloi-

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TABLE I. Experimental groups.

Cell stimulation groups (stimulated with)
Lipopolysaccharide (50 μg/well) (positive control)
*Gymnophalloides seoi** antigen (100 μg/well)
Nippostrongylus brasiliensis† antigen (100 μg/well)
Sparganum‡ antigen (100 μg/well)
Each antigen plus human recombinant IFN-γ (1,000 units/well)
Each antigen plus human recombinant IL-4 (1,000 units/well)
Monoclonal antibodies§ (mAbs) against *G. seoi* 46 kDa antigen (100 μg/well)
Cell pre-incubation groups (incubated with)
Anti-human TLR2 (10 μg/well)
Anti-human TLR4 (10 μg/well)
Non-stimulated cells (negative control)

* Known to cause GC hyperplasia in rodent models (Seo et al., 2003).
† Known to cause GC hyperplasia in rodent models (Onah and Nawa, 2000).
‡ A tissue-invading larval tapeworm (Beaver et al., 1984), no relation with mucosal GC responses.
§ A possible pathogen-associated molecule (unpublished).

TABLE II. Primers used in this study.

Gene	Primer
TLR2	GATGCCTACTGGGTGGAGAA (sense)
	CGCAGCTCTCAGATTTACCC (antisense)
TLR4	CAACAAAGGTGGGAATGCTT (sense)
	TGCCATTGAAAGCAACTCTG (antisense)
MUC2	CTGCACCAA-GACCGTCCTCATG (sense)
	GCAAGGACTGAACAAAGACTCAGAC (antisense)
MUC3	AGTCCACGTTGACCACTGC (sense)
	TGTTCACATCCTGGCT-GGCG (antisense)
MUC4	CGCGGTGGTGGAGGCGTTCTT (sense)
	GAAGAA-TCCTGACAGCCTTCA (antisense)
MUC5AC	TGATCATCCAGCAGCAGGGCT (sense)
	CCGAGCTCAGAGGACATATGGG (antisense)
MUC5B	CTGCGA-GACCGAGGTCAACATC (sense)
	TGGGCAGCAGGAGCACGGAG (antisense)
*GAPDH**	CTACTGGCGCTGGCAAGGCTGT (sense)
	GCCATGAGGTCCACCA-CCCTGCTG (antisense)

* Housekeeping gene.

diasis (Lee et al., 1994). The shell was removed and the oyster was digested slightly in an artificial digestive juice (0.5% pepsin 1:10,000 in 0.6% HCl solution; Sigma, St. Louis, Missouri) at 37 C for 3 min. The digested material, which contained free metacercariae of *G. seoi*, was washed several times with physiological saline. After repeated sedimentation and washing, the metacercariae were collected using a stereomicroscope. Immunosuppressed ICR mice were orally infected with metacercariae. Mice were killed at day 14 post-infection (PI) by cervical dislocation, and adult flukes were harvested using the Baermann's apparatus (Beaver et al., 1984). Adult worms of *N. brasiliensis* were harvested from Sprague-Dawley rats injected with 2,000 infective larvae cultured from eggs on charcoal granules. Spargana, the plerocercoid stage of *Spirometra erinacei*, were harvested from the subcutaneous tissues and viscera of naturally infected grass snakes, *Rhabdophis tigrina*. The worms were washed 3–5 times with sterile phosphate-buffered saline (PBS, pH 7.4), homogenized, sonicated, and centrifuged at 4 C for 30 min at 15,000 *g*. The resulting supernatant was used as the crude antigen. Animal experiments were carried out in accordance with the guidelines of our Institutional Animal Care and User Committee.

Cell line and cell stimulation

A human IEC line, HT29, was obtained from the Korean Cell Line Bank (Seoul, Korea). The HT29 cells were grown in RPMI 1640 media (Gibco BRL, Grand Island, New York) supplemented with 5% heat-inactivated FBS (Gibco BRL). For assays, cells were transferred into a 6-well plate. HT29 cells were divided into 3 groups (Table I): (1) cell stimulation groups with LPS (Sigma) (positive control); *G. seoi*, *N. brasiliensis*, or sparganum antigen; each antigen plus human recombinant IFN-γ or IL-4 (PharMingen, San Diego, California); or mAbs against *G. seoi* 46 kDa antigen; (2) cell pre-incubation groups with anti-human TLR2 or TLR4 (Imgenex, San Diego, California); and (3) non-stimulated cells (negative control). To prepare the mAbs, BALB/c mice were immunized with the crude antigen of *G. seoi* and B-lymphocyte clones were prepared. Among them, a significant clone, producing mAbs against *G. seoi* 46 kDa antigen, was selected and cultured, and has been maintained (unpubl. obs.). All reagents were used after filtering through a 0.45-μm syringe filter (Sigma). Cells were incubated for 24 hr at 37 C in a 5% CO_2 incubator and detached from the plate bottom using 0.25% trypsin-EDTA (Gibco BRL).

Extraction of total RNA

Total RNA was isolated from HT29 cells using the TRIzol reagent (Gibco BRL), following a protocol based on the single-step acid guanidinium thiocyanate-phenol-chloroform RNA isolation method (Chomczynski and Sacchi, 1987). The RNA pellet was briefly air-dried, then dissolved in RNase-free distilled water and stored at −70 C. Reverse transcription for the first-strand DNA was performed using the SuperScript First-strand Synthesis system for reverse-transcriptase-polymerase chain reaction (RT-PCR) kit (Gibco BRL), according to the manufacturer's instructions.

Quantitative RT-PCR

The primers for all genes (Table II) previously reported (Gouyer et al., 2001; Chen et al., 2005) were prepared for the quantitative RT-PCR. The ready-to-use PCR reaction mixture (Bioneer, Daejon, the Republic of Korea) was used and 1 μl of cDNA and 10 *p*moles of each primer set were added to the reaction tube. Both positive and negative controls were included in each assay to confirm that only cDNA PCR products were detected and that none of the reagents was contaminated with cDNA or a previous PCR product. To verify that reagents were not contaminated, an equal amount of cDNA was added to each PCR reaction within an experiment; to verify a uniform amplification process, the housekeeping gene *GAPDH* was also amplified in each assay.

Quantitation of PCR products

Ten microliters of amplified PCR products were electrophoresed in 2% agarose gel. The density of the bands was analyzed from photographs using the densitometrical analysis program, TINA (Raytest, Straubenhardt, Germany). The optical densities of the bands were normalized by compensation with the amplified product of the housekeeping gene, *GAPDH*, and the quantity of naïve mRNA was determined.

Flow cytometric analysis

A total of 10^6 cells were washed and resuspended in staining buffer (PBS containing 1% FBS and 0.1% sodium azide). Nonspecific Fc-binding was blocked using an antibody specific for FcγII/III receptors for 15 min at 4 C. Cells were washed twice with PBS. The mAbs, human TLR2-FITC (IgG2a) (Imgenex), and TLR4-FITC (IgG2a) (Imgenex) were used for single fluorescence flow cytometry to analyze the expression of TLRs on the cell surface. Labeled cells were assayed with a fluorescence-activated cell sorter (FACScan) (Becton Dickinson, Sparks, Maryland). The proportion (%) of cells expressing a given molecule was determined as the average of 3 sample replicates.

Statistical analysis

Data were compared by 1-way analysis of variance and Student's *t*-tests. P values <0.05 were considered statistically significant.

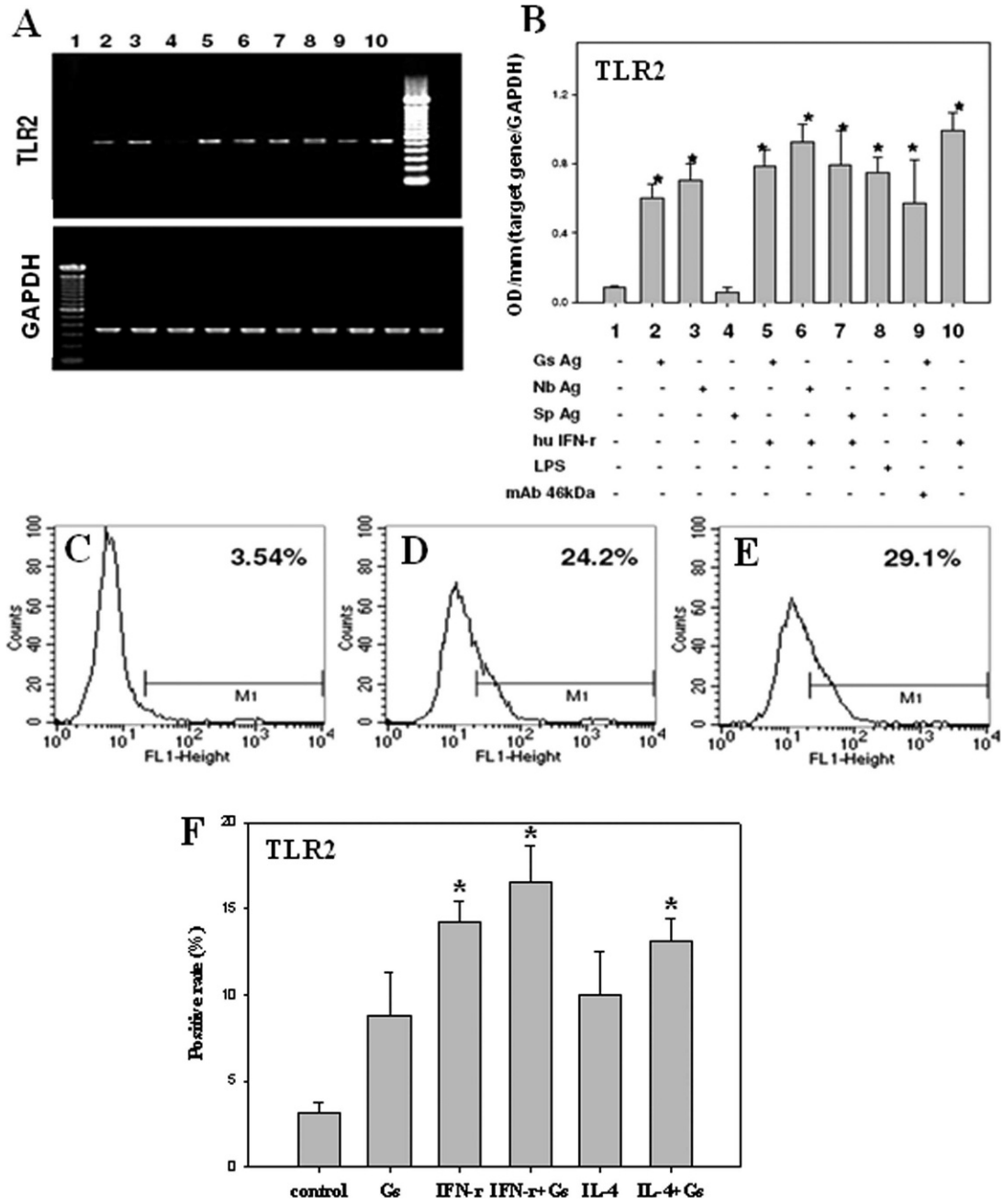

FIGURE 1. *TLR2* expression on HT29 cells by RT-PCR and flow cytometry. (**A**) Quantification of mRNA expression. Products of RT-PCR were separated by 2% agarose gel electrophoresis (left). Marker = 100 bp DNA ladder. (**B**) Values were calculated from OD/mm of each group divided by that of the housekeeping gene (*GAPDH*) (right). Significantly (*, $P < 0.05$) enhanced compared to the negative control. Gs, *Gymnophalloides seoi*; Nb, *Nippostrongylus brasiliensis*; Sp, sparganum. (**C–E**) Flow cytometric analysis of TLR2 protein expression on the surface of HT29 cells. (**C**) Non-stimulated cells. (**D**) Cells stimulated with *G. seoi* antigen. (**E**) Cells stimulated with *G. seoi* antigen plus human IFN-γ. **(F)** Comparison of TLR2 protein expression on IECs under various culture conditions. Significantly (*, $P < 0.05$) enhanced compared to cells stimulated by *G. seoi* antigen. Gs, *G. seoi*.

RESULTS

Expression of *TLR2* and *TLR4* on HT29 cells

The mRNA expression of both *TLR2* (Fig. 1) and *TLR4* (Fig. 2) on HT29 cells stimulated with *G. seoi* or *N. brasiliensis* antigen increased significantly ($P < 0.05$) compared to non-stimulated cells or cells stimulated with sparganum antigen. The enhancement of *TLR2* mRNA expression was greater ($P < 0.05$) than that of *TLR4* mRNA expression. Cells incubated with LPS, a positive control, expressed significant increases of *TLR2* and *TLR4*. However, sparganum antigen did not induce expression of *TLR2* and *TLR4* in human IECs. In addition, the protein expression of TLR2 (Fig. 1) and TLR4 (Fig. 2) on the surface of cells stimulated with *G. seoi* antigen increased significantly ($P < 0.05$) compared to negative controls.

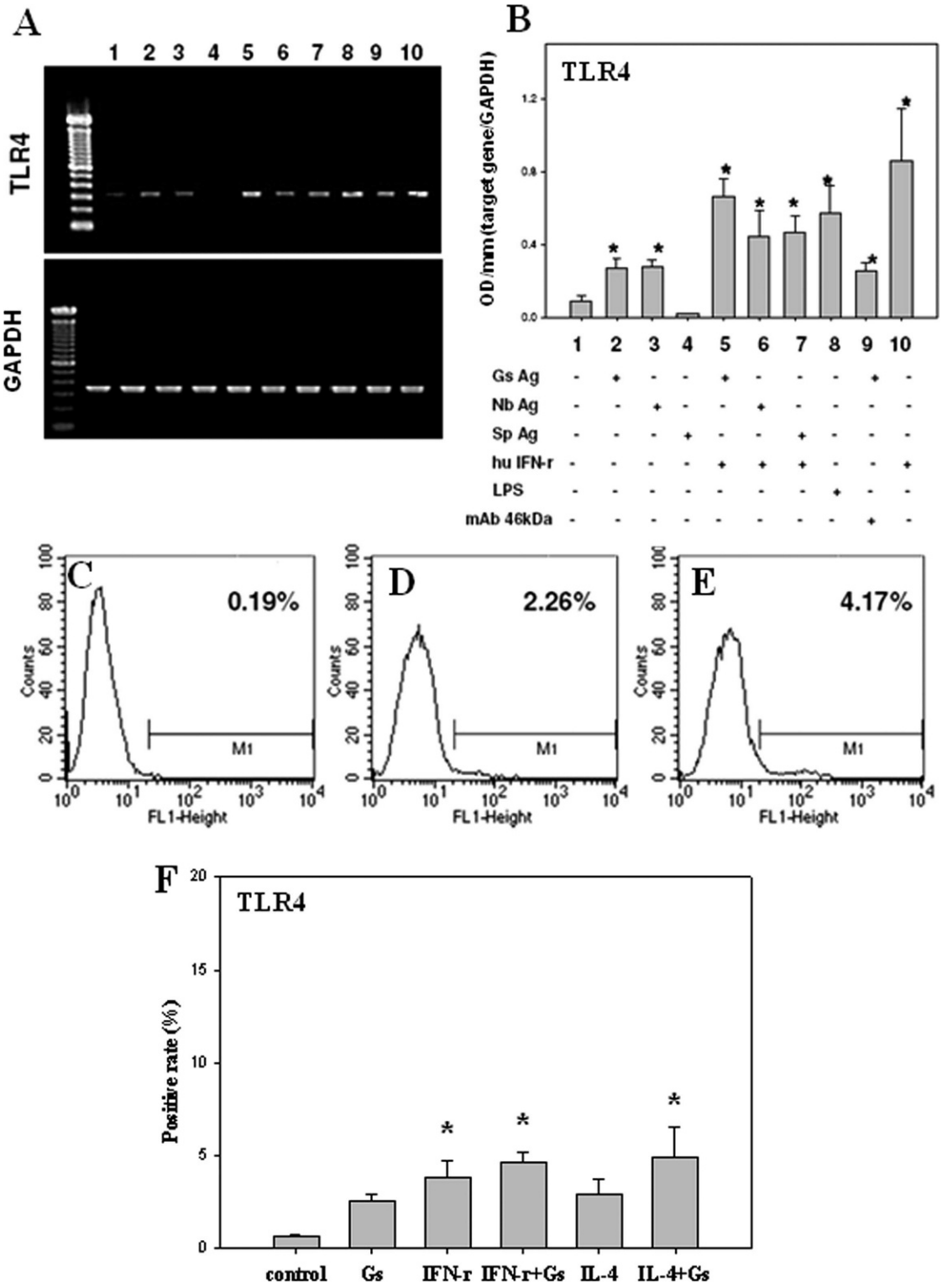

FIGURE 2. *TLR4* expression on HT29 cells by RT-PCR and flow cytometry. (**A**) Quantification of mRNA expression. Products of RT-PCR were separated by 2% agarose gel electrophoresis (left). Marker = 100 bp DNA ladder. (**B**) Values were calculated from OD/mm of each group divided by that of the housekeeping gene (*GAPDH*) (right). Significantly (*, $P < 0.05$) enhanced compared to the negative control. Gs, *Gymnophalloides seoi*; Nb, *Nippostrongylus brasiliensis*; Sp, sparganum. (**C–E**) Flow cytometric analysis of TLR4 protein expression on the surface of HT29 cells. (**C**) Non-stimulated cells. (**D**) Cells stimulated with *G. seoi* antigen. (**E**) Cells stimulated with *G. seoi* antigen plus human IFN-γ. **(F)** Comparison of TLR4 protein expression on IECs under various culture conditions. Significantly (*, $P < 0.05$) enhanced compared to cells stimulated by *G. seoi* antigen. Gs, *G. seoi*.

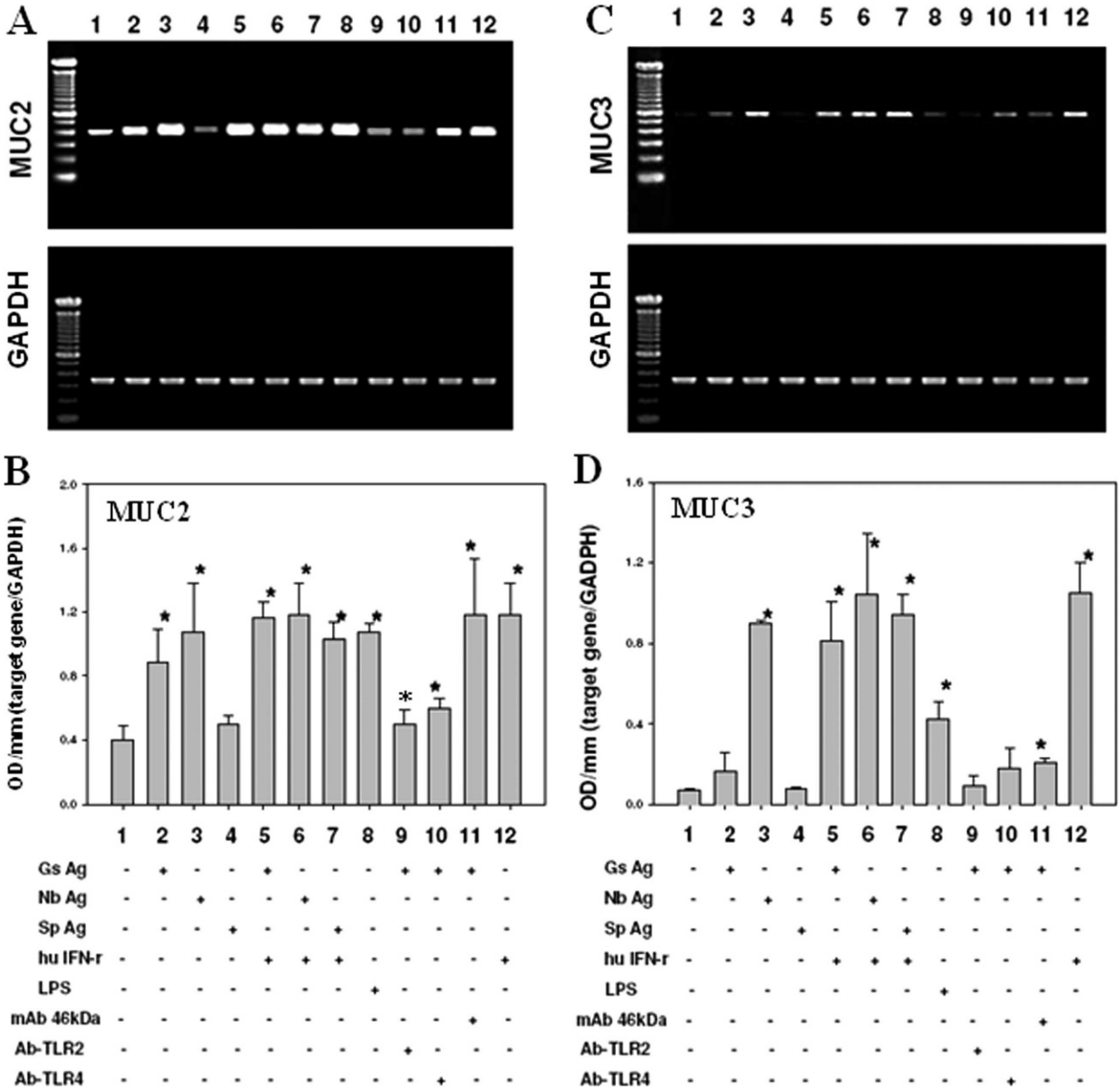

FIGURE 3. Expression of *MUC2* (**A, B**) and *MUC3* (**C, D**) in HT29 cells. (**A, B**) Products of RT-PCR were separated by 2% agarose gel electrophoresis. Marker = 100 bp DNA ladder. (**C, D**) Values were calculated from OD/mm of each group divided by that of the housekeeping gene (*GAPDH*) (right). Significantly (*, $P < 0.05$) enhanced compared to the negative control. Gs, *Gymnophalloides seoi*; Nb, *Nippostrongylus brasiliensis*; Sp, sparganum.

Effects of IFN-γ, IL-4, and mAbs against *G. seoi* 46 kDa on the expression of TLRs

IFN-γ significantly ($P < 0.05$) upregulated the mRNA expression of *TLR2* (Fig. 1) and *TLR4* (Fig. 2) in response to parasite antigens. IFN-γ also significantly ($P < 0.05$) upregulated protein expression of TLR2 (Fig. 1) and TLR4 (Fig. 2) on the HT29 cell surface. The increase of mRNA expression by *G. seoi* antigen was similar to the increase by *N. brasiliensis* antigen.

We also compared the effects of IFN-γ and IL-4 on *TLR* expression. Only IFN-γ significantly ($P < 0.05$) stimulated *TLR* expression, even in the absence of the *G. seoi* antigen. These results showed that IFN-γ upregulated TLR2 and TLR4 expression on HT29 cells stimulated with *G. seoi* antigen at 24 hr after incubation. TLR2 expression was more marked than TLR4 expression. To determine whether *G. seoi* 46 kDa antigen acts as a TLR ligand, mAbs against this antigen were used; however, no significant effects of these mAbs were observed (Figs. 1, 2).

Expression of *MUC* mRNA and effects of IFN-γ

Among the 5 kinds of *MUC* genes (*MUC2*, *MUC3*, *MUC4*, *MUC5AC*, and *MUC5B*), only *MUC2* expression was significant ($P < 0.05$) in cells stimulated with *G. seoi* antigen (Figs. 3, 4). In cells stimulated with *N. brasiliensis* antigen, the mRNA expression of *MUC2*, *MUC3*, and *MUC4* was enhanced ($P < 0.05$) compared to negative controls. Sparganum antigen did not induce significant expression of *MUC* mRNAs. In cells co-

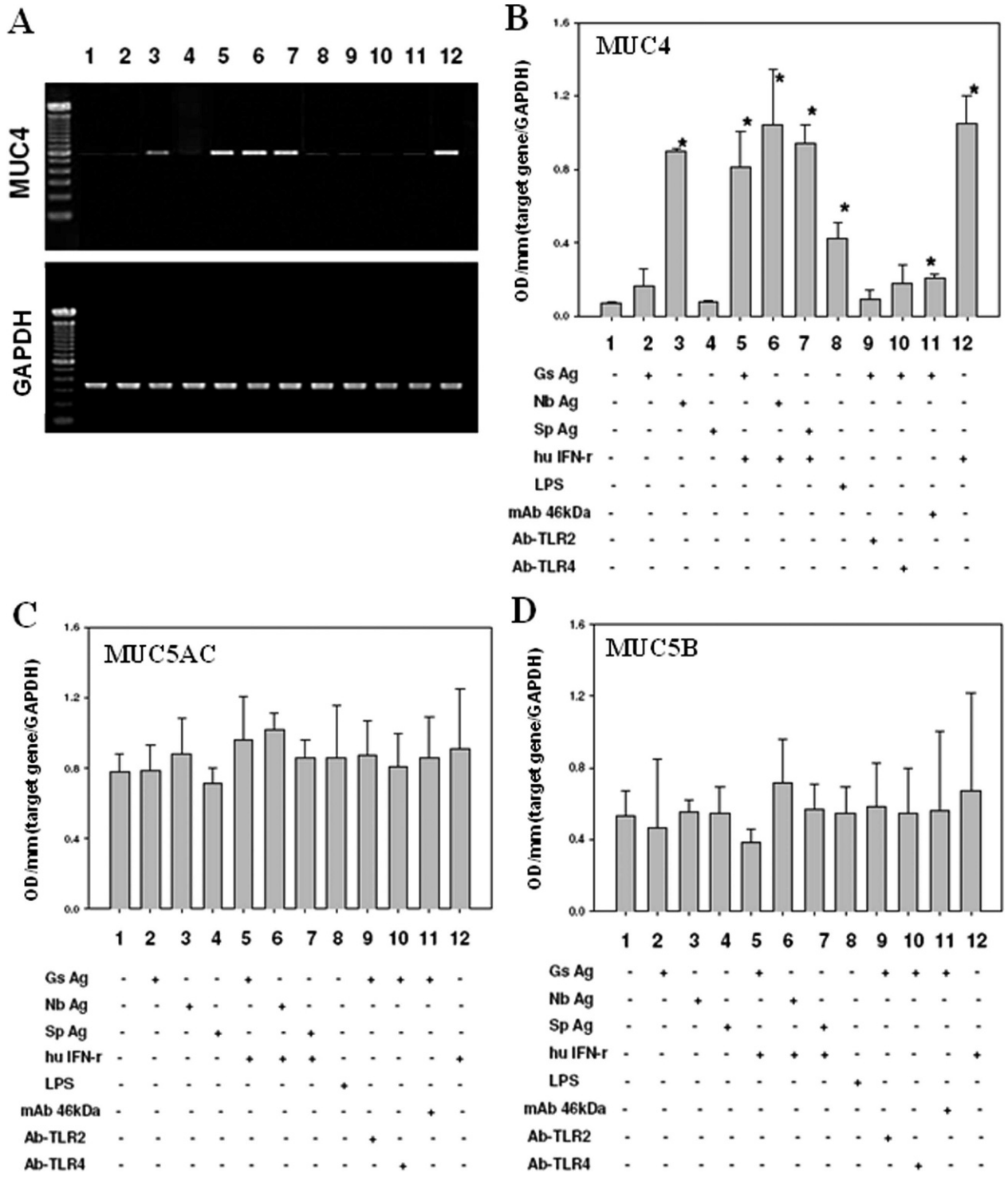

FIGURE 4. Expression of *MUC4* (**A, B**), *MUC5AC* (**C**), and *MUC5B* (**D**) in HT29 cells. (**A**) Products of RT-PCR were separated by 2% agarose gel electrophoresis (left). Marker = 100 bp DNA ladder. (**B–D**) Values were calculated from OD/mm of each group divided by that of the housekeeping gene (*GAPDH*) (right). Significantly (*, $P < 0.05$) enhanced compared to the negative control. Gs, *Gymnophalloides seoi*; Nb, *Nippostrongylus brasiliensis*; Sp, sparganum.

stimulated with parasite antigen and human IFN-γ, the expression of *MUC2*, *MUC3*, and *MUC4* was significantly ($P < 0.05$) higher than it was in negative controls. However, *MUC2* expression in the *G. seoi*–stimulated group was not significantly ($P > 0.05$) different from the *G. seoi* and IFN-γ co-stimulated group (Fig. 3). In contrast, the mRNA expression of *MUC3* (Fig. 3) and *MUC4* (Fig. 4) was significantly ($P < 0.05$) increased by the addition of IFN-γ. The mRNA expression of *MUC5AC* and *MUC5B* was independent of IFN-γ, and there were no significant differences among the experimental groups (Fig. 4).

Effects of TLRs on the expression of MUC proteins

We also determined whether blocking TLRs with mAbs revealed effective regulation of MUC expression (Figs. 3, 4). In cells co-stimulated with *G. seoi* antigen and mAb-TLR2 or mAb-TLR4, the mRNA expression of *MUC2* decreased significantly ($P < 0.05$) compared with cells stimulated only with *G. seoi* antigen. However, other *MUC* expression was not significantly affected by treatment with mAbs (Figs. 3, 4).

DISCUSSION

We demonstrated that *G. seoi* stimulated *TLR2* and *MUC2* mRNA expression on HT29 cells. Similarly, *N. brasiliensis* increased the expression of *TLR2*, *TLR4*, *MUC2*, *MUC3*, and *MUC4* genes on human IECs, while spargana had little effect. *Nippostrongylus brasiliensis* is a well-studied intestinal nematode that elicits extensive mucus production and initiating protective responses in the intestinal epithelial cells of rodent hosts (Nawa et al., 1994; Onah and Nawa, 2000). In contrast, the sparganum invades tissues of mammalian hosts and does not inhabit the intestinal lumen (Beaver et al., 1984). Because of this, *TLR* and *MUC* expression on IECs was unaffected by stimulation with sparganum antigen.

Various intestinal pathogens, including parasites (Akira and Takeda, 2004; Harris et al., 2006), elicit TLR responses on host cells. In human cholangiocytes stimulated with *Cryptosporidium parvum*, all known TLRs were expressed, and it was suggested that TLR2 and TLR4 mediate cholangiocyte defense responses (Chen et al., 2005). In addition, Gal-lectin of *Entamoeba histolytica* strongly induced TLR2 expression in murine macrophages, and this response may contribute to defense against amebic pathogenesis (Kammanadiminti et al., 2004). This kind of innate, inflammatory, and adaptive immune response can eliminate *E. histolytica* in resistant individuals (Campos-Rodriguezp and Jarillo-Luna, 2005). We also found in our study that the enhanced expression of TLR2 on IECs may contribute to innate immune responses, including GC hyperplasia and mucin hypersecretion, which are elicited by *G. seoi* infection in the host intestine (Chai et al., 2003). Our results showed that mAbs against TLR2 and TLR4 inhibited the expression of *MUC* genes in human IECs. Therefore, TLRs and mucin-secreting cells may have a positive association mediated through direct or indirect mechanisms.

To date, 11 different TLR genes have been identified (Harris et al., 2006). *TLR1*, *TLR2*, *TLR4*, and *TLR6* have lipid ligands, *TLR3*, *TLR7* (or *TLR8*), and *TLR9* have nucleic acid ligands, *TLR5* has a protein ligand, and *TLR10* and *TLR11* have unknown ligands (Harris et al., 2006). Emerging experimental and clinical results have shown that different TLR expression and activation are selectively regulated in the gastrointestinal tract, probably due to its unique environment (Harris et al., 2006; Mueller et al., 2006). However, surprisingly few data are available regarding how these receptors affect intestinal helminths. In addition, questions remain with respect to how each type of TLR regulates human gut responses to specific pathogens (Harris et al., 2006; Mueller et al., 2006).

In murine models of *G. seoi* infection, Th1 cytokines (IL-2 and IFN-γ) increased on day 1 PI, and Th2 cytokines (IL-4 and IL-5) increased during the worm expulsion (Guk et al., 2009). TLR expression was upregulated by IFN-γ when stimulated with *G. seoi* or other parasitic antigens. However, IL-4 had a weak influence on TLR expression on HT29 cells stimulated with *G. seoi*. Many studies have focused on the role of TLRs, but there are few reports on cytokines regulating TLR expression by parasites. Th2 cytokines, in particular IL-4 and IL-13, decreased the mRNA and protein expression of TLR3 and TLR4 in human IECs; however, these cytokines also enhanced TLR signaling that is induced by stimulation with IFN-γ, a Th1 cytokine (Mueller et al., 2006). Similarly, IFN-γ may induce the mRNA of TLR2, TLR4, and TLR6 and upregulate protein expression of TLR2 and TLR4 on macrophages (Schroder et al., 2006). Our results suggest that IFN-γ is one of the accelerators of TLR expression in the human gut during the early phase of *G. seoi* infection.

The present study has demonstrated that *G. seoi* antigen can induce significant expression of the *MUC2* gene in human IECs. Our previous reports indicate that GC hyperplasia and mucin hypersecretion may act as defense mechanisms in mice experimentally infected with *G. seoi* (Seo et al., 2003; Guk et al., 2009). MUC2 was upregulated as early as days 2–4 PI in rats infected with *N. brasiliensis*, suggesting that they were associated with an early innate protective response (Yamauchi et al., 2006). In addition, at day 7 PI and thereafter, when the nematodes reach maturity, significant upregulation of MUC3 and MUC4 was observed. This means that the expression level of each GC-related or glycosylation-related gene is altered differently during the time course of infection and indicates a progression of sequential qualitative changes in the mucus layer after *N. brasiliensis* infection (Yamauchi et al., 2006). In our study, *MUC2*, *MUC3*, and *MUC4* gene expression increased in human IECs after *N. brasiliensis* antigen stimulation. In cytokine-deficient mice infected with *T. spiralis*, the total mucin protein and specific *Muc2* and *Muc3* mucin genes of mice were upregulated and the induction was independent of 3 specific cytokines, IFN-γ, TNF-α, and IL-4 (Shekels et al., 2001). However, the present study showed that all 3 mucin genes (*MUC2*, *MUC3*, and *MUC4*) were upregulated by IFN-γ in human IECs stimulated by parasite antigens. Therefore, *G. seoi* antigen can induce the expression of the *MUC2* gene, and IFN-γ may work as an accelerator of *MUC* gene expression in human IECs.

Mucin-like glycoproteins were derived from *Trypanosoma cruzi* after IFN-γ stimulation (Coelho et al., 2002), and IFN-γ upregulated MUC1 expression in hematopoietic and epithelial cancer cell lines (Reddy et al., 2003). In addition, IFN-γ–induced expression of MUC4 in pancreatic cancer cells is mediated by upregulation of signal transducer and activator of transcription-1 (STAT-1) (Andrianifahanana et al., 2007). Our results agree with previous reports in that IFN-γ can upregulate some types of *MUC* genes, although the expression levels of *MUC5AC* and *MUC5B* genes were found to be independent of IFN-γ.

When human IECs were co-stimulated with *G. seoi* antigen and mAb-TLR2 or mAb-TLR4, the expression of the *MUC2* gene decreased significantly compared to cells stimulated only with *G. seoi* antigen. These results suggest a possibility that expression of the *MUC2* gene may be closely correlated with TLR pathways. TLR2 signaling may enhance the physical barrier function of IECs (Akira and Takeda, 2004; Cario et al., 2004). Interestingly, Ikeda et al. (2007) reported that pre-incubation of rat biliary epithelial cells with anti-TLR4 or anti-TLR2 antibodies down-regulated MUC2 expression induced by LPS. In contrast, TLR2

and bacterial components induced expression of MUC2 in cultured gastric epithelial cell lines and were activated by nuclear factor (NF)-κB (Ding et al., 2005; Ikeda et al., 2007). All of these results, including ours, suggest that *G. seoi* antigen induces MUC2 expression via the activation of the TLR pathways in human IECs.

In summary, the intestinal trematode *G. seoi* induces expression of TLR2 and TLR4 in a human IEC line. Furthermore, IFN-γ upregulates TLR2 expression on the surface of the cells. In addition, *G. seoi* stimulated significant expression of the *MUC2* gene on HT29 cells, which was mediated by IFN-γ. We suggest that MUC2 upregulation is related to TLR pathways.

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