

Original

Reduced production of polymeric immunoglobulin receptor in murine dextran sodium sulfate-induced colitis

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Abstract: Polymeric immunoglobulin receptor (pIgR) plays an intrinsic role in protecting the intestinal tract from invading pathogens. In the present study, we observed a decrease in pIgR in colon lysate from mice with dextran sodium sulfate (DSS) colitis. A decrease in pIgR was detected in both mRNA and protein levels. Histologic examinations revealed marked destruction of intestinal epithelial cells (IECs), and only a small number of regenerating IECs expressed pIgR. These results suggest that the decrease in pIgR was due to the destruction of IECs. Because activation of toll-like receptor 3 slows the progression of DSS colitis, we injected polyriboinosinic: polyribocytidylic acid (poly I:C) intraperitoneally and observed the correlation between pIgR level and severity of DSS colitis. Poly I:C markedly decreased progression of DSS colitis, and pIgR levels significantly recovered. Furthermore, we found that expressions of IFN- γ and TNF- α were higher in DSS colitis. These results indicate that the decrease in pIgR was not compensated for by increased expression of these cytokines. In sum, our findings show that pIgR levels vary according to the severity of DSS colitis and that these changes might be useful as a biomarker of the severity of inflamma-

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Introduction

The mucosal immune system is the first line of defense against a variety of antigens (1-3). The main agents in this system are the polymeric immunoglobulins (pIgs). In order to exert their protective effects against environmental antigens, pIgs produced by lamina propria lymphocytes are transported across epithelial cells. This protein transport step is called transcytosis and is mediated by the polymeric immunoglobulin receptor (pIgR) (4) expressed on the basolateral surface of intestinal and glandular epithelial cells.

Inflammatory bowel diseases (IBDs), including Crohn's disease and ulcerative colitis, are chronic inflammatory disorders of the gastrointestinal tract (5). The exact causes of IBD are not known, and many factors have been reported to be involved in the development of disease, including epithelial cell destruction, genetic susceptibility, and bacterial flora in the intestinal environment (5). Several models of experimental colitis have been reported, and they display various pathophysiologic aspects of human IBD (6). The dextran sodium sulfate (DSS) model, originally reported by Okayasu et al. (7), has been used widely to investigate the mechanisms of IBD. The clinical features of the DSS model include weight loss, loose stool/diarrhea, and occult and gross rectal bleeding. Histologically, the DSS model shows

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extensive crypt and epithelial damage and significant infiltration of immune cells.

Recently, we generated monoclonal antibodies against mouse pIgR and developed an enzyme-linked immunosorbent assay (ELISA) system (8) that enabled measurement of pIgR concentration in biological samples. Although the role of pIgR in IBD is not fully understood, Murthy et al. (9) reported that pIgR had functional importance in preventing DSS-induced colitis in pIgR-disrupted mice. To date, no report has examined pIgR levels in the DSS model. In the present study, we found that pIgR was markedly decreased in DSS colitis. The extent of this reduction correlated with the severity of epithelial damage, which suggests that pIgR could be useful as a new biomarker of IBD severity.

Materials and Methods

Reagents

DSS was purchased from TdB Consultancy AB (Uppsala, Sweden). Poly I:C was purchased from Sigma (St. Louis, MO, USA). The use of anti-mouse pIgR monoclonal antibody was described previously (8). Recombinant interleukin-10 (IL-10) was purchased from R&D system (Minneapolis, MN, USA).

Animals

Female C57BL6 mice (age, 8-10 weeks) were purchased from SRL (Tokyo, Japan). Throughout this study, age- and sex-matched mice were used. All experiments were approved by the Nihon University School of Dentistry Animal Ethical Committee and performed according to legal requirements.

DSS colitis

Mice were given water containing 1.5% DSS for 7 days; control mice received only distilled water for the

same period of time. After 7 days on DSS, the mice were killed, and body weight and colon length were measured. In the poly I:C injection experiments, 20 µg of poly I:C in 200 µl of phosphate-buffered saline (PBS) was injected intraperitoneally. After pretreatment, the mice were treated with DSS for 7 days. The controls received an intraperitoneal injection of 200 µl of PBS.

Lysates of the small and large intestines were prepared using a Dounce homogenizer. The protein concentrations of these samples were determined with a protein assay kit (BioRad, Hercules, CA, USA) and subjected to ELISA and Western blotting.

Histopathology and immunohistochemical staining

For histologic examinations, the colons were removed, and frozen sections were prepared. The sections were fixed with 5% acetic acid/95% ethanol for 10 min at room temperature. After fixation, the sections were stained by hematoxylin and eosin. For immunohistochemical staining, endogenous peroxidase activity was blocked by 0.3% H₂O₂-methanol and non-specific staining with 1% bovine serum albumin in PBS (BSA-PBS) for 1 h at room temperature, and the sections were incubated with biotinylated anti-mouse pIgR monoclonal antibody (clone No. 7, 1:10 diluted with 1% BSA-PBS) for 18 h at room temperature. After washing with PBS 3 times, the specimens were incubated with horseradish peroxidase (HRP)-conjugated streptavidin (Zymed, CA, USA; 1:100 diluted with 1% BSA-PBS) for 1 h at room temperature. The color reaction was performed in 50 mM Tris-HCl buffer, pH 7.6, containing 0.05% 3, 3'-diaminobenzidine tetrahydrochloride (DAB; Sigma) and 0.05% hydrogen peroxidase for 10 min. Counterstaining was performed with Mayer's haematoxylin. The images were viewed and photographed using a light microscope (Olympus,

Table 1 Real-time PCR primer sequences for genes of interest (GOI)

GOI	Accession no.	Sequence	Size (bp)
IL-10	NM_010548	F: 5'-TCCTTAATGCAGGACTTTAAGGGTTACTTG-3' R: 5'-GACACCTTGGTCTTGGAGCTTATTAATAATC-3'	156
IFN-γ	NM_008337	F: 5'-GCATCTTGGCTTGCAGCT-3' R: 5'-CCTTTTTTCGCCTTGCTGTTG-3'	125
TNF-α	NM_013693	F: 5'-TGTGCTCAGAGCTTTCAACAA-3' R: 5'-CTTGATGGTGGTGCATGAGA-3'	88
IL-4	NM_021283	F: 5'-ACAGGAGAAGGGACGCCAT-3' R: 5'-GAAGCCCTACAGACGAGCTCA-3'	95
pIgR	NM_011082	F: 5'-ATGAGGCTCTACTTGTTCACG-3' R: 5'-GACGTGGGTGCTACTCGGCAA-3'	417
β-actin	NM_007393	F: 5'-TTCCAGCCTTCCTTCCTGG-3' R: 5'-TTGCGCTCAGGAGGAGCAA-3'	225

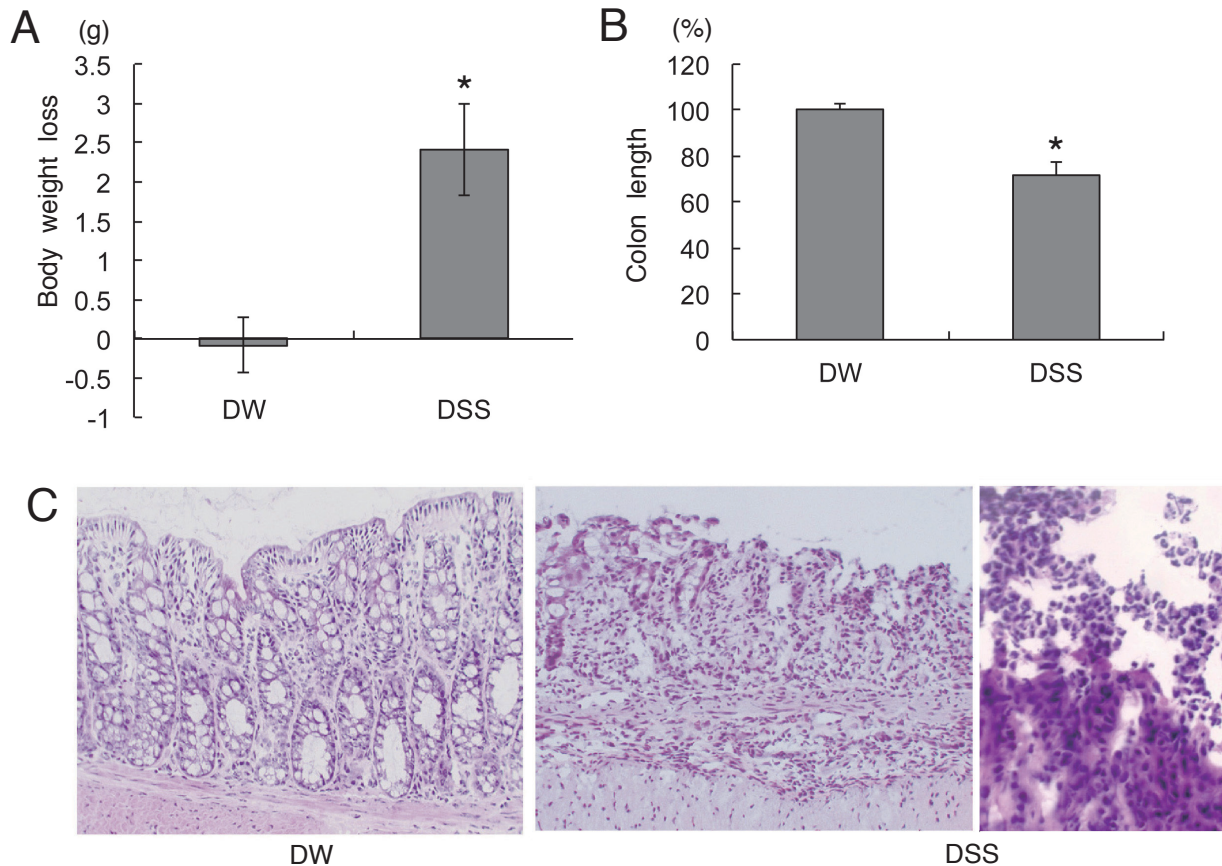


Fig. 1 Successful induction of dextran sodium sulfate (DSS) colitis in mice. Water with 1.5% DSS was given to mice for 7 days; regular drinking water (DW) was fed to control mice. After DSS treatment (A), loss of body weight and (B) length of the entire colon were measured. (C) Histologic examination was performed with hematoxylin and eosin staining. Data are means \pm SE; $n = 5$ to 9 mice per experimental group. * $P < 0.05$.

Tokyo, Japan).

ELISA

ELISA was performed using 100 μ g of tissue lysates, as described previously (8).

Western blotting

For detection of pIgR by Western blotting, 500 μ g of total protein was incubated with 5 μ l of heparin-agarose (GE Healthcare, Tokyo, Japan) (10) and rotated for 1 h at 4°C. The samples were washed with 0.5% Triton X-100/TBS three times and subjected to 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The separated protein was electrophoretically transferred to an Immobilon membrane (Millipore, Tokyo, Japan), and Western blotting was performed as described previously (8). The clone No. 7 monoclonal antibody was diluted to 1:1000 with 1% BSA-PBST (0.1% Tween 20-PBS) and used as the first antibody. HRP-conjugated goat anti-mouse IgG (H+L; 1:5000 dilution with 1% BSA-PBST)

was used as the secondary antibody. The bands were detected by using enhanced chemiluminescence (ECL kit; GE Healthcare).

Total RNA extraction and real-time polymerase chain reaction

Total RNA was purified using an RNeasy mini kit (QIAGEN, Tokyo, Japan). One μ g of total RNA was subjected to first strand cDNA synthesis as described previously (11). The cDNA for IL-10, INF- γ , TNF- α , IL-4, pIgR, and β -actin was amplified by real-time polymerase chain reaction (PCR). The primers used in this study are listed in Table I. Real-time PCR was performed by using a SYBR Green I reagent (Roche, Tokyo, Japan), according to the manufacturer's instructions. Values presented for the real-time PCR experiments are expressed as fold induction relative to basal levels of wild-type (water without DSS) mouse. The expression level of each mRNA was normalized to β -actin mRNA.

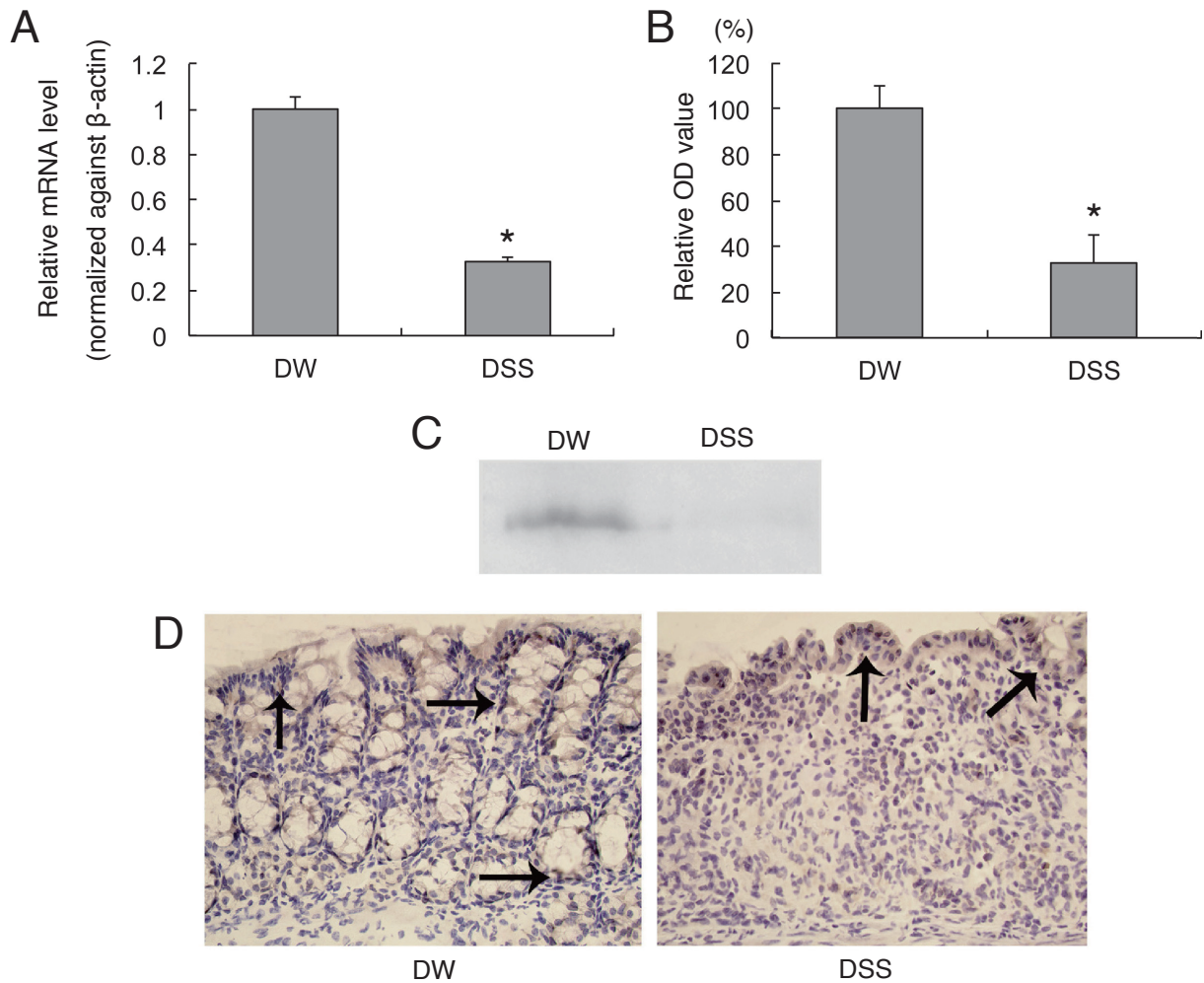


Fig. 2 pIgR level was significantly lower in mice with DSS colitis. After 7 days of DSS colitis induction, the colon was resected, and tissue lysates and total RNA were extracted. (A) One μ g of total RNA was used for cDNA synthesis and subjected to real-time PCR. (B) For ELISA, 100 μ g of tissue lysates was used, and the level of pIgR was measured. The OD value of the normal colon sample was defined as 100%. (C) Five hundred μ g of colon lysates was incubated with heparin-agarose, and the samples were subjected to Western blotting. (D) Immunohistochemical staining was performed as described in the Materials and Methods. Positive staining for pIgR is indicated by the arrows. Data are means \pm SE; $n = 5$ to 7 mice per experimental group. * $P < 0.05$.

Statistical analysis

Data are presented as means \pm SE. Statistical analysis were performed using Student's t test. Data were considered statistically significant when $P < 0.05$.

Results

Induction of DSS colitis in mice

Colitis was quantified by measuring body weight and colon length. In contrast to the slight increase in body weight among wild-type mice, an average body weight loss of more than 2.5 g was observed in DSS mice (Fig. 1A). DSS treatment resulted in apparent thickening/shortening of the colon, and average colon length decreased to approximately 70% of that of the control mice (Fig. 1B).

Neutrophil infiltration of the intestinal mucosa is the hallmark of active IBD. Therefore, we examined the extent of neutrophil infiltration histologically (Fig. 1C). In addition to severe destruction of surface-lining epithelial cells, marked infiltration of neutrophils was observed in the lamina propria of the colons of DSS mice. These results confirm the successful induction of colitis in DSS mice.

Reduced production of pIgR in DSS colitis

Next, we attempted to examine whether DSS treatment influenced the production of pIgR in the colon. For this purpose, total RNA was extracted from wild-type and DSS mice and subjected to real-time PCR. As shown

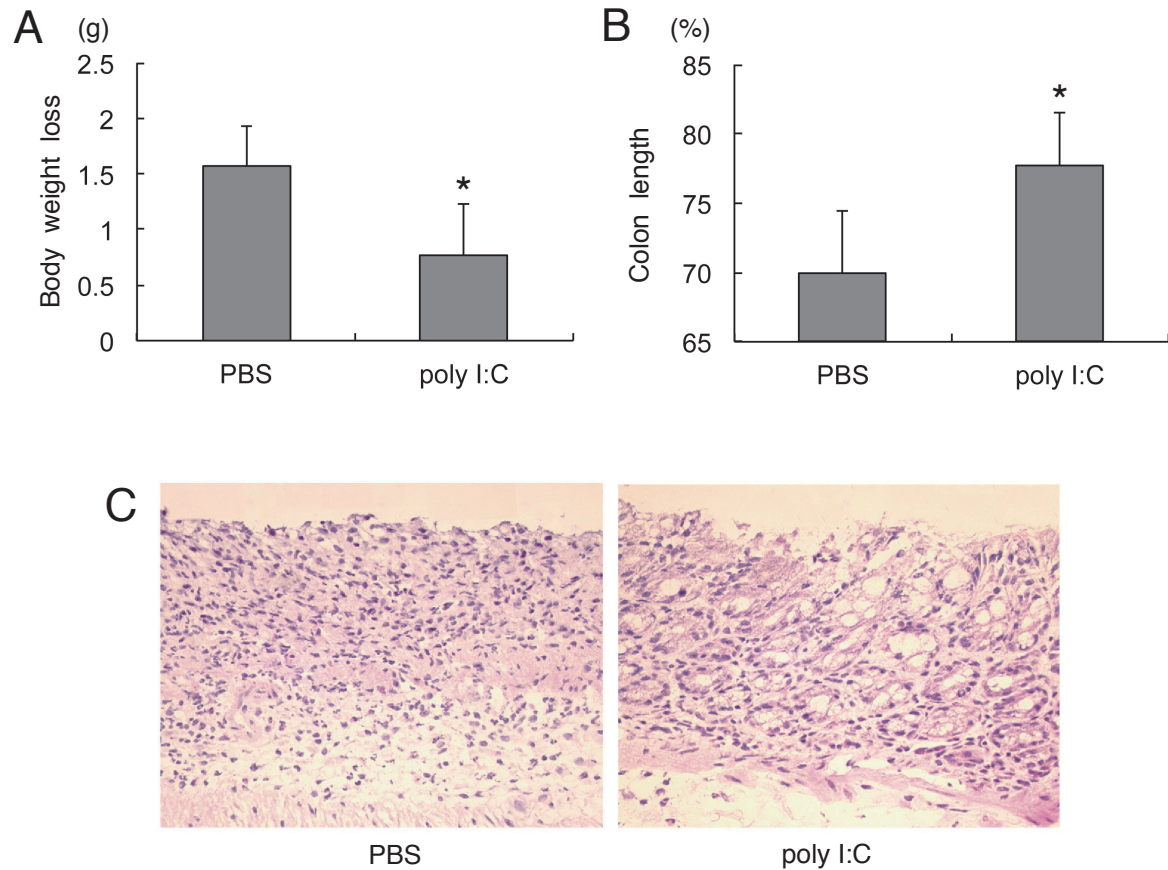


Fig. 3 Poly I:C injection attenuated the progression of DSS colitis. Mice were intraperitoneally injected with 20 μ g of poly I:C at 2 h before induction of DSS colitis. After 7 days, (A) loss of body weight and (B) colon length were measured. (C) Histologic examination was performed with hematoxylin and eosin staining. Data are means \pm SE; $n = 5$ to 9 mice per experimental group. * $P < 0.05$.

in Fig. 2A, expression of pIgR mRNA in DSS mice was reduced to 30% of that in wild-type mice.

To confirm these results at the protein level, we used ELISA to measure the level of pIgR in colon lysates. An apparent reduction in pIgR concentration was observed in DSS mice. With the pIgR level in wild-type mice set to 100%, the average pIgR in DSS mice was approximately 30% (Fig. 2B). No changes were observed in the small intestine (data not shown), indicating the specific induction of colitis by DSS treatment.

The decrease in pIgR was examined by heparin-agarose precipitation followed by Western blotting. A decrease in pIgR was observed in DSS mice (Fig. 2C). Immunohistochemical stainings (Fig. 2D) revealed severe damage in intestinal epithelial cells (IECs), and only the surface-lining epithelial cells were stained by anti-mpIgR monoclonal antibody. These results indicate that the pIgR level was significantly lower in DSS mice.

Reduction of pIgR and the extent of DSS colitis

To determine if there was a linear relationship between the magnitude of pIgR production and the extent of colitis, we examined whether poly I:C injection influenced the level of pIgR in DSS mice. Two hours before induction of DSS colitis, mice were injected intraperitoneally with 20 μ g of poly I:C or PBS. After pretreatment, mice were treated with DSS for 7 days. Poly I:C injection significantly reduced progression of DSS colitis, as determined by body weight loss (Fig. 3A) and colon length (Fig. 3B). Body weight loss was reduced to 0.75 g, and colon length was 80% of that of wild-type mice. Neutrophil infiltration was markedly lower, and damage to IECs was significantly less extensive, in mice receiving poly I:C injection (Fig. 3C).

Under these preventive conditions, pIgR level was measured in colon lysates by ELISA. In contrast to the markedly lower pIgR in DSS mice, pIgR levels remained normal after intraperitoneal injection of poly I:C (Fig. 4A). PBS and poly I:C injection had no effect on pIgR

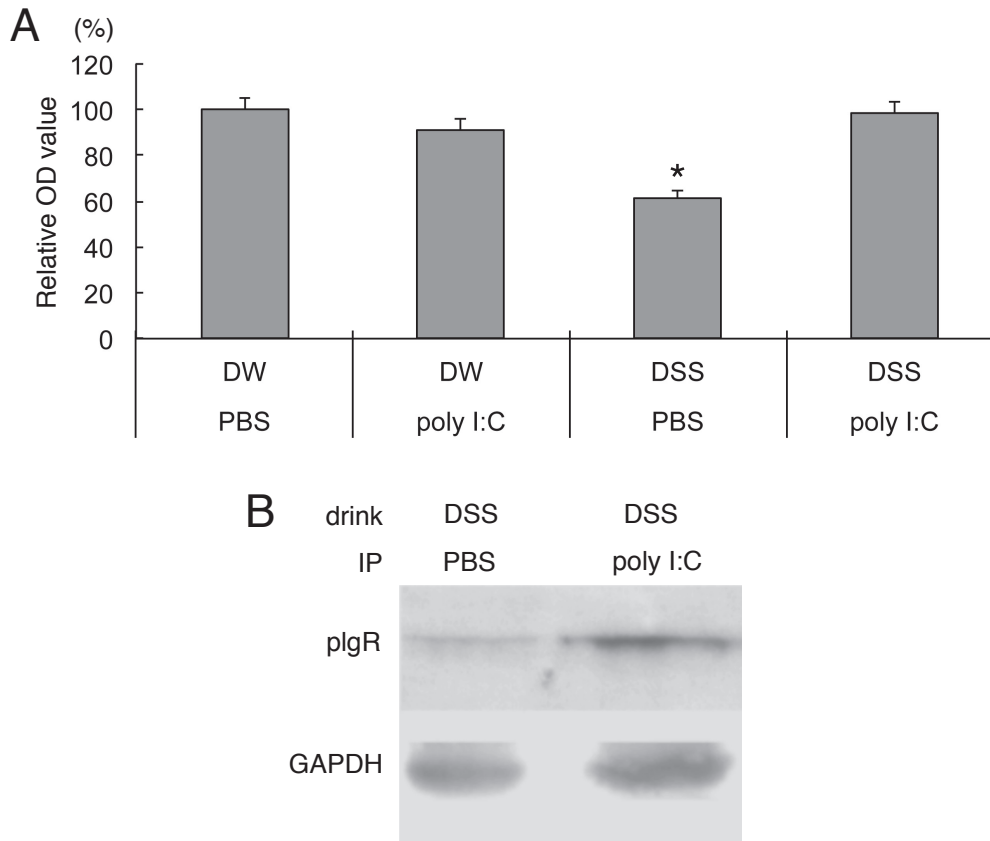


Fig. 4 Poly I:C injection normalized production of pIgR.

DSS colitis was induced in untreated mice and mice injected with poly I:C. The pIgR level was measured by ELISA (A) and Western blotting (B), as in Fig. 2. Data are means \pm SE; $n = 6$ to 10 mice per experimental group. * $P < 0.05$.

levels in colon lysates from wild-type mice, which indicates that poly I:C has a specific effect on DSS mice. Western blotting confirmed the presence of normal pIgR levels in DSS mice pretreated with poly I:C (Fig. 4B). These results suggest that the severity of DSS colitis correlates with the extent of pIgR reduction in colon lysates.

Cytokine profiles

The reduction of pIgR in DSS mice may be due to changes in cytokine levels. To examine this possibility, we measured cytokine levels by real-time PCR. Because IL-10 expression was shown to be higher after DSS treatment (12), we first examined IL-10 expression and found that it was indeed higher in DSS colitis (Fig. 5A). IFN- γ , TNF- α , and IL-4 induce expression of pIgR; therefore, expressions of these cytokines were also examined. IL-4 expression changed only slightly (Fig. 5D); however, IFN- γ and TNF- α (Fig. 5, B and C) were significantly induced by DSS treatment. These results indicate that reduction of pIgR in DSS colitis was not due to changes

in the expression of cytokines. The effect of poly I:C injection on the expression of these cytokines was then examined. Increased expression of all these cytokines was reversed when mice were pretreated with poly I:C (Fig. 5), indicating that the severity of DSS colitis was markedly reduced by poly I:C injection.

Discussion

In the present study, we observed a reduction in pIgR in DSS colitis on real-time PCR, ELISA, and immunohistochemical staining. These results were likely due to the destruction of IECs because they are the only cells that produce pIgR. In fact, HE staining revealed severe destruction of IECs (Fig. 1C), and small numbers of regenerating IECs were observed on immunohistochemical staining (Fig. 2D). These results suggest that pIgR levels can recover when destruction of IECs is prevented. To test this possibility, we used poly I:C treatment to attenuate the progression of DSS colitis. Poly I:C is an artificial analogue of double-stranded RNA, which is a byproduct of RNA virus replication, and is

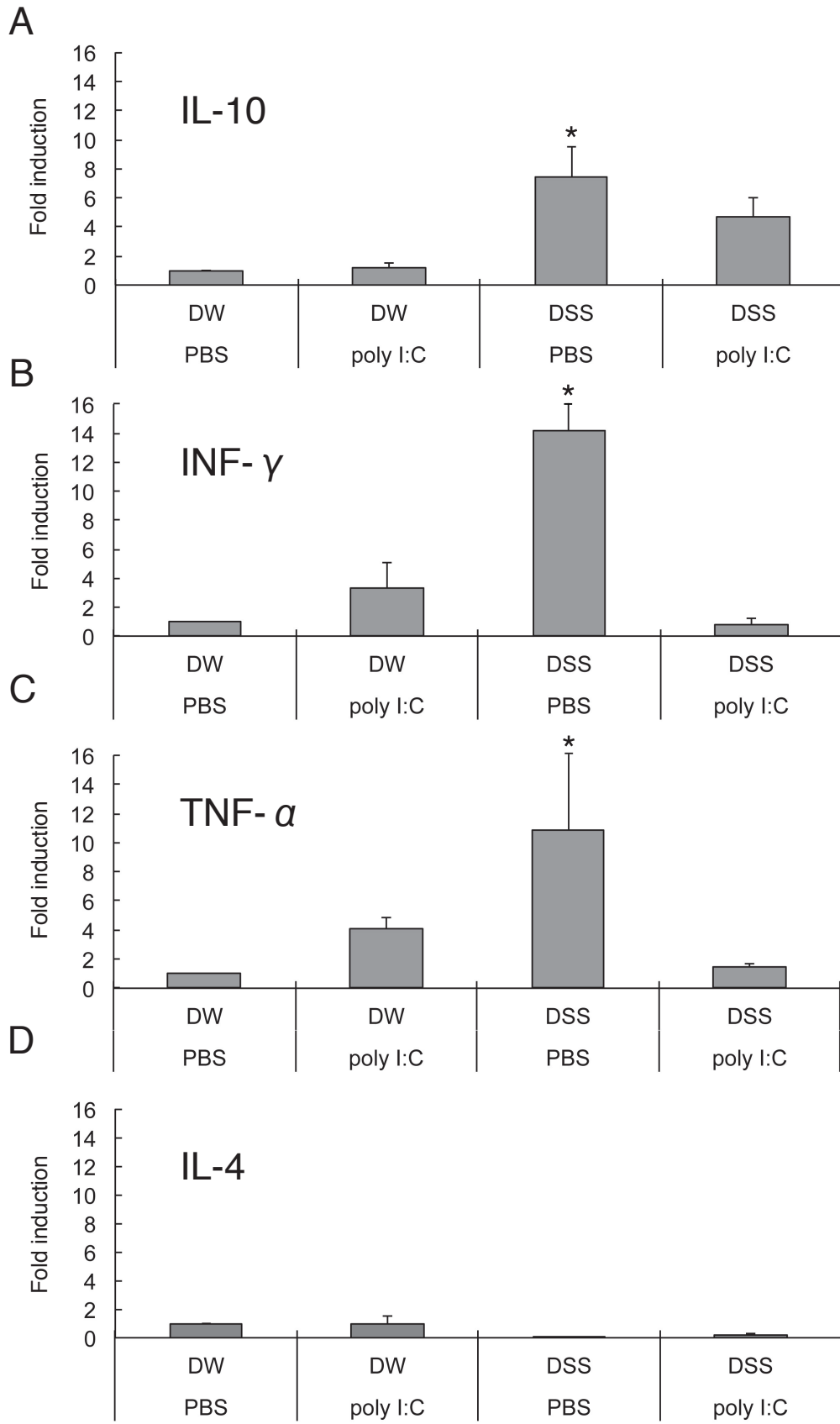


Fig. 5 Poly I:C injection attenuated cytokine profiles in DSS colitis. Total RNA was extracted from colon tissue and subjected to real-time PCR. The expression of (A) IL-10, (B) IFN- γ , (C) TNF- α , and (D) IL-4 are shown. Data are means \pm SE; $n = 6$ to 7 mice per experimental group. * $P < 0.05$.

widely used in viral research (13). The preventive effect of poly I:C against DSS colitis was reported by Vijay-Kumar et al. (14). Poly I:C reduced the severity of acute colitis by activating TLR3, and this effect was abrogated in TLR3 knockout mice. We recently reported poly I:C-induced production of intercellular adhesion molecule-1 (ICAM-1) in a human colon-derived adenocarcinoma cell line, HT-29 (11). The poly I:C signal was sensed by TLR3, and preincubation of the cells with anti-TLR3 antibody inhibited expression of ICAM-1. On the basis of these findings, we examined the relation between the preventive effect of poly I:C against DSS colitis and poly I:C-induced ICAM-1 expression. While 24 h of poly I:C injection up-regulated expression of ICAM-1 mRNA, as observed by real-time PCR, augmented expression of ICAM-1 was not observed after 7 days of DSS treatment. The absence of ICAM-1 overexpression was confirmed by immunohistochemical staining (data not shown). The molecular mechanisms responsible for the preventive effect of poly I:C injection against DSS colitis are unclear, and the possible contribution of poly I:C-induced ICAM-1 expression should be investigated.

Consistent with the reports of Vijay-Kumar et al. (14), poly I:C injection prevented development of DSS colitis, as indicated by shortening of the colon, loss of body weight, and normal values for histopathologic parameters and pIgR level. The relation between pIgR production and the extent of colitis suggests that pIgR level is an appropriate indicator of the severity of damage to IECs and DSS colitis.

Schneeman et al. reported that poly I:C induced production of pIgR in HT-29 cells (15) and that poly I:C treatment evoked TLR3 signaling and induced pIgR mRNA expression. As compared with induction of proinflammatory mediators, induction of pIgR mRNA was delayed. The gene expression and production of pIgR is regulated by several cytokines. Among these, IL-4 (16,17), IFN- γ (18-20), and TNF- α (21) have the most prominent effects on inducing pIgR expression. On the basis of those findings, Schneeman et al. examined the effect of soluble factors produced by poly I:C treatment on pIgR mRNA induction and found that incubation of HT-29 cells with anti-TNF- α antibody abrogated the induction of pIgR mRNA (15). These results suggest the possible contribution of these cytokines to the reduction of pIgR production in DSS colitis. Surprisingly, despite the reduction in pIgR, expressions of IFN- γ and TNF- α , both of which can activate pIgR production, were significantly increased. In contrast, IL-4 production did not markedly change after DSS treatment or poly I:C injection. These results indicate that increased expression

of pIgR-inducible cytokines does not compensate for the reduced production of pIgR due to the destruction of IECs. IL-10 is reported to be induced in DSS colitis, (12) and IL-10-disrupted mice can spontaneously develop chronic colitis (22). We consistently observed augmented IL-10 expression in DSS-mice, and poly I:C injection reduced the expression of IL-10. Therefore, IL-10 might suppress induction of pIgR mRNA, even if production of IFN- α and TNF- α is increased. To date, there is no evidence that IL-10 has a role in controlling pIgR expression.

The preventive role of pIgR in the development of DSS colitis was demonstrated by Murthy et al. (9). They used IgA- and pIgR-disrupted mice to evaluate the roles of IgA and pIgR in maintaining intestinal inflammatory responses in DSS colitis. Interestingly, DSS colitis was particularly severe in pIgR-disrupted mice. The importance of commensal bacterial flora in the maintenance of intestinal homeostasis is well established (23). pIgR is indispensable in the localization of secretory IgA to epithelial-surface mucous, which can capture commensal bacteria. Such captured commensal bacteria could evoke an innate immune response, resulting in the production of cytoprotective factors. On the basis of these facts, Murthy et al. (9) concluded that pIgR may be necessary for maintaining the basal tone of innate immunity in the intestinal environment. The importance of secretory antibodies in the maintenance of commensal microbiota in the intestine was challenged by Sait et al. (24). They compared the composition of the bacterial species in the terminal ileum of wild-type and pIgR-disrupted mice and found no systematic differences between the dominant components of the mucosa-associated bacterial communities. They concluded that secretory antibodies do not control the composition of intestinal microflora. However, in a study using pIgR-disrupted mice, Wijburg et al. (25) noted the importance of secretory antibodies against natural infection by *Salmonella typhimurium* (25). The intrinsic role of pIgR or secretory antibodies on the maintenance of intestinal homeostasis remains controversial.

In conclusion, our results indicate that pIgR levels change according to the severity of damage to IECs and DSS colitis. Thus, pIgR might be useful as a new biomarker of IBD severity.

Acknowledgments

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References

1. Eckmann L, Kagnoff MF (2005) Intestinal mucosal responses to microbial infection. *Springer Semin Immunopathol* 27, 181-196.
2. Kaetzel CS (2005) The polymeric immunoglobulin receptor: bridging innate and adaptive immune responses at mucosal surfaces. *Immunol Rev* 206, 83-99.
3. Mestecky J, Russell MW (1997) Mucosal immunoglobulins and their contribution to defence mechanisms: an overview. *Biochem Soc Trans* 25, 457-462.
4. Mostov KE (1994) Transepithelial transport of immunoglobulins. *Annu Rev Immunol* 12, 63-84.
5. Bouma G, Strober W (2003) The immunological and genetic basis of inflammatory bowel disease. *Nat Rev Immunol* 3, 521-533.
6. Wirtz S, Neurath MF (2007) Mouse models of inflammatory bowel disease. *Adv Drug Deliv Rev* 59, 1073-1083.
7. Okayasu I, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, Nakaya R (1990) A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology* 98, 694-702.
8. Omagari D, Iijima M, Suguro H, Sato I, Asano M, Moro I (2008) Differential distribution of mouse polymeric immunoglobulin receptor (mPIgR): establishment of enzyme-linked immunosorbent assay system for mPIgR. *Scand J Immunol* 68, 543-551.
9. Murthy AK, Dubose CN, Banas JA, Coalson JJ, Arulanandam BP (2006) Contribution of polymeric immunoglobulin receptor to regulation of intestinal inflammation in dextran sulfate sodium-induced colitis. *J Gastroenterol Hepatol* 21, 1372-1380.
10. Boesman-Finkelstein M, Finkelstein RA (1982) Sequential purification of lactoferrin, lysozyme and secretory immunoglobulin A from human milk. *FEBS Lett* 144, 1-5.
11. Omagari D, Mikami Y, Suguro H, Sunagawa K, Asano M, Sanuki E, Moro I, Komiyama K (2009) Poly I:C-induced expression of intercellular adhesion molecule-1 in intestinal epithelial cells. *Clin Exp Immunol* 156, 294-302.
12. Rogler G, Andus T (1998) Cytokines in inflammatory bowel disease. *World J Surg* 22, 382-389.
13. Matsumoto M, Seya T (2008) TLR3: interferon induction by double-stranded RNA including poly(I:C). *Adv Drug Deliv Rev* 60, 805-812.
14. Vijay-Kumar M, Wu H, Aitken J, Kolachala VL, Neish AS, Sitaraman SV, Gewirtz AT (2007) Activation of toll-like receptor 3 protects against DSS-induced acute colitis. *Inflamm Bowel Dis* 13, 856-864.
15. Schneeman TA, Bruno MEC, Schjerven H, Johansen FE, Chady L, Kaetzel CS (2005) Regulation of polymeric Ig receptor by signaling through TLRs 3 and 4: linking innate and adaptive immune responses. *J Immunol* 175, 376-384.
16. Schjerven H, Brandtzaeg P, Johansen FE (2003) Hepatocyte NF- κ B and STAT6 cooperate with additional DNA-binding factors to activate transcription of the human polymeric Ig receptor gene in response to IL-4. *J Immunol* 170, 6048-6056.
17. Schjerven H, Brandtzaeg P, Johansen FE (2000) Mechanism of IL-4-mediated up-regulation of the polymeric Ig receptor: role of STAT6 in cell type-specific delayed transcriptional response. *J Immunol* 165, 3898-3906.
18. Blanch VJ, Piskurich JF, Kaetzel CS (1999) Cutting edge: coordinate regulation of IFN regulatory factor-1 and the polymeric Ig receptor by proinflammatory cytokines. *J Immunol* 162, 1232-1235.
19. Kaetzel CS, Blanch VJ, Hempen PM, Phillips KM, Piskurich JF, Youngman KR (1997) The polymeric immunoglobulin receptor: structure and synthesis. *Biochem Soc Trans* 25, 475-480.
20. Piskurich JF, Youngman KR, Phillips KM, Hempen PM, Blanchard MH, France JA, Kaetzel CS (1997) Transcriptional regulation of the human polymeric immunoglobulin receptor gene by interferon- γ . *Mol Immunol* 34, 75-91.
21. Schjerven H, Brandtzaeg P, Johansen FE (2001) A novel NF- κ B/Rel site in intron 1 cooperates with proximal promoter elements to mediate TNF- α -induced transcription of the human polymeric Ig receptor. *J Immunol* 167, 6412-6420.
22. Kühn R, Löhler J, Rennick D, Rajewsky K, Müller W (1993) Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 75, 263-274.
23. Round JL, Mazmanian SK (2009) The gut microbiota shapes intestinal immune response during health and disease. *Nat Rev Immunol* 9, 313-323.
24. Sait L, Galic M, Strugnell RA, Janssen PH (2003)

Secretory antibodies do not affect the composition of the bacterial microbiota in the terminal ileum of 10-week-old mice. *Appl Environ Microbiol* 69, 2100-2109.

25. Wijburg OLC, Uren TK, Simpfendorfer K,

Johansen FE, Brandtzaeg P, Strugnell RA (2006) Innate secretory antibodies protect against natural *Salmonella typhimurium* infection. *J Exp Med* 203, 21-26.