PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 February 21; 13(7): 1085-1089
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Possible role of human cytomegalovirus in pouchitis after proctocolectomy with ileal pouch-anal anastomosis in patients with ulcerative colitis

Damian Casadesus, Tatsuo Tani, Toshifumi Wakai, Satoshi Maruyama, Tsuneo Iiai, Haruhiko Okamoto, Katsuyoshi Hatakeyama

Damian Casadesus, Tatsuo Tani, Toshifumi Wakai, Satoshi Maruyama, Tsuneo Iiai, Haruhiko Okamoto, Katsuyoshi Hatakeyama, Division of Digestive and General Surgery, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan
Correspondence to: Tatsuo Tani, MD, PhD, Division of Digestive and General Surgery, Niigata University Graduate School of Medical and Dental Sciences, 1-757 Asahimachi-dori, Niigata City 951-8510, Japan. ttani@med.niigata-u.ac.jp
Telephone: +81-25-2272228 Fax: +81-25-2270779
Received: 2006-10-24 Accepted: 2006-11-11

Abstract

AIM: To detect the presence of human cytomegalovirus (HCMV) proteins and genes on the ileal pouch of patients with ulcerative colitis who have undergone proctocolectomy with ileal pouch-anal anastomosis (IPAA).

METHODS: Immunohistochemistry, polymerase chain reaction (PCR) and PCR sequencing methods were utilized to test the presence of HCMV in pouch specimens taken from 34 patients in 86 endoscopies.

RESULTS: HCMV genes and proteins were detected in samples from 12 (35.2%) patients. The rate of detection was significant in the endoscopies from patients diagnosed with pouchitis (5 of 12, 41.6%), according to the Japanese classification of pouchitis, in comparison to patients with normal pouch (7 of 62, 11.2%; P = 0.021). In all patients with pouchitis in which the HCMV was detected, it was the first episode of pouchitis. The virus was not detected in previous biopsies taken in normal endoscopies of these patients. During the follow-up, HCMV was detected in one patient with recurrent pouchitis and in 3 patients whose pouchitis episodes improved but whose positive endoscopic findings persisted.

CONCLUSION: HCMV can take part in the inflammatory process of the pouch in some patients with ulcerative colitis who have undergone proctocolectomy with IPAA.

Key words: Human cytomegalovirus; Pouchitis; Inflammatory bowel disease; Ileal pouch-anal anastomosis

Casadesus D, Tani T, Wakai T, Maruyama S, Iiai T, Okamoto H, Hatakeyama K. Possible role of human cytomegalovirus in pouchitis after proctocolectomy with ileal pouch-anal anastomosis in patients with ulcerative colitis. *World J Gastroenterol* 2007; 13(7): 1085-1089

http://www.wjgnet.com/1007-9327/13/1085.asp

INTRODUCTION

Human cytomegalovirus (HCMV) is a ubiquitous, species-specific beta-herpes virus that can establish lifelong latency in the host after the primary infection. HCMV infection is endemic within the human population, and its role as a pathogen in the colon and ileum is still unclear. Early studies suggested that HCMV infection initiates some cases of ulcerative colitis (UC)[1,2], plays a role in UC exacerbation[3], causes self-limited colitis[4], and increases the incidence of complications, emergency surgery or death in patients with UC[3,5,6].

Pouchitis is a frequent complication in patients with UC who have undergone colectomy with ileal pouch-anal anastomosis (IPAA). The etiology of pouchitis is still unknown, but several theories have been proposed, such as genetic susceptibility, a possible novel third form of inflammatory bowel disease (IBD) in the pouch, recurrence of UC in the pouch, misdiagnosis of Crohn's disease, ischemic complication of surgery, fecal stasis, and bacterial overgrowth. Recent studies have reported HCMV infection as a cause of pouchitis in 3 immunocompetent patients[7,8]. The diagnosis of this specific infectious agent as a possible cause of pouchitis is crucial before initiating immune modifier therapy, fecal diversion, or pouch excision. We explored the presence of viral gene products and proteins in the pouches of a series of patients who have undergone proctocolectomy with IPAA.

MATERIALS AND METHODS

Patients

Enrolled in this study were 34 Japanese patients (17 females, 17 males) who underwent proctocolectomy

with IPAA at the Department of General and Digestive Surgery, Niigata University Hospital between 1990 and 2003. The patients' age ranged from 24 to 68 years (mean 34.8 ± 15.4 years). Oral and written informed consent was obtained from each patient. The study protocol adhered to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institution's ethical committee. We reviewed the clinical and endoscopic records of all the enrolled patients. The patients had a history of UC between 1 to 5 years and underwent the operation in two or three steps. The diagnosis of pouchitis was based on clinical and endoscopic criteria according to the modified pouchitis disease activity index (mPDAI)[9] and the Japanese classification of pouchitis (JCP)[10]. JCP defines pouchitis as a condition with severe endoscopic findings, or with two or more clinical symptoms and moderate endoscopic findings. Eighty-six endoscopies were performed in 34 patients. Twenty-eight endoscopies were performed in patients complaining of symptoms, 46 endoscopies were carried out in routine controls after surgery, and 12 were performed during pouchitis follow-up. Biopsies were performed both in areas with pathological findings (edema, granularity, friability, erythema, loss of vascular pattern, mucous exudates, erosion or ulceration) and in areas with mucosa of normal appearance.

Immunohistochemistry (IHC)

Immunostaining for HCMV was performed on paraffin-embedded sections with a cocktail of two monoclonal mouse antibodies against human cytomegalovirus (clone CCH2 & DDG9, Dako Cytomation, CA, USA) at a dilution of 1:100. All paraffin-embedded samples from each patient were used for IHC. According to the company specifications, antibody CCH2 reacts with an early nuclear protein identical with non-structural DNA-binding protein p52 (UL44), whereas antibody DDG9 reacts with an immediate-early nuclear protein with a molecular weight of about 76 kDa. For both antibodies, the reactivity persists also at later stages during HCMV infection where the localization is less distinctly nuclear and appears to be in the cytoplasm. Five-μm thick sections were deparaffinized and rehydrated using graded alcohol concentrations, then the sections were digested with trypsin (Sigma Chemicals, Germany) at 37℃ for 20 min. After endogenous peroxidase was blocked by incubation with 30 mL/L hydrogen peroxide for 20 min, the sections were incubated overnight at 4℃ with the cocktail of anti-human cytomegalovirus antibodies. Control slides from the same biopsy block were incubated with PBS without the primary antibody. They were then incubated at room temperature for 30 min with goat anti-mouse immunoglobulin conjugated to a peroxidase-labeled amino-acid polymer, Simple Stain Max PO (Nichirei Histofine, Tokyo, Japan). The sections were reacted with diaminobenzidine in 50 mol/L Tris-HCL (pH 7.5) with 0.3% (vol/vol) hydrogen peroxide for 5 min and counterstained with hematoxylin.

Polymerase chain reaction (PCR) and PCR sequencing

To confirm that our probe was specific for HCMV nucleic acids, DNA samples were extracted from two paraffin sections 20 μm in size, and cut from the same biopsy specimens described above with a QIAamp DNA minikit (Qiagen, Tokyo, Japan). From each sample, 200 ng of DNA was amplified by PCR with the primer 5'-TGCAGTTTGGTCCCTTAAAG-3' and 5'-AAGAATCCTCACCTGGCTTA-3' from the HCMV large structural phosphoprotein (UL32) and the primer 5'-TCCAACACCCACAGTACCCGT-3' and 5'-CGGAAACGATGGTGTAGTTCG-3' from the HCMV glycoprotein B (UL55), using a method specific for UL32 gene[11] and UL55 gene[12]. The amplified DNA products were visualized on 2% agarose gel (NuSieve GTG agarose, FMC Bio Products, Rockland, USA) stained with 0.01% (vol/vol) ethidium bromide and visualized under ultraviolet light. The bands of UL32 gene were cut out and the DNA was analyzed by automated sequencing (ABI Prism 310 Diagnostics Systems, Applied Biosystems, Tokyo, Japan). The HCMV sequence was confirmed by NCBI Blast, and found to be identical to that of UL32. To avoid potential PCR contamination, all preparations were processed masked, no positive controls were used in any PCR reactions and a separated room was used for the preparation of the reaction mixture. The distilled water control gave negative results in each assay run, and confirmed the efficiency of these preventive measures. When IHC and/or PCR were positive, the sample was considered to be HCMV-positive, and when both of them were negative, the sample was considered to be HCMV-negative.

Statistical analysis

The results of HCMV detection are expressed in numbers of patients and percentages. The results of the cumulative-life steroid dose are given as means ± SD. Statistical significance was calculated with SPSS 13 (SPSS Inc., Chicago, IL, USA) using Fisher's exact test and Student's *t*-test when appropriate, and the results were considered significant at $P \leqslant 0.05$.

RESULTS

Twenty-eight endoscopies were performed in patients complaining of symptoms and 46 endoscopies were performed in routine controls at different time points after surgery. Four hundred and seventy-three specimens were evaluated, 103 from patients with pouchitis and 370 from patients with a normal pouch. We detected HCMV in samples from 12 endoscopies using IHC and PCR. These endoscopies were performed because the patients were complaining of symptoms (8 endoscopies) or during routine controls after surgery (4 endoscopies). Immunoreactivity was observed in the submucosal layer with predominant nuclear staining of cells (Figure 1). There were not any differences in the staining pattern between the HCMV-positive patients with and without pouchitis either using mPDAI or JCP. We performed PCR for all samples, and amplified products from both genes were detected in the same sample with positive IHC (Figure 2). No HCMV was detected in the distilled water control that was run through the same PCR reaction.

HCMV was significantly detected in endoscopies of patients diagnosed with pouchitis (5 of 12, 41.6%)

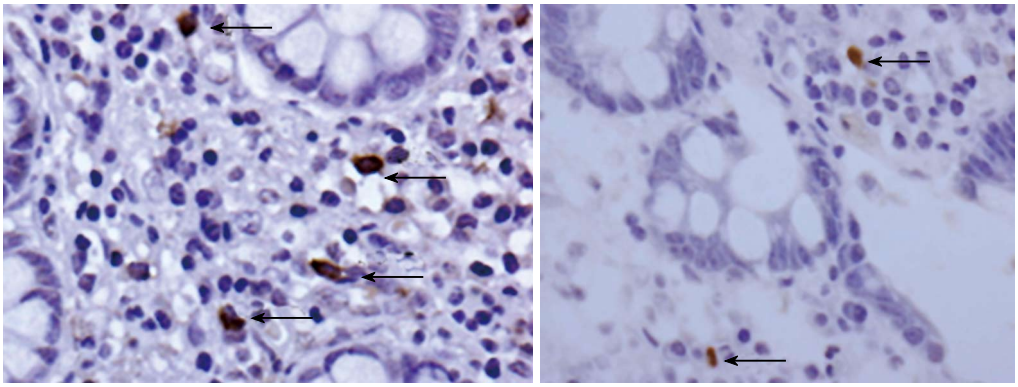

Figure 1 Immunohistochemical staining for HCMV of biopsy samples from ileoanal pouch. Cells in the submucosa (arrows) show strong nuclear staining. Original magnification x 600.

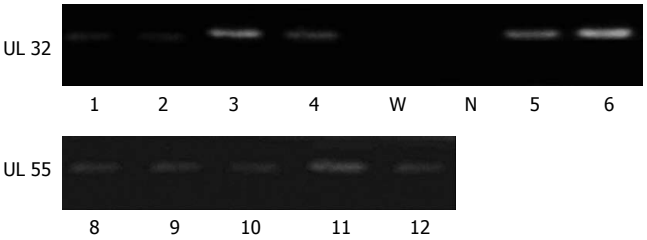

Figure 2 Agarose gel showing UL32 gene products (lanes 1-4, 5 and 6) and UL 55 gene products (lanes 8-12). W: Water; N: HCMV-negative patient.

Table 1 HCMV detection during endoscopic examinations of UC patients with proctocolectomy and ileal pouch-anal anastomosis *n* (%)

	HCMV positive	HCMV negative	*P*
mPDAI[10]			
Pouchitis	3 (27.2)	8 (72.7)	NS
No pouchitis	9 (14.2)	54 (85.7)	
JCP[11]			
Pouchitis	5 (41.6)	7 (58.3)	0.021
No pouchitis	7 (11.2)	55 (88.7)	

mPDAI: modified pouchitis disease activity index; JCP: Japanese classification of pouchitis, NS: not significant.

compared to those with normal pouch (7 of 62, 11.2%; $P = 0.021$, Table 1). According to mPDAI, HCMV was more frequently detected in patients with pouchitis (3 of 11, 27.2%) than in those with normal pouch (9 of 63, 14.2%), but this result was not statistically significant. In all patients with pouchitis in which the HCMV was detected in endoscopy, it was the first episode of pouchitis. In these patients HCMV was not detected in biopsies taken in previous normal endoscopies. The odds ratio suggested that the presence of HCMV was 5 times more frequent in patients with episodes of pouchitis than in patients without.

There was not correlation between the presence of HCMV and the duration of UC, the period of pouchitis, the number of operations, the age, and the gender of the patients. There was no significant difference between the cumulative-life steroid dose and HCMV presence. The mean of the cumulative-life steroid dose was 17152.5 mg ± 16999.0 mg in HCMV-negative patients, and 13347.8 mg ± 12966.7 mg in HCMV-positive patients ($P = 0.52$). After the diagnosis of pouchitis, all patients began treatment with oral metronidazole (500 mg/d). During the follow-up, 2 patients diagnosed with pouchitis were lost and one of them was HCMV-positive. In the samples from 12 endoscopies performed during follow-up, HCMV was detected using IHC and PCR in 4 (4 of 10, 40%) patients. One of these patients had recurrent pouchitis and 3 showed improvement of pouchitis episodes but persistent positive endoscopic findings.

DISCUSSION

It has been suggested that HCMV plays a role in the onset, exacerbation and complication of inflammatory bowel disease (IBD)[2-6,13-18]. However, the etiology of pouchitis is still unknown and the role of HCMV as a possible etiologic factor has not been studied yet. This is the first report exploring the correlation between HCMV and pouchitis in a group of patients with UC who underwent proctocolectomy with IPAA. There are three possible causes for this association: (1) the virus is a simple bystander in the inflammatory process of the pouch, (2) the virus takes part in the inflammatory process after reactivation and productive infection due to another pathogen infection, (3) the virus induces the inflammatory process after infection.

The differentiation of latently infected monocytes[19-23] into tissue macrophages, as occurs with intercurrent infections, could lead to the productive infection and dissemination of HCMV in the digestive tract mucosa of infected patients. No single identifiable causative organism has been detected as the cause of pouchitis, but the infiltration of neutrophils and the proven response to antibacterial therapy suggest that pouchitis could have a bacterial cause[24-27]. However, different facts suggest that HCMV is a real gastrointestinal pathogen and that it can be partly related to the development of pouchitis in some patients. HCMV is often detected in the absence of other pathogens, and some UC complications have been associated with the presence of HCMV[3,5,6]. Moreover, colitis and pouchitis caused by HCMV infection are known to respond to antiviral therapy[8,9,15], and immediate-early HCMV gene products enhance cytokine production and cytokine gene expression[28,29] which *in vivo* would lead to the pronounced inflammation in UC

and pouchitis. Although the pathway of HCMV infection and inflammation is still unclear, the increased production of different cytokines and arachidonic acid, as well as the increased activity of cyclooxygenase 2 after HCMV infection[30-34], could explain the inflammatory response in IBD and pouchitis. In our study on a small series of patients with pouchitis, we hypothesized that HCMV may have played an important role in the etiology of pouchitis in those cases. We detected by IHC an early protein and an immediate-early protein present during an early stage of viral infection. This result corresponds to the detection by PCR of genes that appear early after infection and then frequently decline to undetectable levels with the passage of time[35]. The presence of HCMV turned positive in the episode of pouchitis. However, other factors such as bacterial overgrowth and fecal hydrogen sulfide production[36] could also be implicated in the etiology of pouchitis episode in patients with no detectable HCMV.

The true incidence of HCMV infection in pouchitis as well as in IBD may be underestimated, as diagnostic evaluation for HCMV is not pursued in many of these patients. In our series, the patients did not receive antiviral therapy and HCMV antigenemia was not measured because a diagnosis of HCMV pouchitis was not sought on the occasion of endoscopy. However, after the antibacterial treatment, HCMV persisted in 4 patients with positive endoscopic findings, confirming the possibility of a possible role of HCMV in the etiology of pouchitis in those patients. Since multiple factors play a role in pouchitis, the clinician must exclude HCMV infection as the cause of pouchitis, especially in patients resistant to treatment, before performing fecal diversion, or pouch excision. Concomitant evaluation of the presence of HCMV may be a clinically significant component of the successful treatment of pouchitis.

In conclusion, the presence of HCMV in pouchitis could partly explain the inflammatory response in some patients with UC who have undergone proctocolectomy with IPAA. Therefore, prospective studies with a large number of patients and an analysis of the correlation between antigenemia of HCMV and immunohistological data are definitely needed to identify the specific role of this virus.

ACKNOWLEDGMENTS

The authors thank Mr. Takashi Hatano for his technical assistance. DC is in receipt of a grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

1 **Diepersloot RJ**, Kroes AC, Visser W, Jiwa NM, Rothbarth PH. Acute ulcerative proctocolitis associated with primary cytomegalovirus infection. *Arch Intern Med* 1990; **150**: 1749-1751
2 **Lortholary O**, Perronne C, Leport J, Leport C, Vildé JL. Primary cytomegalovirus infection associated with the onset of ulcerative colitis. *Eur J Clin Microbiol Infect Dis* 1993; **12:** 570-572
3 **Berk T**, Gordon SJ, Choi HY, Cooper HS. Cytomegalovirus infection of the colon: a possible role in exacerbations of inflammatory bowel disease. *Am J Gastroenterol* 1985; **80**: 355-360
4 **Surawicz CM**, Myerson D. Self-limited cytomegalovirus colitis in immunocompetent individuals. *Gastroenterology* 1988; **94**: 194-199
5 **Cooper HS**, Raffensperger EC, Jonas L, Fitts WT. Cytomegalovirus inclusions in patients with ulcerative colitis and toxic dilation requiring colonic resection. *Gastroenterology* 1977; **72**: 1253-1256
6 **Eyre-Brook IA**, Dundas S. Incidence and clinical significance of colonic cytomegalovirus infection in idiopathic inflammatory bowel disease requiring colectomy. *Gut* 1986; **27:** 1419-1425
7 **Moonka D**, Furth EE, MacDermott RP, Lichtenstein GR. Pouchitis associated with primary cytomegalovirus infection. *Am J Gastroenterol* 1998; **93**: 264-266
8 **Muñoz-Juarez M**, Pemberton JH, Sandborn WJ, Tremaine WJ, Dozois RR. Misdiagnosis of specific cytomegalovirus infection of the ileoanal pouch as refractory idiopathic chronic pouchitis: report of two cases. *Dis Colon Rectum* 1999; **42**: 117-120
9 **Shen B**, Achkar JP, Connor JT, Ormsby AH, Remzi FH, Bevins CL, Brzezinski A, Bambrick ML, Fazio VW, Lashner BA. Modified pouchitis disease activity index: a simplified approach to the diagnosis of pouchitis. *Dis Colon Rectum* 2003; **46:** 748-753
10 **Inflammatory bowel disease study group**. Atlas of endoscopic diagnosis of pouchitis. Tokyo: Kyoubundou, 2003
11 **Ashshi AM**, Klapper PE, Cooper RJ. Detection of human cytomegalovirus, human herpesvirus type 6 and human herpesvirus type 7 in urine specimens by multiplex PCR. *J Infect* 2003; **47:** 59-64
12 **Kühn JE**, Wendland T, Eggers HJ, Lorentzen E, Wieland U, Eing B, Kiessling M, Gass P. Quantitation of human cytomegalovirus genomes in the brain of AIDS patients. *J Med Virol* 1995; **47**: 70-82
13 **Cottone M**, Pietrosi G, Martorana G, Casà A, Pecoraro G, Oliva L, Orlando A, Rosselli M, Rizzo A, Pagliaro L. Prevalence of cytomegalovirus infection in severe refractory ulcerative and Crohn's colitis. *Am J Gastroenterol* 2001; **96**: 773-775
14 **Loftus EV**, Alexander GL, Carpenter HA. Cytomegalovirus as an exacerbating factor in ulcerative colitis. *J Clin Gastroenterol* 1994; **19:** 306-309
15 **Papadakis KA**, Tung JK, Binder SW, Kam LY, Abreu MT, Targan SR, Vasiliauskas EA. Outcome of cytomegalovirus infections in patients with inflammatory bowel disease. *Am J Gastroenterol* 2001; **96**: 2137-2142
16 **Pfau P**, Kochman ML, Furth EE, Lichtenstein GR. Cytomegalovirus colitis complicating ulcerative colitis in the steroid-naive patient. *Am J Gastroenterol* 2001; **96:** 895-899
17 **Vega R**, Bertrán X, Menacho M, Domènech E, Moreno de Vega V, Hombrados M, Cabré E, Ojanguren I, Gassull MA. Cytomegalovirus infection in patients with inflammatory bowel disease. *Am J Gastroenterol* 1999; **94:** 1053-1056
18 **Maté del Tío M**, Peña Sánchez de Rivera JM, Larrauri Martínez J, Garcés Jiménez MC, Barbado Hernández FJ. Association of cytomegalovirus colitis and the 1st episode of ulcerative colitis in an immunocompetent patient. *Gastroenterol Hepatol* 1996; **19**: 206-207
19 **Dankner WM**, McCutchan JA, Richman DD, Hirata K, Spector SA. Localization of human cytomegalovirus in peripheral blood leukocytes by in situ hybridization. *J Infect Dis* 1990; **161:** 31-36
20 **Ibanez CE**, Schrier R, Ghazal P, Wiley C, Nelson JA. Human cytomegalovirus productively infects primary differentiated macrophages. *J Virol* 1991; **65:** 6581-6588
21 **Sinzger C**, Plachter B, Grefte A, The TH, Jahn G. Tissue macrophages are infected by human cytomegalovirus in vivo. *J Infect Dis* 1996; **173:** 240-245
22 **Taylor-Wiedeman J**, Sissons JG, Borysiewicz LK, Sinclair JH. Monocytes are a major site of persistence of human cytomegalovirus in peripheral blood mononuclear cells. *J Gen*

Virol 1991; **72** (Pt 9): 2059-2064
23 **Yurochko AD**, Huang ES. Human cytomegalovirus binding to human monocytes induces immunoregulatory gene expression. *J Immunol* 1999; **162:** 4806-4816
24 **Iwaya A**, Iiai T, Okamoto H, Ajioka Y, Yamamoto T, Asahara T, Nomoto K, Hatakeyama K. Change in the bacterial flora of pouchitis. *Hepatogastroenterology* 2006; **53**: 55-59
25 **Lim M**, Sagar P, Finan P, Burke D, Schuster H. Dysbiosis and pouchitis. *Br J Surg* 2006; **93**: 1325-1334
26 **Abdelrazeq AS**, Kelly SM, Lund JN, Leveson SH. Rifaximin-ciprofloxacin combination therapy is effective in chronic active refractory pouchitis. *Colorectal Dis* 2005; **7**: 182-186
27 **Gosselink MP**, Schouten WR, van Lieshout LM, Hop WC, Laman JD, Ruseler-van Embden JG. Eradication of pathogenic bacteria and restoration of normal pouch flora: comparison of metronidazole and ciprofloxacin in the treatment of pouchitis. *Dis Colon Rectum* 2004; **47**: 1519-1525
28 **Geist LJ**, Monick MM, Stinski MF, Hunninghake GW. The immediate early genes of human cytomegalovirus upregulate tumor necrosis factor-alpha gene expression. *J Clin Invest* 1994; **93**: 474-478
29 **Iwamoto GK**, Monick MM, Clark BD, Auron PE, Stinski MF, Hunninghake GW. Modulation of interleukin 1 beta gene expression by the immediate early genes of human cytomegalovirus. *J Clin Invest* 1990; **85:** 1853-1857
30 **Rahbar A**, Boström L, Lagerstedt U, Magnusson I, Söderberg-Naucler C, Sundqvist VA. Evidence of active cytomegalovirus infection and increased production of IL-6 in tissue specimens obtained from patients with inflammatory bowel diseases. *Inflamm Bowel Dis* 2003; **9**: 154-161
31 **Compton T**, Kurt-Jones EA, Boehme KW, Belko J, Latz E, Golenbock DT, Finberg RW. Human cytomegalovirus activates inflammatory cytokine responses via CD14 and Toll-like receptor 2. *J Virol* 2003; **77**: 4588-4596
32 **Redman TK**, Britt WJ, Wilcox CM, Graham MF, Smith PD. Human cytomegalovirus enhances chemokine production by lipopolysaccharide-stimulated lamina propria macrophages. *J Infect Dis* 2002; **185**: 584-590
33 **Zhu H**, Cong JP, Yu D, Bresnahan WA, Shenk TE. Inhibition of cyclooxygenase 2 blocks human cytomegalovirus replication. *Proc Natl Acad Sci USA* 2002; **99**: 3932-3937
34 **Nokta MA**, Hassan MI, Loesch K, Pollard RB. Human cytomegalovirus-induced immunosuppression. Relationship to tumor necrosis factor-dependent release of arachidonic acid and prostaglandin E2 in human monocytes. *J Clin Invest* 1996; **97:** 2635-2641
35 **Greijer AE**, van de Crommert JM, Stevens SJ, Middeldorp JM. Molecular fine-specificity analysis of antibody responses to human cytomegalovirus and design of novel synthetic-peptide-based serodiagnostic assays. *J Clin Microbiol* 1999; **37**: 179-188
36 **Ohge H**, Furne JK, Springfield J, Rothenberger DA, Madoff RD, Levitt MD. Association between fecal hydrogen sulfide production and pouchitis. *Dis Colon Rectum* 2005; **48**: 469-475

S- Editor Wang J **L- Editor** Wang XL **E- Editor** Ma WH