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• *BRIEF REPORTS* •

Appendix is a priming site in the development of ulcerative colitis

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Abstract

AIM: The role of the appendix has been highlighted in the pathogenesis of ulcerative colitis (UC). The aims of this study were to elucidate the immuno-imbalances in the appendix of UC patients, and to clarify the role of the appendix in the development of UC.

METHODS: Colonoscopic biopsy specimens of the appendix, transverse colon, and rectum were obtained from 86 patients with UC: active pancolitis (A-Pan; *n* = 15), active left-sided colitis (A-Lt; *n* = 25), A-Lt with appendiceal involvement (A-Lt/Ap; *n* = 10), inactive pancolitis (I-Pan; *n* = 14), and inactive left-sided colitis (I-Lt; *n* = 22), and from controls. In the isolated mucosal T cells, the CD4/CD8 ratio and proportion of activated CD4+ T cells were investigated, and compared with controls.

RESULTS: In the appendix, the CD4/CD8 ratio significantly increased in A-Lt and A-Lt/Ap. The ratio in the appendix also tended to increase in A-Pan. In the rectum, the ratio significantly increased in all UC groups. In the appendix, the proportion of CD4+CD69+ (early activation antigen) T cells significantly increased in all UC groups. In the rectum, the proportion of CD4+CD69+ T cells significantly increased only in A-Pan. The proportion of CD4+HLA-DR+ (mature activation antigen) T cells significantly increased only in the rectum of A-Pan, but not in the other areas of any groups.

CONCLUSION: The increased CD4/CD8 ratio and predominant infiltration of CD4+CD69+ T cells in the appendix suggest that the appendix is a priming site in the development of UC.

Key words: Appendix; Appendectomy; Ulcerative colitis; Activated T cell; CD4+ T cell

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INTRODUCTION

Although the triggering factor for ulcerative colitis (UC) is still unknown, cytokine imbalance and the production of inflammatory mediators by activated CD4+ T cells play an important role in the pathogenesis of UC. T helper type 2 cells and their cytokines, particularly interleukin (IL)-4, have been suggested to enhance the development of UC[1]. Recently, regulatory T cells, characterized by the expression of cell surface markers CD4 and CD25, have been shown to actively suppress immune responses, and lack of regulatory T cells leads to organ-specific autoimmunity[2]. On the other hand, a sub-population of CD8+ T cells also suppresses the response of activated CD4+ T cells and B cells through an interaction that depends on expression of the major histocompatibility complex class Ib molecule Qa-1, the mouse homolog of human leukocyte antigen (HLA)-E[3]. However, the precise role of these regulatory T cells in UC remains unclear.

Although human appendix is considered as a vestigial remnant[4], recent observations have focused attention on the role of the appendix in the pathogenesis of UC. Many case-control studies suggest that previous appendectomy is rare in UC patients[5-7], raising the possibility that appendectomy protects against the subsequent development of UC[8-11]. Patients with previous appendectomy also have a delayed onset of UC[8,9], a reduced need for immunosuppression therapy and proctocolectomy[8,10], and a reduced relapse rate and extent of UC[11]. Appendectomy in T-cell receptor (TCR)-α deficient mice suppresses the development of experimental colitis[12]. Moreover, we first reported a patient with improvement of left-sided UC after appendectomy[13]. Although these findings support that the appendix may be

related to the pathogenesis of UC, the immunological role of human appendix is unknown.

Extensive infiltration of lymphocytes, especially CD4+ T cells[14], has been observed in the inflamed mucosa of UC patients[15]. Activated CD4+ T cells exhibit increased cytotoxic activity[16] and secrete cytokines that enhance the inflammatory state resulting in tissue injury[17,18]. Several studies concerning T-cell subsets in the resected appendix have been performed previously[19], but very few have focused on the activation status of the immune cells in the appendix as well as in the uninflamed mucosa. In this study, we investigated the CD4/CD8 ratio and proportion of activated CD4+ T cells in the inflamed and uninflamed colonic mucosa, especially in the appendiceal mucosa, of UC patients in order to clarify the role of the appendix in the development of UC.

MATERIALS AND METHODS

Subjects

UC patients with toxic megacolon, coexistence of known cancer, complication of extra-intestinal disease, past colectomy, poor general condition, and no consent to participate in the study were excluded. A total of 86 patients with UC and 27 control subjects were subsequently enrolled in the study. Informed consent to participate in this study was obtained from each patient. The diagnosis of UC was based on the established clinical, endoscopic, histological, and/or radiological criteria[20]. Patients with no malignant or inflammatory colonic disorders, including adenomatous polyps (n = 8), diverticular disease (n = 6), non-specific abdominal pain (n = 5), family history of colorectal cancer (n = 4), and chronic constipation (n = 4) served as the control subjects. Patients receiving non-steroidal anti-inflammatory drugs or immuno-regulatory drugs, such as corticosteroids and azathioprine were excluded from controls. There was no history of appendectomy both in the patients and controls.

Scoring system of disease activity

The disease activity was evaluated based on the endoscopic findings according to the scoring system as reported previously[21]: grade 0, normal vascular pattern; grade 1, erythema with loss of vascular pattern; grade 2, grade 1 plus contact bleeding; grade 3, grade 1 plus spontaneous bleeding; and grade 4, grade 1 plus obvious ulceration. We defined the grades 0 and 1 as inactive disease, and the other grades as active disease. The disease extent was also classified endoscopically into three subgroups: pancolitis and left-sided colitis (involvement up to the splenic flexure) with and without appendiceal involvement (skipped erosions in the appendiceal orifice).

Disease activity and patients

A total of 86 patients with UC were divided into six groups according to the activity and extent of the disease: active pancolitis (A-Pan; n = 15), active left-sided colitis without appendiceal involvement (A-Lt; n = 25), active left-sided colitis with appendiceal involvement (A-Lt/Ap; n = 10), inactive pancolitis (I-Pan; n = 14), inactive left-sided colitis without appendiceal involvement (I-Lt; n = 22), and inactive left-sided colitis with appendiceal involvement (I-Lt/Ap; n = 0). Because the inflamed mucosa in the appendiceal orifice may be restructured by the normal mucosa after treatment, there were no patients with I-Lt/Ap. The characteristics of each group are summarized in Table 1. There was no significant difference in sex (the χ^2 test or Fisher's exact test) and age (Student's t-test) among groups.

At the time of the study, 27 patients with active UC and 3 patients with inactive UC had received no medications. Twenty-five patients were treated with salazosulfapyridine (SASP) (1 000-6 000 mg/d) only, 12 were treated with mesalazine (5-ASA) (1 000-2 250 mg/d) only, 2 were treated with prednisolone (PDN) (10 mg/d) only, 12 were treated with PDN (5-20 mg/d) plus SASP (1 500-4 500 mg/d), and 5 were treated with PDN (5-20 mg/d) plus 5-ASA (1 000-2 250 mg/d).

Cell isolation

We obtained colonic mucosal samples from UC patients and control subjects. At diagnostic or follow-up total colonoscopy when the activity and extent of the disease were evaluated, biopsy specimens from the appendix near the appendiceal orifice, transverse colon, and rectum (four specimens in each area) were obtained. We isolated mucosal mononuclear cells from biopsy specimens, as previously described[22]. The initial viability of the cellular suspensions exceeded 95% in all instances when estimated by the trypan blue dye exclusion test. The viability was maintained at >80% during the entire assay period.

Flow cytometric analysis

Mucosal mononuclear cells were analyzed by two-color or three-color flow cytometry with the following mAbs: anti-CD4-PE/anti-CD8-FITC (Coulter Immunology, Tokyo, Japan), anti-CD45 (leukocyte common antigen)-PE-Cy5 (Immunotech, Cedex, France), anti-CD4-PE (Nichirei, Tokyo, Japan), anti-CD69 (early activation antigen)-FITC (Becton-Dickinson, Tokyo, Japan), and anti-HLA-DR (mature activation antigen)-FITC (Immunotech). The isolated cells were incubated with antibodies at 4 ℃ for 30 min, and washed thrice in FACS buffer (PBS, sodium azide 0.01%, and bovine serum albumin 0.1%, Sigma, St. Louis, USA), and applied for flow cytometry (EPICS XL system II, Coulter Company).

We first analyzed the phenotyped cells by two-color flow cytometry. After the cell suspensions were initially visualized in the forward scatter/side scatter profile, lymphocyte populations were gated to exclude monocytes. The proportion of CD4+ and CD8+ T cells in the total lymphocyte populations was expressed as CD4/CD8 ratio.

By three-color flow cytometry, we also analyzed the activated CD4+ T cells. In the process of T cell activation, CD69, CD25, CD71, and HLA-DR antigens are serially expressed on the surface of T cells. To identify this process, we investigated the expressions of CD69 (early activation antigen) and HLA-DR (mature activation antigen). After the cell suspensions were visualized, lymphocyte populations were gated as defined by CD45 (leukocyte common antigen) expression. The proportion of CD4+CD69+ and CD4+HLA-

Table 1 Characteristics of patients with ulcerative colitis and normal subjects

	A-Pan	A-Lt	A-Lt/Ap	I-Pan	I-Lt	Normal subjects
Number of patients	15	25	10	14	22	27
Sex (M/F)	7/8	13/12	6/4	9/5	11/11	15/12
Age (yr)						
Mean	36.4	40.3	35.5	41.4	50.7	43.6
Range	11-69	22-76	18-64	17-78	23-73	16-65
Medications						
None	7	11	9	1	2	27
SASP	2	7	0	5	11	0
5-ASA	2	5	1	1	3	0
PDN	1	0	0	0	1	0
SASP+PDN	3	2	0	3	4	0
5-ASA+PDN	0	0	0	4	1	0

A-Pan, active pancolitis; A-Lt, active left-sided colitis; A-Lt/Ap, active left-sided colitis with appendiceal involvement; I-Pan, inactive pancolitis; I-Lt, inactive left-sided colitis; PDN, prednisolone; SASP, salazosulfapyridine; 5-ASA, mesalazine.

Table 2 CD4/CD8 ratio in the colon (mean±SD)

	A-Pan	A-Lt	A-Lt/Ap	I-Pan	I-Lt	Controls
Rectum	2.3±2.0[a]	3.6±1.6[b]	4.9±2.2[b]	1.8±1.0[a]	2.4±2.0[b]	1.0±0.6
Transverse	1.5±0.7	1.1±0.4	1.8±0.4[a]	0.9±0.5	1.3±0.9	1.2±0.8
Appendix	3.2±1.3	3.5±1.3[a]	3.9±0.9[a]	2.9±1.9	2.6±1.4	2.6±1.1

[a]$P<0.05$, [b]$P<0.01$ *vs* controls. A-Pan, active pancolitis; A-Lt, active left-sided colitis; A-Lt/Ap, active left-sided colitis with appendiceal involvement; I-Pan, inactive pancolitis; I-Lt, inactive left-sided colitis.

DR+ T cells in the total lymphocyte populations was calculated.

Prior to the present study, we performed a pilot study with immuno-flow cytometric analysis in UC patients (A-Pan: $n = 7$ and A-Lt/Ap: $n = 3$), ascending colon cancer patients ($n = 5$), and ascending colon diverticulitis patients ($n = 5$). The population of T cells, expressing CD4, CD8, CD69, and HLA-DR antigens, in pre-operative biopsy samples of the appendix near the appendiceal orifice and in mucosal samples of resected appendix, was similar in each patient. The results of the pilot study suggested that the analysis of biopsy samples of the appendix near the appendiceal orifice represented those of the appendix.

Statistical analysis

All values were expressed as mean±SD. The Student's *t*-test for comparisons among groups was used for statistical analysis. $P<0.05$ (two-tailed) was considered statistically significant.

RESULTS

CD4/CD8 ratio in appendix

In the appendix, the CD4/CD8 ratio both in A-Lt ($P = 0.0166$) and A-Lt/Ap ($P = 0.0445$) significantly increased compared with that in controls (Table 2). Moreover, the CD4/CD8 ratio significantly increased both in A-Lt ($P = 0.0452$) and A-Lt/Ap ($P = 0.0445$) compared with that in I-Lt. The ratio in A-Pan tended to increase compared with that in controls ($P = 0.1654$) and in I-Pan ($P = 0.6890$). The ratio tended to increase in A-Lt/Ap compared with that in A-Lt ($P = 0.7370$). Interestingly, as the CD4/CD8 ratio in the appendix increased, the ratio in the rectum tended to increase, suggesting that some relations might be present in the immune responses between the appendix and the rectum.

CD4/CD8 ratio in transverse colon

In the normal appearance transverse colon of A-Lt/Ap, the CD4/CD8 ratio significantly increased compared with that in controls ($P = 0.0374$, Table 2). The ratio tended to increase in A-Lt/Ap compared with that in I-Lt ($P = 0.3051$). The ratio significantly increased in A-Lt/Ap compared with that in A-Lt ($P = 0.0064$), and in A-Pan compared with that in I-Pan ($P = 0.0442$).

CD4/CD8 ratio in rectum

In the rectum, the CD4/CD8 ratio significantly increased in all UC groups (A-Pan; $P = 0.0102$, A-Lt; $P<0.0001$, and A-Lt/Ap; $P<0.0001$), even in I-Pan ($P = 0.0109$) and I-Lt ($P = 0.0046$), compared with that in controls (Table 2). The ratio also significantly increased both in A-Lt ($P = 0.0343$) and A-Lt/Ap ($P = 0.0236$) compared with that in I-Lt. The ratio tended to increase in A-Lt/Ap compared with that in A-Lt ($P = 0.1436$), and in A-Pan compared with that in I-Pan ($P = 0.4682$). These findings suggested that the CD4/CD8 ratio might represent the inflammation degree in the mucosa.

Early activated T cells

In the appendix, the proportion of CD4+CD69+ (early activation antigen) T cells significantly increased in all UC groups (A-Pan; $P = 0.0013$, A-Lt; $P = 0.0042$, and A-Lt/Ap; $P = 0.0245$), even in I-Pan ($P<0.0001$) and I-Lt ($P = 0.0357$), compared with that in controls (Table 3), but there were no significant differences among UC groups. In the transverse colon, the proportion did not significantly increase in any UC groups compared with that in controls. In the rectum, the proportion significantly increased only in A-Pan ($P = 0.0497$), but not in the other groups, compared with that in controls.

Table 3 Activated T cells in the colon (mean±SD, %)

	A-Pan	A-Lt	A-Lt/Ap	I-Pan	I-Lt	Controls
CD4+CD69+ T cells						
Rectum	27.8±4.6[a]	23.2±5.0	24.0±7.6	24.5±6.2	28.2±8.6	23.1±4.1
Transverse	25.0±5.7	20.4±7.8	23.4±4.3	21.5±7.3	22.1±6.2	21.0±9.1
Appendix	26.6±3.0[b]	28.0±7.0[b]	26.0±4.0[a]	28.9±3.9[b]	25.4±7.3[a]	19.6±4.1
CD4+HLA-DR+ T cells						
Rectum	15.2±6.2[a]	10.1±3.8	7.3±2.4	11.6±4.1	11.1±6.0	8.6±4.7
Transverse	13.2±5.0	7.9±4.0	9.1±4.9	14.9±6.7	9.5±3.7	9.5±5.3
Appendix	8.9±2.4	9.9±4.1	9.9±2.4	12.6±2.8	10.9±4.3	8.8±3.6

[a]$P<0.05$, [b]$P<0.01$ *vs* controls. A-Pan, active pancolitis; A-Lt, active left-sided colitis; A-Lt/Ap, active left-sided colitis with appendiceal involvement; I-Pan, inactive pancolitis; I-Lt, inactive left-sided colitis.

Late activated T cells

The proportion of CD4+HLA-DR+ (mature activation antigen) T cells in the rectum significantly increased only in A-Pan compared with that in controls ($P = 0.0299$), while the proportion of CD4+HLA-DR+ T cells in the appendix did not significantly increase in any UC groups, compared with that in controls (Table 3). The proportion in the transverse colon also did not significantly increase in any UC groups compared with that in controls.

Effects of drugs on CD4/CD8 ratio and activated T cells

To identify the effects of drug treatment on the profiles of T cells, we analyzed the CD4/CD8 ratio and proportions of CD4+CD69+ and CD4+HLA-DR+ T cells in active UC patients with medication (A-Pan; $n = 8$, and A-Lt; $n = 14$) and those without medication (A-Pan; $n = 7$, and A-Lt; $n = 11$). Although the CD4/CD8 ratio in the transverse colon of A-Pan significantly increased in the patients without medication compared with those with medication ($P = 0.0306$), the other ratios and proportions were not significant between the patients with medication and those without medication. Our results were therefore not so influenced with medical therapy.

DISCUSSION

Although the pathogenesis of UC has not been determined, an abnormal mucosal immune response plays a major role in the development and pathophysiology of UC[23,24]. There are few studies investigating the immune-regulatory cells in the appendix of patients with UC, especially with left-sided UC. Most studies have used cells isolated from colectomy specimens involving the disease to detect local immune abnormality[23,24]. Because colectomy is usually performed in severe and refractory cases, mostly in pancolitis cases, but not in other active or inactive cases after a period of medical therapy, these studies may not cover the whole spectrum of disease activity[25]. We therefore used cells isolated from colonoscopic biopsy specimens of UC patients with a wide range of disease activity, but the appendiceal mucosa is usually hard to obtain by biopsy. In this study, biopsy specimens of the appendix near the appendiceal orifice were used, instead of specimens of the appendix itself, because our prior pilot study showed that the analysis of biopsy samples of the appendix near the appendiceal orifice represented those of the appendix.

Although CD4+ and CD8+ T cells contain counterpart functions, such immuno-activation as helper/inducer T cells and immuno-suppression as regulatory T cells, the CD4/CD8 ratio is one of the most reflective markers for immune activation[14,26]. In the present study, the CD4/CD8 ratio in the appendix significantly increased both in A-Lt and A-Lt/Ap compared with that in controls (Table 2). The ratio in the appendix also tended to increase in A-Pan compared with that in controls. Interestingly, as the CD4/CD8 ratio in the appendix increased, the ratio in the rectum tended to increase, suggesting that some relationships might be present in the immune responses between the appendix and the rectum.

In the normal appearance transverse colon of A-Lt/Ap, the CD4/CD8 ratio significantly increased compared with that in controls (Table 2). In the entire colon, the CD4/CD8 ratio tended to increase in A-Lt/Ap compared with that in A-Lt, but it was significant only in the transverse colon. Matsumoto *et al*.[27], also reported that the histological inflammation grade in the entire colon was higher in A-Lt/Ap than that in A-Lt. The grade was significant both in the inflamed appendiceal orifice ($P<0.001$) and in the uninflamed ascending colon ($P<0.05$). The CD4/CD8 ratio therefore may represent the inflammation degree in the mucosa.

Even in the inactive UC groups, the CD4/CD8 ratio significantly increased in the rectum compared with that in controls. Most patients with inactive UC have low-grade inflammation, and it is possible that symptomatic relapse occurs only when the inflammatory process reaches a critical intensity[28]. Also, because inflammation is a continuous process, direct assessment of the level of inflammatory activity may provide a quantitative pre-symptomatic measure of imminent clinical relapse of the disease[28]. In our study, the increased CD4/CD8 ratio suggested that the significant immuno-imbalance was persistent in the inactive rectum. Because patients with inactive UC even receiving maintenance therapy are easy to relapse[29], we suspect that the disease can relapse when the immuno-imbalance is persistent in the rectum.

Recent investigations including TCR-α deficient mice colitis models suggest that non-pathogenic enteric bacterial flora may be involved in the induction of colitis[12,30]. However, it is unclear which part of the colon is involved in priming luminal antigens as the inductive site. To identify the priming site, we compared early- and late-activated CD4+ T cells with CD69 as an early activation antigen and HLA-DR as a late activation antigen, respectively. In the

appendix, the proportion of CD4+CD69+ T cells significantly increased in all UC groups, even in the inactive UC groups, compared with that in controls (Table 3). In the transverse colon, the proportion did not significantly increase in any UC groups compared with that in controls. In the rectum, the proportion significantly increased only in A-Pan, but not in the other groups, compared with that in controls. The proportion of CD4+HLA-DR+ T cells significantly increased only in the rectum of A-Pan, but not in the other areas of any groups compared with that in controls (Table 3). These findings suggest that the appendix may be a priming site in the development of UC. In A-Pan, the CD4/CD8 ratio tended to increase in all areas compared with that in controls, but it was significant only in the rectum (Table 2), where the proportions of CD4+CD69+ and CD4+HLA-DR+ T cells significantly increased compared with those in controls (Table 3). The findings suggest that the appendix may not play a major role in extended colitis.

In TCR-α-deficient mice, the pathological T cells are initially concentrated in the appendix[31]. Mucosal TCR-αβ+ T cells, including CD4+ T cells, in IL-2-deficient mice appear in the colon prior to the manifestation of colitis[16]. An increase of identical T cell clones involved in the development of inflammation is detectable in the uninflamed appendix and the inflamed colon of UC patients as well as in TCR-α-deficient mice[32,33]. Therefore, the increased CD4+CD69+ T cells indicate that CD4+ T cells may be initially activated in the appendix, and may re-circulate to the entire colon and rectum (increased CD4/CD8) prior to the manifestation of UC, and inflammation originating from the rectum extends to the entire colon. The reason why the inflammation begins in the rectum is unknown.

We first reported the improvement of UC (A-Lt/Ap) without medication during the 3 years after appendectomy in a young patient (21-year-old), and proposed that appendectomy may have a place as a therapeutic strategy in UC patients[13]. Ja"rnerot *et al*.[34], also performed laparoscopic appendectomy in six patients with refractory UC (two A-Pan and four A-Lt), and found that one young patient (26-years old) was in remission with continued maintenance treatment, but five patients (mean age: 50.8 years, range: 44-56 years) had relapse of the disease. Histological analysis of the resected appendix showed mucosal erosions and moderate infiltrations of CD4+ T cells in our patient[13], but did not show any inflammation in all patients as reported by Ja"rnerot *et al*.[34]. They concluded that appendectomy does not influence the course of established UC in a consistent way, which supports our results in this study. Eri *et al*.[35], also reported the clinical course of six patients (mean age: 30.5 years) with refractory UC (five A-Lt and one A-Lt/Ap) after laparoscopic appendectomy, and found that five patients were in complete clinical remission, and one patient had improved. Histological analysis of the resected appendix showed colitis-type inflammation (ulcerative appendicitis), containing a highly activated lymphocyte population, in the five patients. Recently, Jo *et al*.[19], reported the clinical course of nine patients (mean age; 32.5 years, range; 13-48 years) with mildly activated UC (four A-Pan and five A-Lt) after appendectomy, and found that two A-Lt patients with ulcerative appendicitis had improved, but the disease remained active in the other patients (three A-Lt without ulcerative appendicitis and four A-Pan). Hallas *et al*.[36], reported the nationwide study with complete follow-up of 202 patients (mean age; 43.3 years) with UC who underwent appendectomy after their onset of UC, and concluded that appendectomy has no beneficial effect on admission rates in UC patients. Although appendectomy is associated with a low risk for subsequent UC only in young patients[13,19,34,35], especially before the age of 20 years[7], no stratification of data for any age had been performed[36]. Later, Hallas *et al*.[37], supported that appendectomy would be useful against UC in young subjects by analyzing those who underwent appendectomy before the age of 30 years. These findings and our results indicate that appendectomy may be performed in young UC patients with ulcerative appendicitis.

In conclusion, our study suggests that the CD4/CD8 ratio represents the inflammation degree in the mucosa. Appendectomy may be a benefit therapy in young UC patients with ulcerative appendicitis. Apart from the rectum, the appendix is a priming site in the development of UC, and should no longer be considered as an evolutionary redundancy. Further studies including analysis of CD4+ and CD8+ T cells are necessary to clarify the role of the appendix in the pathogenesis of UC.

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REFERENCES

1 **Rachmilewitz D,** Karmeli F, Takabayashi K, Hayashi T, Leider-Trejo L, Lee J, Leoni LM, Raz E. Immunostimulatory DNA ameliorates experimental and spontaneous murine colitis. *Gastroenterology* 2002; **122**: 1428-1441
2 **Walker MR,** Kasprowicz DJ, Gersuk VH, Be'nard A, Van Landeghen M, Buckner JH, Ziegler SF. Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+CD25- T cells. *J Clin Invest* 2003; **112**: 1437-1443
3 **Hu D,** Ikizawa K, Lu L, Sanchirico ME, Shinohara ML, Cantor H. Analysis of regulatory CD8 T cells in Qa-1-deficient mice. *Nat Immunol* 2004; **5**: 516-523
4 **Panaccione R,** Sandborn WJ. The appendix in ulcerative colitis: a not so innocent bystander. *Gastroenterology* 1999; **117**: 272-273
5 **Rutgeerts P,** D'haens G, Hiele M, Geboes K, Vantrappen G. Appendectomy protects against ulcerative colitis. *Gastroenterology* 1994; **106**: 1251-1253
6 **Russel MG,** Dorant E, Brummer RJ, Van De Kruus MA, Muris JW, Bergers JM, Goedhard J, Stockbru"gger RW. Appendectomy and the risk of developing ulcerative colitis or Crohn's disease: results of a large case-control study. *Gastroenterology* 1997; **113**: 377-382
7 **Andersson RE,** Olaison G, Tysk C, Ekbom A. Appendectomy and protection against ulcerative colitis. *N Engl J Med* 2001; **344**: 808-814
8 **Radford-Smith GL,** Edwards JE, Purdie DM, Pandeya N, Watson M, Martin NG, Green A, Newman B, Florin TH. Protective role of appendicectomy on onset and severity of ulcerative colitis and Crohn's disease. *Gut* 2002; **51**: 808-813
9 **Selby WS,** Griffin S, Abraham N, Solomon MJ. Appendectomy protects against the development of ulcerative colitis but

not affect its course. *Am J Gastroenterol* 2002; **97**: 2834-2838
10 **Cosnes J,** Carbonnel F, Beaugerie L, Blain A, Reijasse D, Gendre JP. Effects of appendicectomy on the course of ulcerative colitis. *Gut* 2002; **51**: 803-807
11 **Naganuma M,** Iizuka B, Torii A, Ogihara T, Kawamura Y, Ichinose M, Kojima Y, Hibi T. Appendectomy protects against the development of ulcerative colitis and reduces its recurrence: results of a multicenter case-controlled study in Japan. *Am J Gastroenterol* 2001; **96**: 1123-1126
12 **Mizoguchi A,** Mizoguchi E, Chiba C, Bhan AK. Role of appendix in the development of inflammatory bowel disease in TCR-α mutant mice. *J Exp Med* 1996; **184**: 707-715
13 **Okazaki K,** Onodera H, Watanabe N, Nakase H, Uose S, Matsushita M, Kawanami C, Imamura M, Chiba T. A patient with improvement of ulcerative colitis after appendectomy. *Gastroenterology* 2000; **119**: 502-506
14 **Mu"ller S,** Lory J, Corazza N, Griffiths GM, Z'graggen K, Mazzucchelli L, Kappeler A, Mueller C. Activated CD4+ and CD8+ cytotoxic cells are present in increased numbers in the intestinal mucosa from patients with active inflammatory bowel disease. *Am J Pathol* 1998; **152**: 261-268
15 **Ueyama H,** Kiyohara T, Sawada N, Isozaki K, Kitamura S, Kondo S, Miyagawa J, Kanayama S, Shinomura Y, Ishikawa H, Ohtani T, Nezu R, Nagata S, Matsuzawa Y. High Fas ligand expression on lymphocytes in lesions of ulcerative colitis. *Gut* 1998; **43**: 48-55
16 **Simpson SJ,** Mizoguchi E, Allen D, Bhan AK, Terhorst C. Evidence that CD4+, but not CD8+ T cells are responsible for murine interleukin-2-dificient colitis. *Eur J Immunol* 1995; **25**: 2618-2625
17 **Fiocchi C.** Inflammatory bowel disease: etiology and pathogenesis. *Gastroenterology* 1998; **115**: 182-205
18 **Bhan AK,** Mizoguchi E, Smith RN, Mizoguchi A. Colitis in transgenic and knockout animals as models of human inflammatory bowel disease. *Immunol Rev* 1999; **169**: 195-207
19 **Jo Y,** Matsumoto T, Yada S, Nakamura S, Yao T, Hotokezaka M, Mibu R, Iida M. Histological and immunological features of appendix in patients with ulcerative colitis. *Dig Dis Sci* 2003; **48**: 99-108
20 **Schachter H,** Kirsner JB. Definitions of inflammatory bowel disease of unknown etiology. *Gastroenterology* 1975; **68**: 591-600
21 **Riley SA,** Mani V, Goodman MJ, Herd ME, Dutt S, Turnberg LA. Comparison of delayed-release 5-aminosalicylic acid (mesalazine) and sulfasalazine as maintenance treatment for patients with ulcerative colitis. *Gastroenterology* 1988; **94**: 1383-1389
22 **Meenan J,** Spaans J, Grool TA, Pals ST, Tytgat GN, van Deventer SJ. Altered expression of α4β7, a gut homing integrin, by circulating and mucosal T cells in colonic mucosal inflammation. *Gut* 1997; **40**: 241-246
23 **Scott IS,** Sheaff M, Coumbe A, Feakins RM, Rampton DS. Appendiceal inflammation in ulcerative colitis. *Histopathology* 1998; **33**: 168-173
24 **Kroft SH,** Stryker SJ, Rao MS. Appendiceal involvement as a skip lesion in ulcerative colitis. *Mod Pathol* 1994; **7**: 912-914
25 **Haruta J,** Kusugami K, Kuroiwa A, Ina K, Shinoda M, Morise K, Iokawa H, Morita M, Ishihara A, Sarai S. Phenotypic and functional analysis of lamina propria mononuclear cells from colonoscopic biopsy specimens in patients with ulcerative colitis. *Am J Gastroenterol* 1992; **87**: 448-454
26 **Sasakawa T,** Takizawa H, Bannai H, Narisawa R, Asakura H. Activated CD4+ and CD8+ cells in the colonic mucosa of ulcerative colitis patients: their relationship to HLA-DR antigen expression on the colonic epithelium and serum soluble CD25 levels. *Digestion* 1995; **56**: 516-522
27 **Matsumoto T,** Nakamura S, Shimizu M, Iida M. Significance of appendiceal involvement in patients with ulcerative colitis. *Gastrointest Endosc* 2002; **55**: 180-185
28 **Tibble JA,** Sigthorsson G, Bridger S, Fagerhol MK, Bjarnason I. Surrogate marker of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease. *Gastroenterology* 2000; **119**: 15-22
29 **Bitton A,** Peppercorn MA, Antonioli DA, Niles JL, Shah S, Bousvaros A, Ransil B, Wild G, Cohen A, Edwardes MD, Stevens AC. Clinical, biological, and histologic parameters as predictors of relapse in ulcerative colitis. *Gastroenterology* 2001; **120**: 13-20
30 **Taurog JD,** Richardson JA, Croft JT, Simmons WA, Zhou M, Ferna'ndez-Sueiro JL, Balish E, Hammer RE. The germfree state prevents development of gut and joint inflammatory disease in *HLA-B27* transgenic rats. *J Exp Med* 1994; **180**: 2359-2364
31 **Takahashi I,** Kiyono H, Hamada S. CD4+ T-cell population mediates development of inflammatory bowel disease in T-cell receptor α chain-deficient mice. *Gastroenterology* 1997; **112**: 1876-1886
32 **Mizoguchi A,** Mizoguchi E, Saubermann LJ, Higaki K, Blumberg RS, Bhan AK. Limited CD4 T-cell diversity associated with colitis in T-cell receptor α mutant mice requires a T helper 2 environment. *Gastroenterology* 2000; **119**: 983-995
33 **Probert CS,** Chott A, Turner JR, Saubermann LJ, Stevens AC, Bodinaku K, Elson CO, Balk SP, Blumberg RS. Persistent clonal expansions of peripheral blood CD4+ lymphocytes in chronic inflammatory bowel disease. *J Immunol* 1996; **157**: 3183-3191
34 **Ja"rnerot G,** Andersson M, Franze'n L. Laparoscopic appendectomy in patients with refractory ulcerative colitis. *Gastroenterology* 2001; **120**: 1562-1563
35 **Eri RD,** Cross S, Misko I, Rickard M, Lumley J, Stitz R, Stevenson A, Lewindon P, Radford-Smith GL. Appendectomy for refractory ulcerative colitis: targeting the right patient. *Gastroenterology* 2002; **122**: A61
36 **Hallas J,** Gaist D, Vach W, Sorensen HT. Appendicectomy has no beneficial effect on admission rates in patients with ulcerative colitis. *Gut* 2004; **53**: 351-354
37 **Hallas J,** Gaist D, Vach W, Sorensen HT. The role of age in the protection of appendicectomy against ulcerative colitis: author's reply. *Gut* 2004; **53**: 1719-1720

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