

mined by PCR. *Clostridium difficile* was detected from samples 1, 4, and 5 but not from samples 2 or 3. All strains were TcdA positive and TcdB positive but binary toxin negative. PCR ribotyping was carried out for the three isolates, and all were classified into ribotype smz. The isolates were also used for *slpA* sequencing.<sup>3</sup> Nucleotide sequences of the *slpA* of the isolates recovered from samples 1, 4, and 5 were compared to representative sequences of each *C. difficile* type, and all of the isolates identified in this study were classified into type smz-1.

*Clostridium difficile* is a causative agent of antibiotics-associated diarrhea. It is most often associated with nosocomial acquisition and is a leading cause of infectious diarrhea among patients in hospitals. So far, low rates of severe disease and death from CDAD as well as the incorrect perception that CDAD is a "self-limited" antibiotics-induced disease on the part of some clinicians in Japan, may have led to an underestimation of the importance of CDAD as a nosocomial infection. CDAD may sometimes resolve without specific treatment after treatment with an antimicrobial agent is ended, but sometimes the symptoms are worsened by the thoughtless use of antimotility agents. Probably, the diarrhea induced by *C. difficile* toxins aids in resolution of the disease by reducing fecal stasis.

In this study, the *C. difficile* ribotype smz was identified in three of the five samples. The PCR ribotype smz is further subdivided into at least three subtypes, named smz-1, -2, and -3, according to *slpA* sequence differences.<sup>4</sup> A previous study reported that ribotype smz strains have caused outbreaks in multiple hospitals in Japan.<sup>5</sup> Type smz also has the unique characteristic that it is not typeable by pulse field gel electrophoresis because of DNA degradation. Among the more than 100 *C. difficile* ribotypes, this degradation character is shared by ribotype gr, serogroup G according to immunoblot analysis, which was reported as the epidemic strain in hospitals in the United States.

Whether patients with *C. difficile* in their stool develop clinically mild disease or severe colitis may relate not only to differences in the colonic environment of the patients but also to differences in the virulence and toxigenicity of various strains of *C. difficile*. Typing of *C. difficile* is indispensable before the transmission route of *C. difficile* can be analyzed in cases of hospital outbreaks of CDAD, and it is important to understand the relationship between strain or type virulence and the severity of the disease. Accumulation of data on *C. difficile* types is required to reveal the relationship between *C. difficile* types and disease severity, and *slpA* sequence typing as well as the LAMP assay are useful tools for those investigations.

Hideaki Kato<sup>1,2</sup>, Haru Kato<sup>3</sup>, Makoto Nakamura<sup>2</sup>, and Atsushi Nakamura<sup>1</sup>

<sup>1</sup>Department of Internal Medicine and Molecular Science, Nagoya City University Graduate School of Medical Sciences, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan

<sup>2</sup>Department of Gastroenterology, Toyokawa City Hospital, Toyokawa, Japan

<sup>3</sup>Department of Bacterial Pathogenesis and Infection Control, National Institute of Infectious Diseases, Tokyo, Japan

## References

- Stubbs SL, Brazier JS, O'Neill GL, Duerden BI. PCR targeted to the 16S-23S rRNA gene intergenic spacer region of *Clostridium difficile* and construction of a library consisting of 116 different PCR ribotypes. *J Clin Microbiol* 1999;37:461-3.
- Warny M, Pepin J, Fang A, Killgore G, Thompson A, Brazier J, et al. Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. *Lancet* 2005;366:1079-84.
- Kato H, Yokoyama T, Arakawa Y. Typing by sequencing the *slpA* gene of *Clostridium difficile* strains causing multiple outbreaks in Japan. *J Med Microbiol* 2005;54:167-71.
- Kato H, Yokoyama T, Kato H, Arakawa Y. Rapid and simple method for detecting the toxin B gene of *Clostridium difficile* in stool specimens by loop-mediated isothermal amplification. *J Clin Microbiol* 2005;43:6108-12.
- Kato H, Kato N, Watanabe K, Yamamoto T, Suzuki K, Ishigo S, et al. Analysis of *Clostridium difficile* isolates from nosocomial outbreaks at three hospitals in diverse area of Japan. *J Clin Microbiol* 2001;39:1391-1395.

Received: December 26, 2006 / Accepted: February 16, 2007

Reprint requests to: H. Kato

DOI 10.1007/s00535-007-2037-9

## Early postoperative application of extracorporeal leukocyte apheresis in ulcerative colitis patients: results of a pilot trial to prevent postoperative septic complications

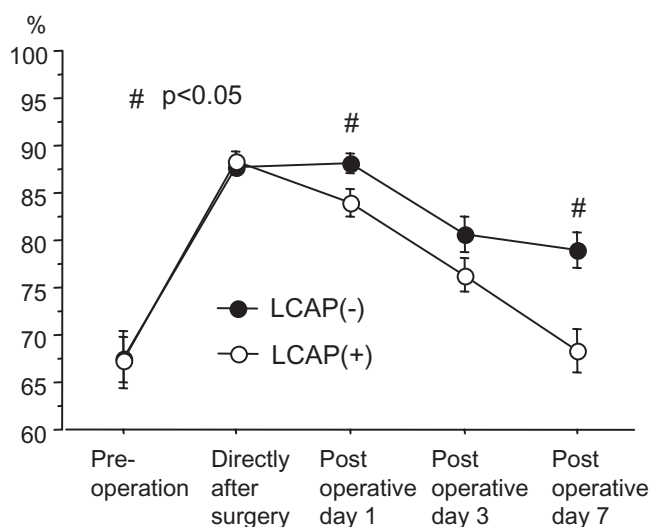
*To the Editor:* Restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) is the current procedure of choice for ulcerative colitis (UC) and familial adenomatous polyposis (FAP). However, the outcome of this procedure is often compromised by various surgical site infections (SSIs), which are known to be a major cause of pouch failure. Interestingly, there is evidence suggesting that UC patients have a higher morbidity rate, up to 60%, and a higher risk of pouch failure than do FAP patients.<sup>1</sup> We analyzed UC patients undergoing IPAA and found that the total amount of steroids administered is a surgery-independent risk factor for septic complications, whereas perioperative neutrophil activation is a surgery-dependent risk factor for septic complications.<sup>2</sup>

In recent years, leukocyte apheresis (LCAP) using a leukocyte removal filter has been applied to the treatment of various autoimmune diseases, including UC. Although its therapeutic mechanism is not yet fully understood, LCAP removes activated leukocytes from the peripheral blood circulation, which is the major source of inflammatory mediators.<sup>3</sup> Since LCAP maintains a high clearance rate of neutrophils, a pilot trial of LCAP was conducted for surgical UC patients in the early postoperative period in an attempt to control the exaggerated postoperative neutrophil activation. LCAP was carried out directly after surgery using a specially designed leukocyte removal column, Cellsorba EX (Asahi Kasei Medical, Tokyo, Japan). In this case-control study, a total of 29 patients undergoing IPAA were enrolled and their clinical outcomes were compared with those of 43 patients not receiving postoperative LCAP. All patients underwent the same protocol for preparation for surgery and received the perioperative cover of prophylactic antibiotics and steroids. All patients were included in the study after informed consent was obtained. The investigations were performed in accordance with the Helsinki Declaration and approved by the Institutional Review Board. LCAP had good feasibility and no adverse events

**Table 1.** Clinical backgrounds of the patients undergoing or not undergoing LCAP

| Factors  | LCAP        |             | P value |
|--|-------------|-------------|---------|
|  | +           | -           |         |
|  | (n = 29)    | (n = 43)    |         |
| Sex (F:M)  | 8:2         | 19:2        | 0.6050  |
| Truelove and Witts' categories<br>(mild:moderate:severe) | 8:17:4      | 18:19:6     | 0.4276  |
| Matts grade classification (1:2:3:4)                     | 4:5:6:2     | 5:12:12:1   | 0.5084  |
| Age at operation (years)                                 | 31.1 ± 2.0  | 34.0 ± 2.4  | 0.5203  |
| Age at disease onset                                     | 25.2 ± 1.8  | 27.0 ± 2.2  | 0.7566  |
| Disease duration (years)                                 | 6.9 ± 0.93  | 6.9 ± 0.95  | 0.5315  |
| Albumin (g/dl)   | 3.5 ± 0.1   | 3.4 ± 0.1   | 0.3614  |
| Hemoglobin (g/l)   | 11.7 ± 0.4  | 11.0 ± 0.4  | 0.2005  |
| Choline esterase (ΔpH)                                   | 0.65 ± 0.05 | 0.72 ± 0.05 | 0.4316  |
| Body mass index (kg/m <sup>2</sup> )                     | 19.1 ± 0.6  | 20.1 ± 0.5  | 0.893   |
| Total amount of steroids (g)                             | 14.7 ± 2.1  | 11.6 ± 1.7  | 0.1795  |
| Surgical duration (min)                                  | 273 ± 13    | 258 ± 12    | 0.1701  |
| Operative blood loss (g)                                 | 358 ± 41    | 620 ± 87    | 0.053   |
| Perioperative blood transfusion (Y:N)                    | 5:24        | 12:31       | 0.2960  |
| Covering ileostomy (Y:N)                                 | 27:2        | 34:9        | 0.1045  |
| Surgical site infection (Y:N)                            | 3:26        | 23:20       | <0.01   |

LCAP, leukocyte apheresis



**Fig. 1.** Serial changes in the percentage of peripheral neutrophils in patients undergoing or not undergoing leukocyte apheresis

were observed. SSIs were identified in 23 (53%) patients without LCAP: 11 had incisional SSIs and 12 organ/space SSIs. By contrast, only three patients undergoing LCAP developed SSIs: two had incisional SSIs and one an organ/space SSI (Table 1). To assess the elimination rate of neutrophils, we counted the numbers of circulating lymphocytes and neutrophils and assessed the changes in the percentage of neutrophils in white blood cells as described by Kobayashi and Yamauchi.<sup>4</sup> Interestingly, the postoperative percentage of peripheral neutrophils was significantly suppressed in the LCAP (+) group, suggestive of some control of the systemic inflammatory response in these patients (Fig. 1). It has been hypothesized that surgical UC patients carry a considerable risk for a neutrophil respiratory burst after intense surgical stress, which results in higher rates of morbidity and complications.<sup>5</sup> Although our study was preliminary, the indications are that early postoperative LCAP therapy may control neutrophil hyperactivation, leading to an improved clinical outcome follow-

ing IPAA. Larger controlled studies are needed to confirm the clinical advantages of this novel strategy.

Chikao Miki, Yoshiki Okita, Shigeyuki Yoshiyama, Toshimitsu Araki, Keiichi Uchida, and Masato Kusunoki  
Department of Gastrointestinal and Pediatric Surgery, Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu 514-8507, Japan

## References

- Fazio VW, Ziv Y, Church JM, Oakley JR, Lavery IC, Milsom JW, et al. Ileal pouch-anal anastomoses complications and function in 1005 patients. *Ann Surg* 1995;222:120-7.
- Miki C, Yoshiyama S, Okita Y, Araki T, Uchida K, Yanagi H, et al. Neutrophil priming as a surgery-related risk factor for postoperative infectious complications in patients with ulcerative colitis. *Dig Surg* 2006;23:179-85.
- Sawada K, Egashira A, Ohnishi K, Fukunaga K, Kusaka T, Shimoyama T. Leukocyte apheresis (LCAP) for management of fulminant ulcerative colitis with toxic megacolon. *Dig Dis Sci* 2005;50:767-73.
- Kobayashi E, Yamauchi H. Interleukin-6 and a delay of neutrophil apoptosis after major surgery. *Arch Surg* 1997;132:209-10.
- Menger MD, Vollmar B. Surgical trauma: hyperinflammation versus immunosuppression? *Langenbecks Arch Surg* 2004;389:475-84.

Received: January 12, 2007 / Accepted: February 22, 2007

Reprint requests to: C. Miki  
DOI 10.1007/s00535-007-2035-y

## IBS and depression in young adults in Turkey: results of a questionnaire survey

*To the Editor:* We read the article by Shiotani et al.<sup>1</sup> with great enthusiasm. These authors stated that epidemiological studies of irritable bowel syndrome (IBS) among young adults are few, especially in Asian countries. We have just finished the first part of a similar study in Abant Izzet Baysal University in Turkey. We