ingestion of bLF increases the number of neutrophil precursor cells in human patients (24, 27). In addition, because LF is an important component of the human immune system (23, 28–31), we also measured the levels of human lactoferrin (hLF) in the serum of trial participants.

At the end of the trial period, the target lesions were removed and examined. The number of neutrophils in these specimens was of particular interest: Several reports indicate that neutrophils can enhance tumor growth (32–35), but in rodents, ingestion of bLF attenuates the movement of neutrophils to the small intestine (36).

Materials and Methods

Trial profile

An outline of the trial profile is shown in Supplementary Fig. S1. The trial was initiated after approval by the Ethical Committee of the National Cancer Center Hospital, Tokyo, Japan, and continued until January 2006. This study is registered in the University Hospital Medical Information Network Clinical Trials Registry (Tokyo, Japan; no. C000000182). The Independent Data Monitoring Committee determined the trial should have approximately 105 participants. Between February 2002 and January 2005, 307 patients scheduled for colonoscopic examination at the National Cancer Center Hospital, Tokyo, Japan, were invited to join the trial; patients were approached before their examinations. Of these, 215 patients provided written informed consent.

The 215 potential trial participants underwent their scheduled colonoscopic examinations, and during the examination, potential target polyps were marked by injection of india ink close to the polyp and photographed. Patients were excluded from the trial who met any of the following criteria: no target lesion present; dairy product allergies; use of nonsteroidal anti-inflammatory drugs (NSAID) or statins for >50 d in the previous 3 mo (use of these drugs could affect polyp size refs. 37, 38); diagnosed as having cancer; a history of colectomy within the previous 3 y; inflammatory bowel disease, familial adenomatous polyposis, or hereditary nonpolyposis colorectal cancer; or active infection such as hepatitis B or C. Patients with a history of cancer were included in the trial only if they were diagnosed as being cured of cancer and unlikely to suffer a relapse for at least 1 y. Ultimately, 108 participants ages 40 to 75 y with ≤5-mm-diameter polyps showing a pit pattern III were enrolled in the trial; the classification of pit patterns was based on Kudo's classification (39).

Participants were randomized (Randomization Center; Japan Clinical Research Support Unit, Tokyo, Japan) and assigned to one of three treatment groups (the placebo group, the 1.5-g bLF group, or the 3.0-g bLF group). Trial participation commenced within 30 d after colonoscopic examination. At commencement, patients underwent their initial trial interviews.

Participants took six tablets orally everyday for 12 mo. One tablet (1.5 g) contained bLF at 0, 250, or 500 mg. In addition, the tablets contained carbohydrate (D-sorbitol, maltitol, and corn starch), but not fat or dietary fiber. The caloric value of six tablets was 36 kcal for all groups. Tablets were designed to be indistinguishable from each other in appearance, smell, and taste. Good compliance was defined as having taken two thirds or more of the tablets prescribed, and poor compliance as having taken less than two thirds of the tablets prescribed. Intake of any product containing bLF was prohibited throughout the entire study period. Participants were verbally instructed to continue with their usual food (especially fat and fiber), alcohol, and supplement intake at trial commencement and 3, 6, and 9 mo, although they were not requested to record their meals during the trial period. Treatment assignments and participant assessments were not revealed to investigators, participants, or the sponsor over the study period.

Colonoscopy

Endoscopists performed initial and final total colonoscopic examinations with zoom colonoscopes (CF-240ZI, PCF-240ZI, and CF-200Z, Olympus). During the initial examination, before the commencement of the trial, all lesions detected by colonoscopy were observed at 100-fold magnification after spraying 0.2% indigo carmine dye over the lesion to easily differentiate polyp from normal mucosa and to more clearly observe the pit patterns of the polyps. Of these, ≤5-mm-diameter polyps with pit pattern III at the most proximal sites of the right colon (cecum to transverse colon) and the left colon (descending colon to rectum) were identified as target lesions. The classification of pit patterns was based on Kudo's classification (39). This classification system assigns colonic lesions into six categories: types I and II are designated nonneoplastic; types IIIS, IIIL, IV, and V are designated neoplastic. Type IIIS shows small tubular or roundish pits; type IIIL shows large tubular or roundish pits. Macroscopically, intramucosal polypoid growths (pedunculated polypoid lesions and sessile and broad-based polypoid lesions) and nonpolypoid growths showing infiltration of tumor cells below the submucosal layer were excluded from being used as target lesions because they would most probably show malignant characteristics (40–43). These lesions and >5-mm-diameter polyps with pit pattern III were removed at the time of detection. A total of 119 lesions in the full analysis set (FAS) population (104 participants) with pit pattern III were identified as target polyps.

Target polyps were photographed and marked by injection of india ink close to the polyp. Target polyp size was estimated using both open biopsy forceps (44–46) and a 5-mm paper disc and open scale forceps (see Supplementary data for a description of the use of the 5-mm paper disc to estimate polyp size). At the end of the trial period, target polyps were identified by location and the presence of the india ink marker. The size and pit pattern of the polyps were measured by endoscopy, and the polyps were excised for examination. (All other premalignant and malignant growths were also removed at this time.) Target polyps were immediately fixed in phosphate-buffered neutral formalin, embedded in paraffin, and stained with H&E.

The Endoscopic Data Adjudication Committee (Drs. Akasu and Gotoda) evaluated the size of the polyps and pit pattern by photographs. Excised lesions were histopathologically diagnosed by a board-certified pathologist.

Assessment of safety

To assess safety, medical health checkups and evaluation of clinical findings through diary writing and patient interviews were done at trial commencement and every 3 mo. Safety analyses were done for all participants who took at least one tablet.

Immunologic parameters

Peripheral blood was collected from participants at trial commencement (before treatment), 3 mo, 6 mo, 9 mo, and 12 mo. Parameters to be measured were selected based on the results of previous experimental studies (12, 14, 15, 47). ELISA, lymphocyte subset (CD4, CD8, CD16, and CD56) measurement, and NK cell activity were measured as described below.

ELISA. Peripheral blood was collected from trial participants as noted above. ELISA for hLF was done as follows. Microtiter plates were coated with a mouse anti-hLF antibody (mouse, clone: 2B8, IgG1, Advanced Immuno Chemical) and incubated at 5°C overnight. To block the wells, 250 μL of 0.5% gelatin in PBS were added to the wells and the plate was incubated at 37°C for 1 h. One hundred microliters of sample were applied to the blocked wells and the plate was incubated at 5°C overnight. Captured hLF was detected using horseradish peroxidase–labeled polyclonal antibodies against hLF (rabbit; Cappel) and visualization was done using *o*-phenylenediamine (Sigma). The minimal detectable concentration of hLF was ~200 pg/mL; the minimal detectable concentration of bLF was >20 μg/mL. An ELISA specific for bLF using a specific monoclonal

antibody against bLF, developed by Morinaga Milk Industry, was done similarly. Briefly, microtiter plates were coated with capture antibody (anti-bLF rabbit polyclonal, Morinaga Milk Industry) and blocked, and then 100-μL sample was applied. Captured bLF was detected using a biotin-labeled anti-bLF polyclonal antibody (rabbit; Nacalai, Japan) and visualized with horseradish peroxidase–labeled streptavidin (Zymed) and *o*-phenylenediamine. The minimal detectable concentration of bLF was ~500 pg/mL; the minimal detectable concentration of hLF was >20 μg/mL. An ELISA kit (minimum detection limit, 25.0 pg/mL; Medical & Biological Laboratories Co. Ltd.) was used to measure mature human interleukin-18. Human IFNγ levels were determined by SRL, Inc.

Lymphocyte subsets (CD4, CD8, CD16, and CD56). Peripheral blood was collected from trial participants as noted above. Blood samples were diluted with saline, and lymphocytes were separated from the diluted blood samples using Ficoll-Conray solution (relative density, 1.077; IBL). Fluorescence-activated cell sorting was used to isolate the different lymphocyte subsets. The following antibodies were used for immunofluorescence staining of lymphocytes: two-color antihuman CD4-FITC (T4, Beckman Coulter), CD8-RD1 (T8, Beckman Coulter), CD16-FITC (Becton Dickinson), and CD56-RD1 (Beckman Coulter). Relative fluorescence intensities of single- or two-color staining were then measured with a FACSCalibur (Becton Dickinson).

NK cell activity. Peripheral blood was collected from trial participants and lymphocytes isolated using Ficoll-Conray solution as noted above. The entire lymphocyte population (containing effector cells) was washed twice with PBS. Cell killing activity was then measured using K-562 cells labeled with ^{51}Cr as target cells. Target cells (1×10^6/mL, 10 μL) and effector cells (1×10^6/mL, 200 μL) were mixed and incubated at 37°C, 5% CO_2 for 3.5 h. The medium was then removed and clarified by centrifugation, and soluble ^{51}Cr released by killed K-562 cells was measured with a gamma-counter (1470 Wizard, Perkin-Elmer Life and Analytical Sciences).

Polymorphonuclear leukocyte infiltration into target polyps

At the end of the trial period, a final colonoscopic examination was done and target polyps (and all other premalignant and malignant growths found) were removed. Target polyps were immediately fixed in buffered formalin, embedded in paraffin, and stained with H&E, followed by histopathologic examination. Polymorphonuclear leukocytes (PMN) were counted in the stroma of adenomatous polyps. Only polyp sections containing at least five atypical adenoma glands and mucosa propria to a depth equal to at least the average gland diameter (*d*) were used. PMNs in the stroma of five glands and the underlying mucosa propria to depth *d* were counted. The area was measured using an image analysis system (Image Processor for Analytical Pathology, Sumika Technos Corp.).

Statistical analyses

Fifteen participants had two target polyps; these participants were assessed according to the change in the average of the diameter of the two polyps. Polyp assessment was done based on the Response Evaluation Criteria in Solid Tumors (48). Analysis of covariance and Dunnett's multiple comparison test were used to compare the change in polyp size in the LF-treated groups with that in the placebo group, using the initial polyp size as a covariate. The assumption for analysis of covariance was checked, and there was no interaction between baseline and treatment. To check the interaction between treatment outcome and each prognostic factor (age, sex, presence or absence of previous colectomy, and site of target lesion), multiple regression analysis was done with the following four variables: treatment, sum of target-lesion diameters by colonoscopy at trial commencement, factor, and factor-by-treatment interaction. For grouping by age, trial participants were divided into two groups: participants at or below the overall median age of the trial participants (≤63) and participants above the overall median age of the trial participants (>63). For prognostic factors showing significant interaction with treatment outcome, subgroup analyses were done separately in each subgroup population. The data for NK activity and hLF levels were analyzed using Dunnett's test. Pearson coefficients of correlation were calculated to determine degrees of association between different variables. Levels of significance were set at 0.05 (two-sided) for all statistical analyses. All calculations were conducted using SAS version 8.2 (SAS Institute).

Results

Disposition of subjects

The trial profile is shown in Supplementary Fig. S1 and detailed in Materials and Methods. Briefly, over the course of ~3 years (February 2002 to January 2005), patients scheduled for a colonoscopic examination were approached before their examinations and invited to join the trial. Each of the 215 patients who agreed to join the trail provided written informed consent and underwent their scheduled examination. During this examination, potential target polyps were marked and photographed for further evaluation by the Endoscopic Data Adjudication Committee. Each of these patients also underwent a pre-trial interview to determine their suitability for inclusion in the trial. For each trial participant, trial commencement began within 30 days after their initial colonoscopic examination.

Of the 215 patients who initially agreed to join the trial, 108 patients met the trial criteria and agreed to continue with the trail. These 108 patients were each enrolled and randomized into one of three treatment groups (the placebo group, the 1.5-g bLF group, or the 3.0-g bLF group). After randomization into treatment groups, two participants originally enrolled into the trial were excluded: One participant assigned to the placebo group did not have a target polyp, as judged by the Endoscopic Data Adjudication Committee after further evaluation of the photographs taken during the initial colonoscopic examination, and during the initial trial interview, one participant assigned to the 3.0-g bLF group was found to have used statins. Therefore, the initial trial population consisted of 106 participants. Table 1 shows the characteristics of this population at trial commencement. The overall age (mean ± SD) was 62.4 ± 6.9 years. Eighty-seven subjects (82.1%) were men. A total of 121 polyps with pit pattern III were identified by colonoscopy in this population, and the estimated diameter (mean ± SD) was 3.5 ± 0.9 mm. All those (n = 27) with a history of colectomy had undergone it as a result of colorectal cancer.

Two participants withdrew from the trial after commencement (both in the placebo group). The FAS population for the trial, therefore, consisted of 104 participants. The per-protocol set (PPS) population included 102 participants after exclusion of two from the FAS population who used NSAIDs (both in the 3.0-g bLF group). The FAS population was used to analyze the effects of bLF treatment on target polyp size, and the PPS population was used to analyze the effects of bLF treatment on immunologic parameters; see Supplementary data for a brief explanation of our use of the FAS and PPS population data.

Compliance

In the FAS population (n = 104), tablet intake rates (mean ± SD) were 92.1 ± 9.0% in the placebo group (n = 33), 94.3 ± 5.0% in the 1.5-g bLF group (n = 37), and 92.1 ± 9.3% in the 3.0-g

Table 1. Characteristics of the initial trial population at trial commencement

	Placebo group (n = 35)	1.5-g bLF group (n = 37)	3.0-g bLF group (n = 34)
Age (y), mean ± SD	63.0 ± 6.4	61.4 ± 7.3	63.0 ± 6.8
Male, n (%)	29 (82.9)	29 (78.4)	29 (85.3)
Height (cm), mean ± SD	165.4 ± 7.4	164.5 ± 7.0	164.4 ± 7.8
Weight (kg), mean ± SD	64.2 ± 7.6	65.9 ± 11.9	63.9 ± 9.0
Previous colectomy, n (%)	10 (28.6)	8 (21.6)	9 (26.5)
Alcohol consumption, n (%)	26 (74.3)	28 (75.7)	26 (76.5)
Smoking, n (%)	5 (14.3)	13 (35.1)	8 (23.5)
Target-lesion diameters (mm) by colonoscopy, mean ± SD	4.0 ± 1.4	3.8 ± 1.4	4.1 ± 1.7
Site of target lesions by colonoscopy			
Right colon, n (%)	23 (65.7)	23 (62.2)	20 (58.8)
Left colon, n (%)	7 (20.0)	9 (24.3)	9 (26.5)
Right and left colon, n (%)	5 (14.3)	5 (13.5)	5 (14.7)

bLF group (n = 34). There was poor compliance (as defined in Materials and Methods) from two participants: one participant of two withdrawing consent from the placebo group (tablet intake rate, 54.6%) and one participant of two excluded from the 3.0-g bLF group due to NSAID use (tablet intake rate, 51.7%).

Pit patterns and histologic diagnosis

A total of 119 polyps in the FAS population with pit pattern III were identified by colonoscopy as target polyps. The pit pattern of two (both in the 3.0-g bLF group) changed into pit pattern I (regular round crypts, normal mucosa) at 12 months. All the others showed pit pattern III. Of these lesions, 91 were histologically diagnosed: 31 in the placebo group, 30 in the 1.5-g bLF group, and 30 in the 3.0-g bLF group. (Twenty-eight polyps were used up during RNA extraction; the analysis of the RNA is ongoing and not part of this report.) Eighty-nine (97.8%) were adenomas and two were hyperplasias (both in the 1.5-g bLF group), verifying our identification of polyps with pit pattern III as a predictor of adenoma.

Safety

One participant in each of the three groups had an adverse event for which a causal relationship with tablets could not be determined: A mild decrease in triacylglycerol levels was observed in a participant in the placebo group; a mild increase in alkaline phosphatase levels was observed in a participant in the 1.5-g bLF group; and a moderate increase in total bilirubin levels (with a mild increase observed at trial commencement) was observed in a participant in the 3.0-g bLF group. Levels of alkaline phosphatase and total bilirubin spontaneously returned to normal after the end of the study treatment. In the 3.0-g bLF group, lung metastases from colorectal cancer were observed in one participant and liver metastases from colorectal cancer were observed in a second participant (both participants had a history of colon cancer). Because both were diagnosed with metastases within 1 week before the end of the treatment and were found to have no laboratory abnormalities associated with them, the treatment was continued and completed. No other serious adverse events occurred during this study, and the safety of the treatment was confirmed.

Efficacy of bLF treatment on polyp size

A comparison of the change in polyp size among treatment groups in the FAS population is shown in Table 2. Although the differences between LF-treated groups and the placebo group were not significant (P = 0.098), some reduction in polyp diameter in the 3.0-g bLF group (−0.2 mm, 4.9% regression) was observed, whereas an increment in polyp size was observed in the placebo group (+0.2 mm, 5.0% increase). Similar results were obtained in the analysis of the PPS population (Table 2).

A comparison of the change in polyp size among groups and in subgroups (age or sex) is shown in Table 2. Multiple regression analysis revealed both age-by-treatment (P = 0.034) and sex-by-treatment (P = 0.043) interactions. Significant retardation of target polyp diameter was found in participants ≤63 years of age ingesting 3.0 g of bLF per day (P = 0.006; Table 2) and possibly in female participants ingesting 3.0 g of bLF per day (P = 0.019; Table 2). The number of female participants, however, was small; therefore, whereas the retardation of target polyp diameter was statistically significant, the effect of bLF in women needs to be confirmed in trials with a larger number of female participants.

The number and site of target lesions and the presence or absence of previous colectomy showed nonsignificant interaction with treatment outcome (P = 0.39 and P = 0.57, respectively).

Efficacy of bLF treatment on immunologic parameters

The effect of bLF ingestion on polyp size was equivalent in the FAS and PPS populations (Supplementary Table S1); therefore, the PPS population was used to examine the effect of bLF treatment on immunologic parameters (see Supplementary data for a brief explanation of our use of the FAS and PPS population data).

Ingestion of 3.0-g bLF resulted in significantly elevated serum hLF levels (P < 0.001; Fig. 1A), suggesting that ingestion of 3.0-g bLF affected the immune system. Multiple regression analysis revealed age-by-treatment interaction on serum levels of hLF (P < 0.001). As with the effect of bLF on polyp size, bLF significantly affected serum levels of hLF only in participants ≤63 years of age. In the ≤63 years age subgroup (n = 54),

serum hLF levels changed by −1.63 ± 10.69 ng/mL in the placebo group and by 25.43 ± 19.35 ng/mL in the 3.0-g bLF group ($P < 0.001$); in the ≥64 years age subgroup ($n = 48$), serum hLF levels changed by −3.18 ± 5.69 ng/mL in the placebo group and by 5.25 ± 14.16 ng/mL in the 3.0-g bLF group ($P = 0.079$). Analysis of serum hLF levels in participants ingesting 3.0-g bLF revealed that in this group, induction of hLF weakened with aging ($r = -0.642$, $P < 0.001$; Fig. 1B). Serum levels of (ingested) bLF were below the limit of detection in all groups (data not shown).

Ingestion of 1.5-g bLF increased NK cell activity ($P = 0.048$; Fig. 2); however, the increase in NK cell activity in participants ingesting 3.0-g bLF did not attain statistical significance ($P = 0.058$). There was no age-by-treatment interaction on NK cell activity ($P = 0.911$).

For all other immunologic parameters measured, no differences between LF-treated groups and the placebo group were observed (data not shown).

Changes in polyp size and possible associated factors

As noted above, the effect of bLF ingestion on polyp size was equivalent in the FAS and PPS populations. Therefore, the PPS population was used to examine correlations between changes in target polyp size and NK cell activity, serum hLF levels, and PMN infiltration into target polyps.

Participants with growth-retarded polyps had significantly higher NK cell activity compared with participants with growing polyps ($P = 0.037$; Supplementary Fig. S2A). In addition, increased NK cell activity correlated with increases in the $CD16^+/CD56^+$ subset of NK cells in the blood ($r = 0.371$,

Table 2. Analysis of the growth of polyps by colonoscopy

	Baseline,* mm ± SD	Change,† mm ± SD (%)	95% CI,‡ mm	P§
Variable				
Full analysis set ($n = 104$)‖				
Placebo group ($n = 33$)	4.0 ± 1.4	0.2 ± 0.8 (5.0)		
1.5-g bLF group ($n = 37$)	3.8 ± 1.4	0.1 ± 0.8 (2.6)	−0.70, 0.26	0.490
3.0-g bLF group ($n = 34$)	4.1 ± 1.7	−0.2 ± 1.3 (−4.9)	−0.91, 0.07	0.098
Per-protocol set ($n = 102$)				
Placebo group ($n = 33$)	4.0 ± 1.4	0.2 ± 0.8 (5.0)		
1.5-g bLF group ($n = 37$)	3.8 ± 1.4	0.1 ± 0.8 (2.6)	−0.70, 0.26	0.500
3.0-g bLF group ($n = 32$)	4.1 ± 1.8	−0.2 ± 1.3 (−4.9)	−0.95, 0.05	0.081
Subgroup				
Age ($n = 104$)				
≤63-year-old ($n = 55$)¶				
Placebo group ($n = 18$)	3.9 ± 1.5	0.5 ± 0.8 (12.8)		
1.5-g bLF group ($n = 21$)	4.0 ± 1.6	−0.1 ± 0.5 (−2.5)	−1.08, 0.03	0.066
3.0-g bLF group ($n = 16$)	4.0 ± 1.7	−0.4 ± 1.3 (−10)	−1.41, −0.22	0.006
≥64-year-old ($n = 49$)				
Placebo group ($n = 15$)	4.1 ± 1.3	−0.1 ± 0.8 (−2.4)		
1.5-g bLF group ($n = 16$)	3.5 ± 1.2	0.3 ± 1.0 (8.6)	−0.66, 1.02	0.840
3.0-g bLF group ($n = 18$)	4.1 ± 1.8	−0.1 ± 1.3 (−2,4)	−0.82, 0.80	>0.950
Sex ($n = 104$)				
Male ($n = 85$)				
Placebo group ($n = 27$)	3.9 ± 1.4	0.2 ± 0.8 (5.1)		
1.5-g bLF group ($n = 29$)	3.9 ± 1.5	0.1 ± 0.9 (2.6)	−0.66, 0.39	0.790
3.0-g bLF group ($n = 29$)	4.1 ± 1.9	−0.1 ± 1.2 (−2.4)	−0.72, 0.33	0.600
Female ($n = 19$)				
Placebo group ($n = 6$)	4.6 ± 1.2	0.3 ± 0.9 (6.5)		
1.5-g bLF group ($n = 8$)	3.4 ± 0.6	0.1 ± 0.4 (2.9)	−2.06, 0.73	0.410
3.0-g bLF group ($n = 5$)	3.7 ± 0.5	−1.1 ± 1.4 (−29.7)	−3.10, −0.29	0.019

Abbreviation: 95% CI, 95% confidence interval.

*Baseline refers to the polyp measurements obtained during the initial examination.

†Difference between initial polyp measurements (baseline) and month 12 values. Percentages show the ratio of mean change to mean baseline values.

‡95% CI with Dunnett's adjustment using the baseline value as a covariate.

§P values versus placebo, calculated by Dunnett's test using the baseline value as a covariate.

‖Two participants in the placebo group who withdrew from the trial after commencement were excluded from the full analysis set population. As a result, 104 participants were analyzed.

¶The overall median age of the trial participants is 63 y.

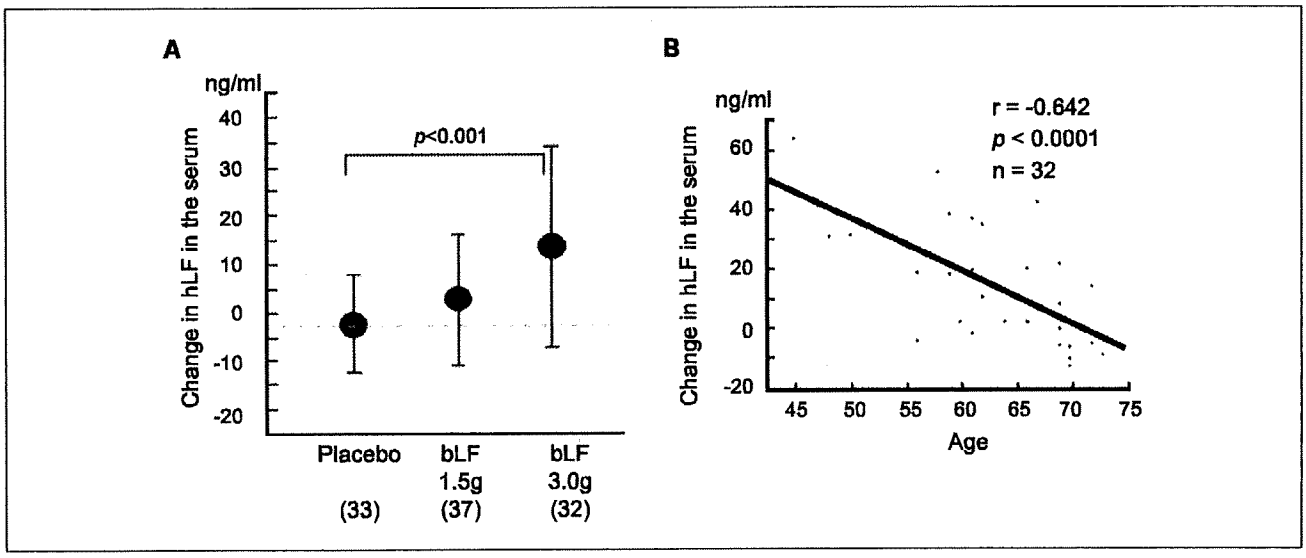

Fig. 1. Serum levels of hLF were measured every 3 mo during the trial period. Changes in serum hLF over the course of the trial period (t = 0 to t = 12 mo) in the PPS population (n = 102) are shown (see Supplementary data for a description of the PPS population). *A*, effect of bLF ingestion on serum levels of hLF in the PPS population. *B*, correlation between age and hLF induction in PPS members ingesting 3.0-g bLF. The numbers in parentheses indicate the number of participants in the separate groups. *Bars*, SD.

$P < 0.001$; Supplementary Fig. S2B). Our data suggest that higher NK cell activity may be associated with retardation of target polyp growth.

Participants with growth-retarded polyps had higher levels of hLF in their serum than participants with growing polyps ($r = -0.279$, $P = 0.005$; Fig. 3A). Our data suggest a possible negative correlation between serum hLF levels and polyp growth; however, because bLF ingestion affects both polyp growth and serum hLF in the ≤63 years age subgroup, the association between hLF and polyp growth is currently unknown.

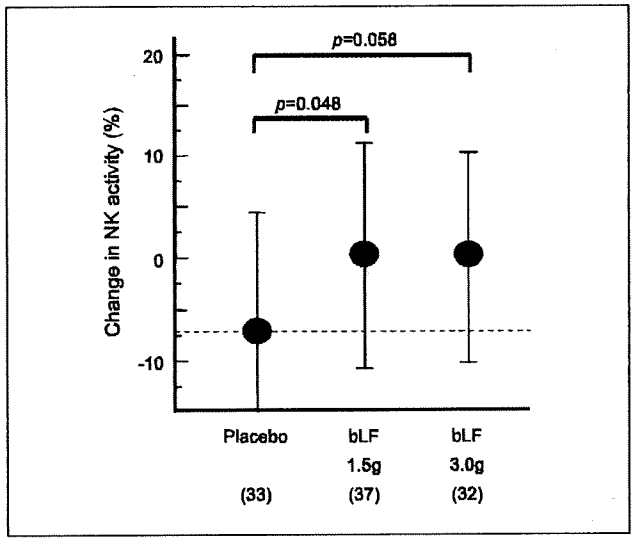

Fig. 2. NK cell activity in the serum was measured every 3 mo during the trial period. Changes in NK cell activity over the course of the trial period (t = 0 to t = 12 mo) in the PPS population (n = 102) are shown (see Supplementary data for a description of the PPS population). The numbers in parentheses indicate the number of participants in the separate groups. *Bars*, SD.

Growth-retarded polyps contained a lower density of PMNs compared with faster-growing polyps. A total of 91 target polyps were histologically diagnosed, and the density of PMNs in the stroma surrounding the target polyps could be determined in 88 of these polyps, defining a subgroup referred to as the PPS population with countable PMNs (PCP). In the PCP subgroup, the stroma surrounding growth-retarded polyps contained a lower density of PMNs than the stroma surrounding growing polyps ($r = 0.440$, $P < 0.001$; Fig. 3B). There was no statistically significant age-by-treatment interaction on PMN infiltration into target polyps ($n = 88$, $P = 0.819$).

Finally, PCP subgroup participants in whom ingestion of bLF resulted in induction of serum hLF levels by more than 5 ng/mL had a lower density of PMNs in the stroma surrounding the target polyps than PCP subgroup participants in whom ingestion of bLF resulted in induction of serum LF levels by 5 ng/mL or less ($P = 0.021$; Fig. 3C). These results suggest a possible negative correlation between serum levels of hLF and PMN infiltration into the stroma surrounding adenomatous polyps; however, because the variables being measured were not independent, the actual association between hLF and PMN infiltration into the target polyp is currently unknown.

Discussion

Diet and dietary supplements are factors in colorectal cancers (7–10). When used as a dietary supplement, bLF isolated from cow milk decreases the incidence of both colorectal cancer and aberrant crypt foci in animal models (12, 14, 15). We conducted a randomized, double-blind, controlled trial with patients ages 40 to 75 years with ≤5-mm-diameter adenomatous colorectal polyps to determine whether supplementation of bLF to the human diet had an effect on these

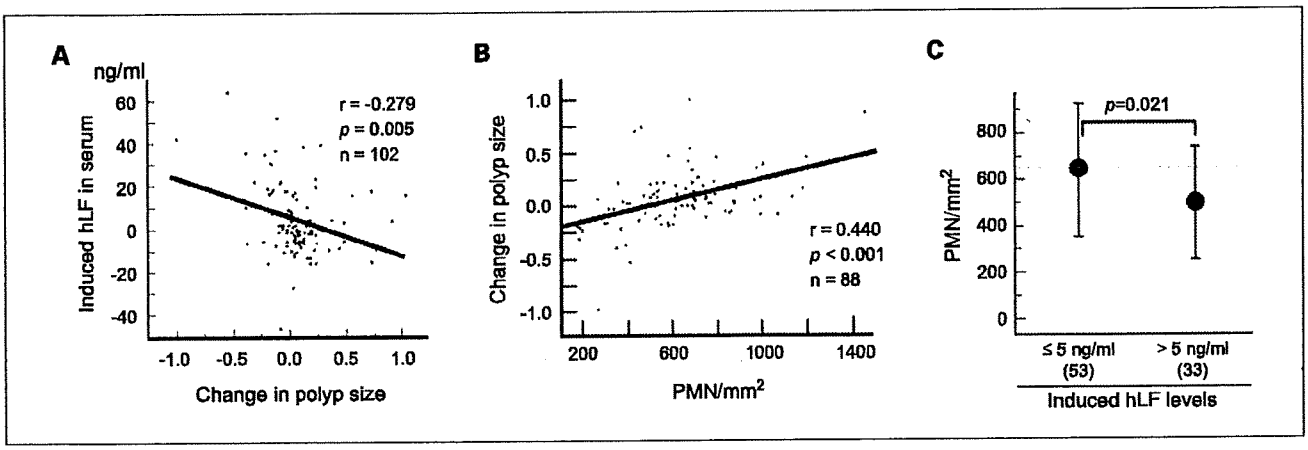

Fig. 3. Comparison of the change in target polyp size, change in serum levels of hLF, and the density of PMNs in the stroma of the target polyps. *A*, changes in serum hLF over the course of the trial period (t = 0 to t = 12 mo) in the PPS population (n = 102) were determined (see Supplementary data for a description of PPS) and the change in hLF levels was compared with the change in the size of the targets polyps in these participants. *B*, a total of 91 target polyps from the PPS population were histologically diagnosed. The density of PMNs in the stroma surrounding the target polyps could be determined in 88 of these polyps; this defines a PPS subgroup referred to as the PPS population with countable PMNs (PCP) subgroup. The change in polyp size was compared with the density of PMNs at the target polyp in the PCP subgroup. *C*, PMN counts at the target polyps of PCP members in whom ingestion of bLF resulted in induction of serum hLF by 5 ng/mL or less and participants in whom ingestion of bLF resulted in induction of serum hLF by more than 5 ng/mL. Relative change in polyp size (*A* and *B*) was determined as [(size at the end of the trial/initial size) − 1]; this method normalizes the changes in polyp sizes, with negative values representing regressing polyps and positive values representing growing polyps. In *C*, 5 ng/mL was the mean change in serum hLF levels among the participants during the course of this study (i.e., $\frac{\sum_{i=1}^{n}(hLF_{after}-hLF_{before})_i}{n}$ = 5 ng/mL. The numbers in parentheses indicate the number of participants in the separate groups. *Bars*, SD.

polyps. Participants were assigned to receive placebo, 1.5-g bLF, or 3.0-g bLF daily for 12 months. In sum, a 1-year oral intake of 3.0 g of bLF per day induced statistically significant retardation of colorectal adenomatous polyp size in participants 63 years old or younger.

The beneficial effect of bLF may be more prominent in women than in men (see Table 2). However, because of the small number of women taking part in the present study, the retardation of polyp size in the 3.0-g bLF group versus the placebo group, although statistically significant, is not a reliable finding. Further studies with an increased number of participants are warranted.

The exact mechanisms by which bLF affects colorectal polyps in humans were not directly addressed in this clinical trial. One possibility is that oral intake of bLF affects human immunologic activities, and that this in turn retards polyp growth; immune modulation mediated by oral administration of bLF has been shown in animal models (12, 22, 47, 49, 50). Notably, at the time of writing of this article, a study sponsored by the National Cancer Institute[8] is recruiting participants to examine the effects of talactoferrin, a recombinant form of hLF that has been successfully tested in phase II clinical trials with patients with refractory metastatic renal cell carcinoma (51), on the immune system and its effectiveness in treating non–small cell lung cancer.

Intake of 3.0 g of bLF increased serum hLF levels (Fig. 1A). Because hLF is an important component of the innate immune system (23, 28–31), the increase in hLF levels suggests that ingestion of bLF did affect the immune system. Importantly, increased serum hLF correlated well with retardation of polyp size (Fig. 3A). However, whereas ingestion of bLF induced serum hLF levels and serum hLF levels correlated with retardation of polyp size, a statistically significant association between bLF ingestion and retardation of polyp size was obtained only in participants 63 years old or younger. Intriguingly, the ability of bLF ingestion to elevate serum hLF weakened with aging (Fig. 1B and C), suggesting that the effect of bLF on immune function may weaken with age, and this weakening may be the reason that bLF inhibition of adenomatous polyp growth seems to be age dependent.

The mechanisms by which ingested bLF exerts its effects on the immune system are currently being studied in several laboratories (see ref. 23). Possibly, ingested bLF peptides interact with gut-associated lymphoid tissue. Several pathways have been proposed, but the exact cell types and the receptors with which ingested bLF interacts remain undefined.

Another consideration is that LF is an iron chelator (23), and the bLF used in this study was approximately 10% to 20% iron saturated. Consequently, this bLF had a high iron chelating ability. Tumor cells, like all cells, require iron, and removal of iron from the tumor environment results in regression of tumor growth (52, 53). However, the ability of bLF peptides to chelate iron after passage of the bLF-containing tablets through the stomach and small intestine has not been determined, and due to the nature of this trial, we were unable to measure iron levels in the target polyp environment. Therefore, the effect, if any, of the iron chelating ability of bLF on target polyp growth is unknown, but it is a possible factor.

LF is found in a variety of exocrine secretions (e.g., tears, nasal exudate, saliva, bronchial mucus, gastrointestinal fluids, bile, cervicovaginal mucus, and seminal fluid); it is also a major component of the secondary granules of neutrophils and is released by activated neutrophils (see ref. 23). Therefore, should ingested bLF cause activation of neutrophils, this would result in elevated serum hLF levels. Due to the nature of this study, however, we could not directly measure neutrophil activity in the serum samples obtained

[8] Available from: http://ClinicalTrials.gov; identifier NCT00923741.

from the participants in the trial. Consequently, bLF-mediated induction of serum hLF levels via activation of neutrophils is hypothetical, and it remains a possibility that induction of serum hLF proceeds wholly or in part by other routes.

The induction of serum hLF is most likely due to the effect of ingested bLF on the immune system. Whether serum hLF itself caused regression of polyp growth or was simply a consequence of bLF treatment is not known, and the exact relationship between tumor growth and serum hLF levels was not elucidated in this study. The major difficulty in determining whether serum hLF affects polyp growth is the lack of knowledge of possible mechanisms by which serum hLF could affect polyp growth.

NK cells are a principal effector of immunosurveillance against tumors (54). The effect of bLF on NK cell activity in this study was inconsistent. There was a tendency for NK cell activity to increase in participants ingesting bLF, and the increases were statistically significant in participants ingesting 1.5 g of bLF per day, but not in participants ingesting 3.0 g of bLF. Importantly, participants with growth-retarded polyps had higher NK cell activity than participants with growing polyps. Therefore, whereas this study did not establish a relationship between ingestion of bLF and NK cell activity, it remains a reasonable possibility that ingestion of bLF is associated with increased NK cell activity, and that this increased NK cell activity may have retarded polyp growth. A second trial with an increased number of participants extended over a longer period of time is needed to resolve these points.

Infiltration of PMNs into a tumor site can enhance tumor growth (32–35). In this study, participants with higher induction of serum hLF levels had both retarded polyp growth (Fig. 3A) and a lower density of PMNs in the stroma surrounding their target polyps (Fig. 3C). In rodents, oral administration of recombinant human LF attenuates neutrophil migration to the intestine (36). Therefore, in our study, bLF acting directly in the colon or possibly through serum hLF may have inhibited PMN infiltration into the target area.

In summary, ingestion of bLF had two significant effects: First, it resulted in regression of polyp growth in participants ≤63 years of age; second, it resulted in increased serum hLF levels in participants ≤63 years of age. In addition, induction of serum hLF was statistically associated with decreased infiltration of PMNs into the target area, and decreased infiltration of PMNs into the target area correlated with decreased polyp growth. Finally, enhanced NK cell activity was associated with decreased polyp growth, and there was a tendency (not consistent statistically) for NK cell activity to be enhanced in participants ingesting bLF. Taken together, our findings suggest that ingested bLF inhibits the growth of adenomatous colon polyps and that this inhibition proceeds via bLF modulation of immune system function.

Colonoscopy with clearing of neoplasms by polypectomy significantly reduces colorectal cancer; however, colorectal cancer incidence after clearing colonoscopy is appreciable (55). Factors considered to be involved in colorectal cancer that arise after clearing colonoscopy include detection failures during colonoscopy and incomplete polyp extraction (55, 56). Agents associated with retardation of adenomas are likely to reduce colorectal cancer risk because the cumulative incidences of progression from ≥10-mm-diameter polyps to cancer at 5, 10, and 20 years are reported to be 2.5%, 8%, and 24%, respectively (57), significantly higher than those for 2- to 5-mm-diameter polyps (58). Therefore, a supplement effective in the retardation of polyp growth would be a clinically useful adjunct to colorectal polyp extraction. Cyclooxygenase-2 inhibitors have been shown both to decrease the incidence of sporadic colorectal polyps (38) and to induce regression of colorectal polyps already present (44, 45); however, these drugs can have severe adverse effects (38, 44). Similarly, NSAIDs can be beneficial, but because of possible severe adverse effects, the U.S. Preventive Services Task Force recommends against their routine use to prevent colorectal cancer in individuals at average risk (59, 60). bLF is well tolerated in preclinical (61) and clinical research studies (16, 17), and no severe adverse effects related to bLF were observed in the present trial. Our study suggests that daily intake of 3.0 g of bLF could be a useful adjunct to colorectal polyp extraction.

Disclosure of Potential Conflicts of Interest

T. Kozu, G. Iinuma, Y. Saito, T. Akasu, D. Saito, and T. Kakizoe report receiving funding from the Morinaga Milk Industry Co. Ltd. in accordance with the provisions of their respective institutions; Y. Ohashi reports receiving consultancy fees from Morinaga Milk Industry. D.B. Alexander, M. Iigo, and H. Tsuda report no potential conflict of interest.

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Clinical outcome of endoscopic submucosal dissection versus endoscopic mucosal resection of large colorectal tumors as determined by curative resection

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Abstract
Background and Aims Endoscopic submucosal dissection (ESD) has recently been applied to the treatment of superficial colorectal cancer. Clinical outcomes compared with conventional endoscopic mucosal resection (EMR) have not been determined so our aim was to compare the effectiveness of ESD with conventional EMR for colorectal tumors ≥20 mm.
Methods This was a retrospective case-controlled study performed at the National Cancer Center Hospital in Tokyo, Japan involving 373 colorectal tumors ≥20 mm determined histologically to be curative resections. Data acquisition was from a prospectively completed database. We evaluated histology, tumor size, procedure time, en bloc resection rate, recurrence rate, and associated complications for both the ESD and EMR groups.
Results A total of 145 colorectal tumors were treated by ESD and another 228 were treated by EMR. ESD was associated with a longer procedure time (108 ± 71 min/29 ± 25 min; $p < 0.0001$), higher en bloc resection rate (84%/33%; $p < 0.0001$) and larger resected specimens (37 ± 14 mm/28 ± 8 mm; $p = 0.0006$), but involved a similar percentage of cancers (69%/66%; p = NS). There were three (2%) recurrences in the ESD group and 33 (14%) in the EMR group requiring additional EMR ($p < 0.0001$). The perforation rate was 6.2% (9) in the ESD group and 1.3% (3) in the EMR group (p = NS) with delayed bleeding occurring in 1.4% (2) and 3.1% (7) of the procedures (p = NS), respectively, as all complications were effectively treated endoscopically.
Conclusions Despite its longer procedure time and higher perforation rate, ESD resulted in higher en bloc resection and curative rates compared with EMR and all ESD perforations were successfully managed by conservative endoscopic treatment.

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Keywords Endoscopic submucosal dissection (ESD) · Endoscopic mucosal resection (EMR) · Recurrence · Colon · Colorectal · Short-term clinical outcome

Abbreviations

B-knife	Bipolar needle knife
CO_2	Carbon dioxide
EMR	Endoscopic mucosal resection
EPMR	Endoscopic piecemeal mucosal resection
ESD	Endoscopic submucosal dissection
IT knife	Insulation-tipped knife
LN	Lymph node
sm	Submucosal
LST	Laterally spreading tumor
LST-G	Laterally spreading tumor granular type
LST-NG	Laterally spreading tumor nongranular type
NS	Not significant
SD	Standard deviation
sm1	Minute submucosal cancer
sm2	Submucosal deep cancer

Endoscopic mucosal resection (EMR) is indicated for the treatment of superficial, early-stage colorectal cancer because of its minimal invasiveness and excellent results in terms of clinical outcomes [1–6]. However, conventional EMR techniques [6–8] currently used for the resection of laterally spreading tumors (LSTs) [7–10] are inadequate for the en bloc resection of flat lesions ≥20 mm because of both incomplete removal [11] and problems with local recurrence [12]. The endoscopic submucosal dissection (ESD) technique, which facilitates en bloc resection of early gastric cancer [11, 13–17], has recently been reported to be useful in the treatment of superficial colorectal tumors [18–28]. Previously, we reported on the effectiveness and safety of ESD for colorectal tumors using a bipolar needle knife (B-knife) (XEMEX Co., Tokyo, Japan) and an insulation-tipped knife (IT knife) (Olympus Optical Co., Ltd., Tokyo, Japan), neither of which produces any coagulation effect at the needle tip [20, 21, 24]. The effectiveness and long-term clinical outcome of ESD compared with conventional EMR is unclear, however, so the purpose of this study was to demonstrate the comparative effectiveness of ESD with conventional EMR for colorectal tumors ≥20 mm

Materials and methods

Originally, 553 large (≥20 mm) colorectal tumors were resected endoscopically between January 2003 and December 2006 at the National Cancer Center Hospital (NCCH) in Tokyo with data acquisition from a prospectively completed database. Twenty-nine lesions that required surgery after endoscopic treatment because of noncurative resections and 151 lesions treated by conventional EMR for which follow-up colonoscopy examinations could not be carried out or ascertained at NCCH were excluded, leaving a final total of 373 large colorectal tumors that were included in this retrospective case-controlled study (Fig. 1). All ESD and EMR procedures were conducted by experienced colonoscopists (three staff doctors and two senior residents), each of whom had performed more than 1,000 colonoscopies annually.

The histology, tumor size, procedure time, en bloc resection rate, recurrence rate, and associated complications were evaluated for both an ESD group and a conventional EMR group. We defined an en bloc resection as a one-piece resection of the entire lesion as observed endoscopically. In assessing for a local recurrence or the presence of a residual tumor, we repeated colonoscopy

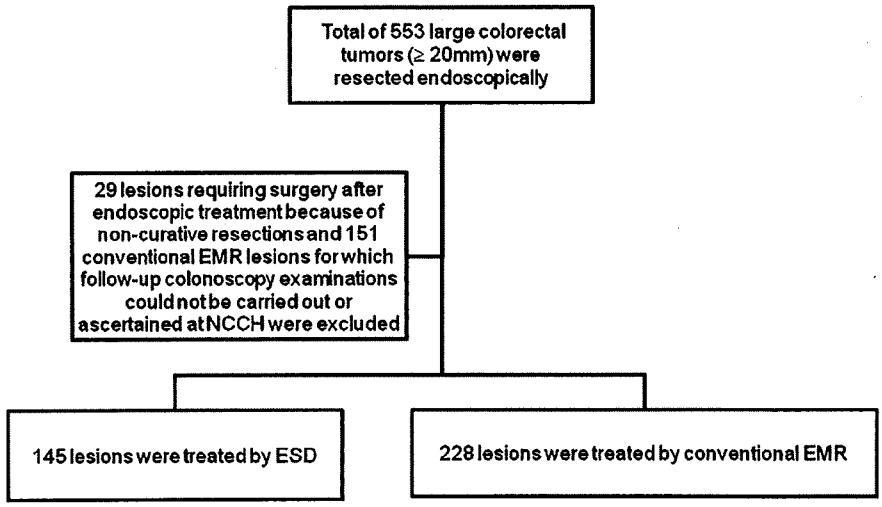

Fig. 1 Flow chart showing the patients in this study

examinations at intervals of 6 months. The procedure time was measured from the injection of a submucosal (sm) injection solution into the sm layer to removal of the colonoscope after the resection of a tumor.

Indication criteria for EMR and ESD

The existence of a noninvasive pattern [10, 24, 29–31] as determined by magnification chromoendoscopy was the minimum requirement for all lesions that were candidates for ESD and EMR. When a lesion was detected by conventional endoscopic examination, surface mucous was washed away with lukewarm water that contained pronase (Pronase MS; Kaken Pharmaceutical Co., Ltd., Tokyo, Japan) and then 0.4% indigo-carmine dye was sprayed over the lesion to enhance its surface detail. High-magnification colonoscopes (CF-240ZI, PCF-240ZI and H260AZI; Olympus Optical Co., Ltd.) were used to evaluate the surface character to differentiate an invasive pattern from a noninvasive pattern. The invasive pattern is characterized by irregular and distorted epithelial crests observed in a demarcated area suggesting that sm invasion is $\geq$1,000 μm while a noninvasive pattern does not have this finding which suggests intramucosal neoplasia or sm invasion <1,000 μm. When high-magnification observation with indigo-carmine dye was insufficient to determine the surface structure, we performed staining with 0.05% crystal violet. Based on extensive clinicopathological analyses [10], we defined the indications for ESD [24] as an LST nongranular (LST-NG)-type lesion >20 mm and an LST granular (LST-G)-type lesion >40 mm because they both had a higher sm invasion rate and were difficult to treat even by endoscopic piecemeal mucosal resection (EPMR) [7]. Some colonoscopists chose to perform EPMR [7] to treat LST-G lesions measuring between 20 and 40 mm with the final decision based on each individual colonoscopist's judgment. Large villous tumors as well as intramucosal lesions, recurrent lesions, and residual intramucosal lesions showing nonlifting sign after EMR were also potential candidates for ESD with the final decision once again made by each colonoscopist (Table 1).

Endoscopic operating systems

ESD and EMR procedures were performed using Olympus PCF-Q240ZI, CF-Q240ZI, and CF-H260AZI video endoscopes.

Bowel preparation

Bowel preparation consisted of a patient drinking 2–3 L of polyethylene glycol (PEG) solution in the morning before the procedure. In an effort to further ensure excellent bowel preparation, stool color was assessed before each colonoscopy by a trained nurse and additional PEG solution was used when necessary.

ESD procedures

The procedures were primarily performed using a B-knife [20] or an IT knife with carbon dioxide (CO_2) insufflation instead of air insufflation to reduce patient discomfort [21]. Lesion margins were delineated before ESD using 0.4% indigo-carmine dye spraying (Fig. 2A, B). Following injection of Glyceol® (Chugai Pharmaceutical Co., Tokyo, Japan) (10% glycerol and 5% fructose in normal saline solution) [32] and sodium hyaluronate acid into the sm layer [33], a circumferential incision was made using the B-knife and an ESD was then carried out using both the B-knife and IT knife (Fig. 2C–F).

Conventional EMR procedures

Conventional EMR procedures were performed using the inject and cut technique with a single-channel colonoscope (PCF-Q240ZI, CF-Q240ZI or CF-H260AZI; Olympus) and

Table 1 Indication criteria for endoscopic submucosal dissection (ESD)/endoscopic mucosal resection (EMR)

Minimum requirement
A noninvasive pattern as determined by magnification chromoendoscopy was required for all lesions that were candidates for ESD and EMR
Definite indication for ESD
LST-NG lesion ≥20 mm
Relative indication for ESD
LST-G lesion ≥40 mm
Large villous tumor, intramucosal lesion, recurrent lesion or residual intramucosal lesion showing nonlifting sign after EMR
Definite indication for EMR/EPMR
Any lesion <20 mm
LST-G lesion ≥20 mm and <40 mm

EMR endoscopic mucosal resection; *EPMR* endoscopic piecemeal mucosal resection; *ESD* endoscopic submucosal dissection; *LST-G* laterally spreading tumor granular type; *LST-NG* laterally spreading tumor nongranular type

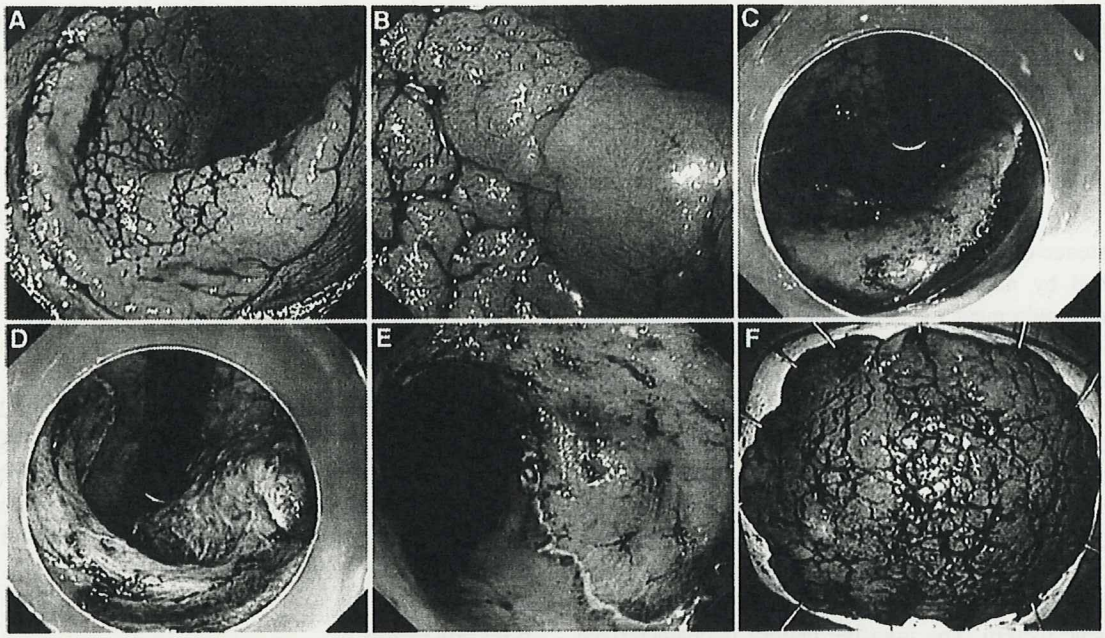

Fig. 2 Endoscopic submucosal dissection (ESD) procedures, primarily performed using a bipolar needle knife (B-knife) and an insulation-tipped knife (IT knife) with carbon dioxide (CO_2) insufflation. **A** Fifty-millimeter laterally spreading tumor nongranular (LST-NG)-type lesion located in the transverse colon. Lesion margins were delineated before ESD using 0.4% indigo-carmine dye spraying. **B** Magnified colonoscopy revealed a noninvasive pattern so the estimated depth of this LST-NG lesion was intramucosal despite its large size. **C** Following injection of Glycerol® (10% glycerol and 5% fructose in normal saline solution) and sodium hyaluronate acid solution into the submucosal layer, a circumferential incision was made using the B-knife. **D** An ESD was then carried out using both the B-knife and IT knife. **E** The ulcer bed is shown here after the successful en bloc resection. **F** The resected specimen was 65 × 50 mm in diameter and histology revealed an intramucosal cancer with a tumor-free margin

snare (10-mm or 25-mm snare master or 20-mm spiral snare; Olympus) as described in previous reports [1–3, 6, 7]. Glyceol® [32] was injected into the sm layer of the lesion with a 23-gauge needle and the lifted lesion was then resected using the snare.

In this study, we distinguished an EMR from an EPMR according to the number of resected pieces as either single or multiple, respectively. An LST-G $\geq$20 mm and <40 mm can usually be treated by EPMR rather than ESD with the area including the large nodule resected first followed by the remaining tumor (Fig. 3A–C). After EMR and EPMR, we confirmed whether or not there was any residual tumor using chromomagnification colonoscopy and performed a hot biopsy as necessary for ablative purposes.

Tumor size was estimated by measuring the resected specimen after retrieval for en bloc resected specimens and by comparing the endoscopic observation with the snare size for piecemeal resected specimens.

Sedation

Midazolam (2 mg/iv) and pentazocin (15 mg/iv) were administered during all ESD procedures. An additional 2 mg midazolam was given as necessary whenever indicated based on the judgment of the colonoscopist. In conventional EMR procedures, midazolam (2 mg iv) was administered to selected patients as determined by the colonoscopist, but only when a patient complained of pain or abdominal distension.

Histological assessment

All specimens were evaluated after being cut into 2-mm slices and examined microscopically for histological type, depth of invasion, lateral resection margin, and vertical resection margin. Resections were considered tumor free when the lateral and vertical margins of a specimen were both negative for tumor cells independent of its histological features.

A curative resection was achieved when both the lateral and vertical margins of the specimen were free of cancer and there was no sm invasion deeper than 1,000 μm from the muscularis mucosae (sm1), lymphatic invasion, vascular involvement or poorly differentiated component [34]. An adenoma with an unknown lateral margin was also considered to be a curative resection provided that such adenoma met all the other criteria. Histological diagnoses

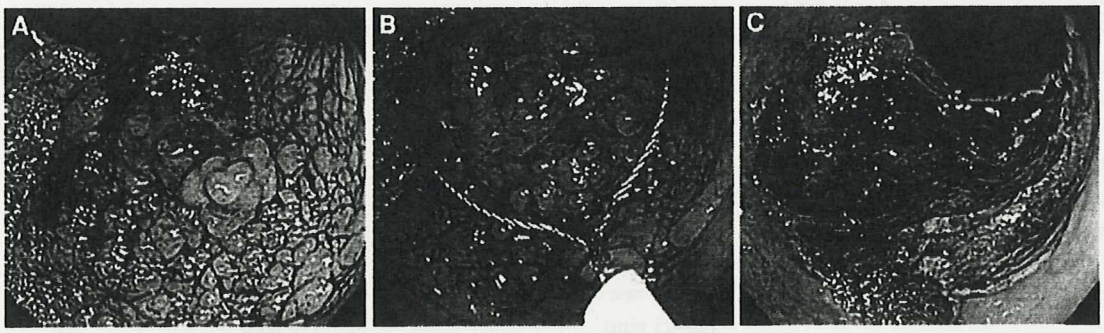

Fig. 3 Conventional endoscopic mucosal resection (EMR) procedures. Conventional EMRs were usually performed using an inject and cut technique with a single-channel colonoscope and snare. Glycerol® was injected into the submucosa of the lesion with a 23-gauge needle and then the lifted lesion was resected using a round snare. **A** A 35-mm laterally spreading tumor granular (LST-G)-type lesion located in the rectum. **B** An LST-G between 20 and 40 mm can be treated by endoscopic piecemeal mucosal resection (EPMR) rather than ESD with the area including the large nodule resected first followed by the remaining tumor. **C** The ulcer bed after a three-piece resection

were based on the Japanese classification of cancer of the colon and rectum [35] and the Vienna classification [36].

Follow-up endoscopic care

In assessing for local recurrence or the presence of a residual tumor, we usually repeated colonoscopic examinations at intervals of 6 months for ESD patients because the technique was still relatively new and indicated for large colorectal lesions that had previously been treated surgically. In most cases, we repeated colonoscopic examinations at intervals of 6 months for EPMRs and at 12-month intervals for EMRs with en bloc resections because of an expected lower risk of recurrence [37] with such examinations performed either by the endoscopy staff at NCCH or the patient's previous hospital.

All ESD and EMR patients with sm1 invasion were followed up regularly with annual computed tomography and endoscopic ultrasonography examinations for the detection of lymph-node metastasis. Complete endoscopic follow-up care was available for all 145 lesions in the ESD group and all 228 lesions in the EMR group. Indigo-carmine dye was sprayed on previously resected areas and high-magnification views were obtained in all cases. Recurrent neoplastic disease was identified as type IIIs, IIIL, IV or V pit pattern according to the criteria established by Kudo and Fujii [6, 9, 10, 30–32, 38–41].

Statistical analysis

All variables in this study are described as mean ± standard deviation (SD). In comparing baseline characteristics between the two groups, we used a *t*-test for continuous variables and a chi-square test for dichotomous variables. All statistical analyses were performed using SAS version 8.0 (SAS Institute Inc., Cary, NC). The p values are two-sided and $p < 0.05$ was used to determine statistical significance.

Ethics

The ethics committee at NCCH approved the study protocol and informed written consent was obtained from all patients in the ESD and EMR groups for each specific colonoscopic treatment and all scheduled follow-up colonoscopy examinations.

Results

During the study period, 145 lesions were treated with ESD and 228 were treated with conventional EMR (Fig. 1). All 373 lesions were eligible for outcome analysis. Clinical characteristics of the patients in the two groups are presented in Table 2. There were no differences between the two groups in terms of age, gender, endoscopic follow-up frequency or follow-up periods (Tables 2 and 3).

En-bloc resection rates

In the ESD group, 122 out of 145 lesions (84%) were completely resected en bloc compared with only 74 of 228 lesions (33%) in the EMR group ($p < 0.0001$), although tumor size was significantly larger in the ESD group ($p < 0.0001$) (Table 3).

Endoscopic characteristics of resected specimens

Regarding macroscopic type, 50% of the EMR group lesions were LST-Gs and 49% of the ESD group lesions were LST-NGs. There were no differences between the two groups in terms of tumor location. The percentage of

Table 2 Clinical characteristics of patients

	EMR/EPMR	ESD	*p*-Value
Number of lesions	228 (74/154)	145	
Pathology	77/151	45/100	NS
(Adenoma/M-SM1; %)	(34%/66%)	(31%/69%)	NS
Macroscopic type	80/114/34/0	5/63/71/6	
(Is/LST-G/LST-NG/recurrence[a])	(35%/50%/15%/0)	(3%/43%/49%/4%)	<0.0001
Location (Rt/Lt/rectum)	89/52/110	44/28/73	
Tumor size (mean ± SD)	28 ± 8 mm	37 ± 14 mm	0.0006
(range)	(20–95 mm)	(20–140 mm)	
Age (mean ± SD; years)	64 ± 4	64 ± 11	NS

[a] Recurrence included local recurrence after EMR and residual tumor after incomplete en bloc resection

EMR endoscopic mucosal resection; *EPMR* endoscopic piecemeal mucosal resection; *ESD* endoscopic submucosal dissection; *M* intramucosal; *SM* submucosal; *LST-G* laterally spreading tumor granular type; *LST-NG* laterally spreading tumor nongranular type; *Rt* right colon; *Lt* left colon; *SD* standard deviation; *NS* not significant

Table 3 Clinical outcomes

	EMR/EPMR	ESD	*p*-Value
Number of lesions	228 (74/154)	145	
Endoscopic follow-up times (mean ± SD; number)	2.4 ± 1.6	2.0 ± 1.1	NS
(range)	(1–8)	(1–5)	
Endoscopic follow-up periods (mean ± SD; months)	26 ± 17	20 ± 13	NS
(range)	(6–68)	(6–61)	
En bloc resection (%)	74 (33%)	122 (84%)	<0.0001
Recurrence rate (%)	33 (14%)	3 (2%)	<0.0001
En bloc/piecemeal recurrences	2/31	0/3	
Complications			
Perforation	3 (1.3%)	9 (6.2%)	NS
Delayed bleeding	7 (3.1%)	2 (1.4%)	NS
Procedure time (mean ± SD; min)	29 ± 25	108 ± 7	<0.0001
(range)	(3–120)	(15–360)	

EMR endoscopic mucosal resection; *EPMR* endoscopic piecemeal mucosal resection; *ESD* endoscopic submucosal dissection; *SD* standard deviation; *NS* not significant

carcinomas was 69% in the ESD group and 66% in the EMR group (p = NS) (Tables 2 and 3).

Local recurrences rates

There were only three cases (2.1%) of local recurrence in the ESD group during a mean endoscopic follow-up period of 20.0 ± 12.9 months (range 6–61 months). In comparison, local recurrence occurred in 14.5% (33/228) of the lesions in the EMR group during a mean endoscopic follow-up period of 25.9 ± 17.0 months (6–68 months). All three recurrences in the ESD group had previously been resected on a piecemeal basis and each recurrence required one additional EMR. Each of these recurrences was diagnosed histologically as a tubular adenoma and curative resections were achieved for all three. The 33 recurrences in the EMR group involved 2/74 (2.7%) en bloc resections and 31/174 (17.8%) piecemeal resections (Table 3). Twenty-six of the 33 EMR recurrent cases were successfully treated by one additional EMR with the other seven cases needing two sessions of repeat EMR. Two EPMRs required surgery because of invasive recurrence (Fig. 4A–F) while a third piecemeal resection also required surgery because of technical difficulty in performing another EMR despite the intramucosal nature of that particular recurrence.

Duration of recurrence detection

Mean duration of recurrence detection was 6 months (2–18 months) in the EMR group and 6 months (4–6 months) in the ESD group (Table 3).

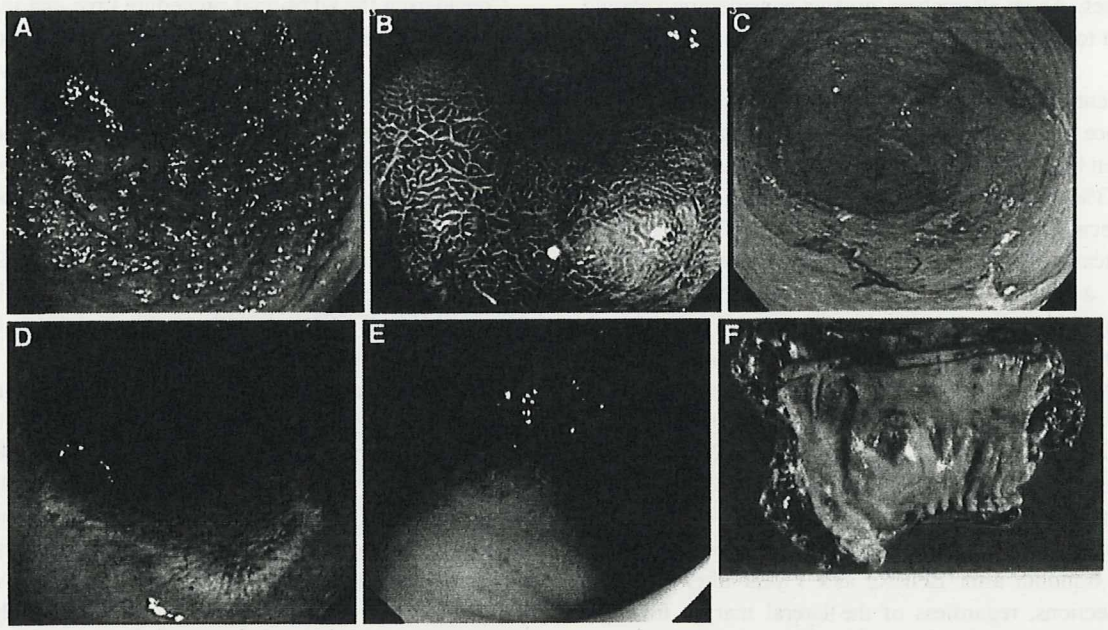

Fig. 4 **A** This case originally involved a large LST-G lesion >3/4 in circumference. **B** Magnified colonoscopy using 0.05% crystal violet staining revealed a noninvasive pattern on the large nodule. **C** An EPMR consisting of more than ten pieces finally resected the entire lesion. Histology revealed an intramucosal carcinoma without any evidence of lymphovascular invasion or a poorly differentiated component so we followed this patient closely without surgery. **D** The third follow-up colonoscopy after 18 months revealed no recurrence. **E** A fourth follow-up was performed 1 year later, at which time a submucosal tumor-like recurrence was found 1 cm from the original EPMR scar. **F** Surgery was performed on this lesion and histology revealed the recurrence of an invasive cancer

Early and late complications

Perforations occurred in 9 out of 145 patients (6.2%) in the ESD group, which was higher compared with the perforation rate of 1.3% (3/228) in the EMR group (p = NS). None of the 12 perforations was delayed and all of them were successfully treated endoscopically using endoclips and managed conservatively.

Minor delayed bleeding occurred in two patients (1.4%) in the ESD group and seven (3.1%) patients in the EMR group (p = NS), but all nine cases were successfully managed conservatively using endoclips with no blood transfusions or additional procedures necessary (Table 3).

Procedure times

The procedure time for ESDs was 108 ± 71 min (15–360 min) compared with 29 ± 25 min (3–120 min) for EMRs, resulting in a significantly shorter procedure time for the EMR group ($p < 0.0001$) (Table 3).

Discussion

This study is, to the best of our knowledge, the first to compare clinical outcomes for colorectal ESD with EMR/EPMR including mid-term follow-up.

For many years, conventional EMR and surgery were the only available treatments for large colorectal tumors, even those detected at an early stage. Conventional EMRs usually resulted in EPMRs particularly for large LSTs ≥20 mm with reports of local recurrence rates ranging from 7.4% to 17% [8, 12, 32]. Most of those recurrences, however, received repeated endoscopic treatment with excellent results regarding preservation of the colorectum [32].

In our series, the introduction of ESD enabled us to effectively treat large colorectal tumors that were LST-NGs and carcinomas, resulting in higher en bloc resection and curability rates compared with conventional EMR. EPMR also was effective in treating many LST-Gs ≥20 mm, with only three cases requiring surgery after such piecemeal resections, including two invasive recurrences cases. Those two cases were originally diagnosed histologically as intramucosal carcinomas without lymphatic or vascular invasion, but both recurrences consisted of invasive carcinomas. We suspect that each case may have originally involved either sm invasion or lymphatic invasion that was not diagnosed histologically because of the increased difficulty in assessing a piecemeal resection. Based on our results, therefore, EPMRs must be performed carefully and close follow-up is required in the event that additional treatment becomes necessary because accurate histological evaluation can be difficult or impossible in

such cases. As an alternative, greater consideration should be given to either ESD or laparoscopic surgery rather than EPMR.

Conventional EMRs in this study had an overall local recurrence rate that was similar to in previous reports [12, 33], as en bloc resection cases resulted in a low recurrence rate of 3%, but piecemeal resections had a considerably higher recurrence rate of 20%. In contrast, ESDs resulted in a significantly higher en bloc resection rate and, consequently, a significantly lower recurrence rate. In those ESDs in which en bloc resections were not achieved, however, the local recurrence rate was approximately 13%, which was much closer to the local recurrence rate for EPMRs. According to our findings, EPMR resulted in a higher recurrence rate compared with ESD, although EPMR produced results similar to those of ESD in relation to preservation of the colorectum.

In this study, we conducted follow-up examinations on patients 6 month after EPMRs and 1 year after EMR en bloc resections, regardless of the lateral margin findings. This was based on our preliminary data [33] indicating that EPMR recurrences were more frequent compared with EMR en bloc resection recurrences and most such EPMR recurrences occurred within 6 months. This current study once again confirmed that most EPMR recurrences were detected after the first 6 months, so such recurrences could continue to be successfully treated endoscopically, supporting the propriety of our follow-up program after EPMR.

As for complications, the perforation rate in the ESD group was 6.2%, which was considerably higher than the 1.3% perforation rate in the EMR group, although there was no statistical difference between the two groups. In other reported series, the perforation rates for colorectal ESDs [8, 27, 28] and EMRs [42] ranged from 1.4% (1/71) to 5.5% (11/200) and 0.31% to 0.93%, respectively, which were similar to our results. The target lesions for ESD in this study, however, were large LSTs that would have been treated by surgery in the past because of the technical difficulty [43]. In fact, the mean tumor size was significantly larger in the ESD group compared with the EMR group so conventional EMRs performed on such lesions undoubtedly would have resulted in a higher complication rate for the EMR group.

All perforation cases were successfully treated conservatively without surgery by endoscopic clipping. As a result, the perforation rate of 6.2% in the ESD group was considered to be acceptable, although further instrument improvements and technique refinements will both be necessary to reduce the perforation rate. The delayed bleeding rate was relatively low in both groups, but particularly in the ESD group, probably because small vessels were coagulated during the ESD procedure.

Considering the additional procedure time and increased cost of ESD devices, it would be difficult to standardize the colorectal ESD procedure on a widespread basis at the present time. We currently select lesions with more serious indications for colorectal ESD that would otherwise be treated surgically. Such ESD patients usually are discharged from the hospital sooner than if surgery had been performed, resulting in reduced medical costs.

Finally, the long-term efficacy of colorectal ESD needs to be established by evaluating an extended follow-up period, although ESD certainly appears to be a feasible alternative to conventional EMR, particularly for certain kinds of colorectal cancers. This study was not a randomized controlled trial, however, and eligibility criteria for the two endoscopy procedures were sometimes unclear for different kinds of lesions. It will be necessary, therefore, to prospectively assess the clinical outcome comparison between ESD and EMR for large colorectal tumors in the future. Another limitation of this study that may have been a source of bias was the exclusion of 40% of the total EMR/EPMR cases from our analysis because follow-up colonoscopy examinations were not carried out at NCCH or could not be ascertained by us.

In conclusion, ESD was selected more often for treating large colorectal tumors because it provided higher en bloc resection and curability rates compared with EMR despite the longer procedure time and higher perforation rate associated with ESD. All ESD perforations, however, could be successfully managed by conservative endoscopic treatment. EMR effectively treated many large colorectal tumors, and only three cases required surgery after EPMRs; such procedures should be carefully performed because it can be more difficult and occasionally impossible to make an accurate histological evaluation, meaning that close follow-up is required in the event that additional treatment is necessary in such cases.

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CURRENT STATUS AND FUTURE PERSPECTIVE OF ENDOSCOPIC TREATMENT FOR COLORECTAL NEOPLASIA

ENDOSCOPIC SUBMUCOSAL DISSECTION (ESD) FOR COLORECTAL TUMORS

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Background: Endoscopic submucosal dissection (ESD) is accepted as a minimally invasive treatment for early gastric cancer, however, it is not widely used in the colorectum because of its technical difficulty.
Objective: To determine the feasibility of using ESD for treating large superficial colorectal tumors.
Patients: A total of 400 consecutive patients were treated by ESD for 405 lesions at National Cancer Center Hospital, Tokyo, Japan.
Interventions: Endoscopic submucosal dissection procedures were performed using a bipolar needle knife (B-knife) or an insulation-tip knife (IT knife).
Results: The en-bloc resection rate was 87% and the curative resection rate was 86% among the 405 ESDs: 101 involved tubular adenomas, 255 intramucosal cancers and minute submucosal cancers, 46 submucosal deep cancers and 3 others (MALT and carcinoid tumors). The median operation time was 90 minutes and the mean size of resected specimens was 40 mm (range: 15 mm-150 mm). Perforations occurred in 14 (3.5%) cases and postoperative bleeding in four (1%) cases, but only one perforation case needed emergency surgery because endoscopic clipping was ineffective.
Limitations: Conducted at single center.
Conclusions: Endoscopic submucosal dissection is a feasible technique for treating large superficial colorectal tumors because it provides a higher en-bloc resection rate and is less invasive than surgical resection.

Key words: colorectum, endoscopic mucosal resection (EMR), endoscopic submucosal dissection (ESD), laterally spreading tumor granular type (LST-G), laterally spreading tumor non-granular type (LST-NG).

INTRODUCTION

Endoscopic mucosal resection (EMR) is indicated for the treatment of adenoma and intramucosal or submucosal superficial (sm1: less than 1000 μm from the muscularis mucosae) colorectal cancer because of its minimal invasiveness, negligible risk of lymph-node (LN) metastasis[1] and excellent results in terms of clinical outcome.[2–4] Conventional EMR techniques, such as strip biopsy, currently used for the resection of laterally spreading tumors (LSTs),[5,6] are inadequate for the en-bloc resection of flat lesions >20 mm because incomplete removal and local recurrence are occasionally observed after conventional EMR.[7,8] In order to completely remove large gastric lesions en bloc, we have developed an insulation-tip (IT) electrosurgical knife, originally reported by Hosokawa and Ono,[9] and obtained good results using it.[10]

Endoscopic submucosal dissection (ESD) for colorectal cancer[11] is not widely accepted, however, because of its technical difficulty and the risk of perforation. In addition, endoscopic piecemeal mucosal resection (EPMR) can treat many LSTs > 20 mm and only a few cases require surgery after such piecemeal resection.[8]

It is important, therefore, to investigate which lesions should be resected en bloc and which lesions can be resected piecemeal.

CLINICOPATHOLOGICAL FEATURES OF LATERALLY SPREADING TUMORS

Based upon clinicopathological analyses of LSTs,[12] LST non-granular type (LST-NG) has a higher rate of submucosal (sm) invasion and diagnosis of tumor depth is more difficult to make endoscopically compared to LST granular type (LST-G) (Tables 1,2). Histologically, 27% of sm invasions are multi-focal in LST-NGs and such invasions are difficult to predict before treatment. In contrast, LST-Gs have a lower rate of sm invasion and most such invasions are found under the largest nodule (Fig. 1).[12]

INDICATIONS FOR COLORECTAL ESD

We have defined the indications for colorectal ESD, therefore, as LST-NG > 20 mm at National Cancer Center Hospital, Tokyo, Japan (NCCH). An LST-G > 20 mm can be treated by planning EPMR rather than ESD with the area including the largest nodule resected first followed by the remaining

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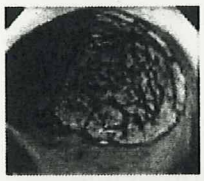
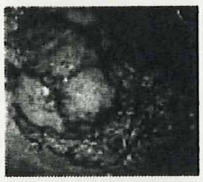
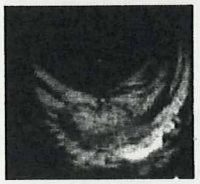
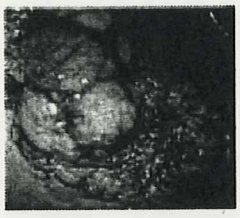
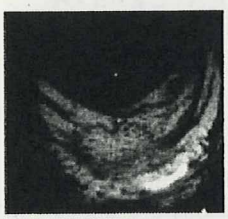
Table 1. Relationship between size of laterally spreading tumors (LSTs) and rate of submucosal (sm) invasion

	10 mm–	20 mm–	30 mm–	40 mm–	Total
 IIa (LST-G)	0/87 (0%)	0/51 (0%)	1/17 (6%)	0/6 (0%)	1/161 (0.6%)
 IS+IIa (LST-G)	3/56 (5%)	6/46 (13%)	2/34 (6%)	8/40 (20%)	19/176 (11%)
 IIa (LST-NG)	11/193 (6%)	16/56 (29%)	7/16 (44%)	3/6 (50%)	37/271 (14%)

LST-G, laterally spreading tumor-granular; LST-NG, laterally spreading tumor-non granular
1999.1–2004.3: National Cancer Center Hospital.

Table 2. Pit pattern diagnosis for laterally spreading tumors (LSTs)

LST-G	Adenoma·m·sm1*	sm2,3	
 Non-Inv. pattern	275	4	98.6% (275/279)
Inv. pattern	3	5	62.5% (5/8)
287	Specificity 98.9%	Sensitivity 55.6%	
LST-NG	Adenoma·m·sm1*	sm2,3	
 Non-Inv. pattern	203	2	99.0% (203/205)
Inv. pattern	2	17	89.5% (17/19)
224	Specificity 99.0%	Sensitivity 89.5%	

LST-G, laterally spreading tumor-granular; LST-NG, laterally spreading tumor-non granular
*sm1: <1000 μm from muscularis mucosae. 1999.1–2003.12 NCCH.

tumor (Fig. 2A,B). However, LST-G > 40 mm are also good candidates for ESD because they have higher sm invasion rates and are difficult to treat even by EPMR.

Recently, similar detailed indications for colorectal ESD have been proposed by Working Group for Standardisation of Colorectal ESD.

DEVELOPMENT OF COLORECTAL ENDOSCOPIC SUBMUCOSAL DISSECTION

The colon mucosa is very thin and the lumen is too narrow to handle the scope freely, so some device is necessary to make the ESD in the colorectum easier and safer.