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Leukocytapheresis therapy, performed with leukocyte removal filter, for inflammatory bowel disease

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Abstract: Leukocytapheresis (LCAP), performed with a leukocyte removal filter, was administered five times, at 1-week intervals, for 5 weeks of intensive therapy and five times, at approximately 1-month intervals, for approximately 5 months of maintenance therapy, to 13 patients with inflammatory bowel disease (IBD) diagnosed as ulcerative colitis (UC) in 8 and Crohn's disease (CD) in 5. Clinical and blood examinations showed no side effects in any of the patients. During the intensive therapy, excellent or moderate clinical response was recognized in 11 of the 13 patients (84.6%), of whom 6 had a dramatic response; the excellent or moderate clinical response continued throughout the maintenance therapy in 8 of the patients (61.5%). Flow cytometry showed that the patients who had improved generally had high values for percentages of $HLADR^+$, $HLADR^+CD3^+$, and $HLADR^+CD8^+$ cells before the first LCAP, and that these values and the C-reactive protein levels and erythrocyte sedimentation rates had decreased to the normal range by the end of both intensive and maintenance therapy. In the patients who showed poor response, in contrast, all the above values had been at or near normal before the initial LCAP administration. The clinical improvement in the absence of any additional medical treatment suggests that LCAP has the capacity to influence the causal mechanism(s) of IBD and that IBD is strongly associated with the cell-mediated immune response.

Key words: leukocytapheresis, inflammatory bowel disease, ulcerative colitis, Crohn's disease

Introduction

Inflammatory bowel disease (IBD) is generally treated medically with drugs such as salazosulfapyridine,[1] corticosteroids, and ACTH,[2,3] and occasionally with immunosuppressive agents.[4] Surgery is often indicated for patients with insufficient response to these conventional therapies, and patient relapse is often noted following a period of effective response. The side effects of drug therapy also present a problem, particularly with long-term use. Alternative therapies are therefore necessary and desirable.

While the etiology of IBD remains unknown, it seems increasingly likely that both ulcerative colitis (UC) and Crohn's disease (CD) are multifactorial in origin, involving environmental, genetic, immune, and microbiological factors.[5] Regardless of its cause, however, the final pathway of tissue damage in IBD is mediated, via white blood cells (WBC), by the cellular immune response in the intestinal mucosa.

Leukocytapheresis (LCAP),[6] performed with a leukocyte removal filter, has been found effective in the treatment of various disorders involving the immune response, such as rheumatoid arthritis (RA),[7,8] ophthalmic Graves' disease,[8] Behcet's disease,[8] and pemphigus vulgaris.[8] We therefore examined its efficacy and safety, as described here, in the treatment of IBD.

Patients and methods

Major inclusion criteria for the LCAP therapy were active clinical condition and active disease stage shown

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by endoscopic findings and/or barium enema X-ray examination, and either insufficient response to conventional therapy, allergic or severe side effect reactions to drugs in conventional therapy, or severe symptoms with no prior treatment by medication. Major exclusion criteria were severe cardiac problems, severe cerebral diseases such as cerebral bleeding or cerebral infarction within the 6 months before LCAP would have been initiated, severe renal and liver failure, pregnancy, drug abuse, dementia, hypotension (less than 80 mmHg systolic pressure), age under 10 or over 75 years, and effective on-going control by other therapeutic means.

Thirteen IBD patients were enrolled in this study (Table 1), 8 with recurring UC and 5 with CD. Of the 8 UC patients, 7 exhibited entire-colon UC, and 1 exhibited proctitis UC.[9] Disease was severe in 4 of the 8 UC patients, moderately severe in 2, and mild in 2.[10] Of the 5 CD patients, the disease in 4 was of moderate severity, with a history of recurrent concomitant ileal and colonic lesions, and 1 patient had experienced a first episode of severe transmural colitis.[11] Informed consent was obtained from all patients prior to their entry into the study.

LCAP was performed with the Plasauto 1000 apheresis unit (Asahi Medical Co. Ltd, Tokyo, Japan)[6] equipped with a Cellsorba (Asahi Medical) leukocyte removal filter.[6–8] Leukocyte removal in LCAP is effected by adherence to fibers in the filter. Three thousand milliliters of whole blood were processed, at

Table 1. Clinical features of patients with ulcerative colitis (*UC*) and Crohn's disease (*CD*)

Patient no	Disease	Age (years)	Sex	Duration of disease (years)	Drugs administered before LCAP	Severity and classification	Major complaints	Response to LCAP		Duration of improvement (months)
								Intensive	Maintenance	
1	UC	26	M	1.5	PI 60 mg/day	Severe Entire colon	WD 7–9 times Fever (38.3°C) Bloody stool	Excellent	Excellent	18
2	UC	27	F	2.5	PI 60 mg/day	Severe Entire colon	WD 6–9 times Fever (38.5°C) Bloody stool	Excellent	Excellent	15.5
3	UC	27	F	2.3	PI 60 mg/day	Severe Entire colon	WD 6–8 times Fever (38.5°C) Bloody stool	Excellent	Excellent	15
4	UC	22	M	3.5	PI 50 mg/day S 3.0 g/day	Severe Entire colon	WD 6–8 times Fever (38.5°C) Bloody stool	Excellent	Excellent	20
5	UC	19	M	5.0	PO 50 mg/day S 3.0 g/day	Moderate Entire colon	WD 4–6 times Bloody stool	Moderate	Moderate	19
6	UC	32	M	6.0	PO 40 mg/day S 3.0 g/day	Moderate Entire colon	WD 6–8 times	Moderate	No change	Drop out
7	UC	40	F	10.0	PO 20 mg/day S 3.0 g/day	Mild Entire colon	WD 1–3 times	No change	Drop out	—
8	UC	47	M	15.0	PO 30 mg/day S 3.0 g/day	Mild Proctitis	WD 1–3 times	No change	Drop out	—
9	CD	19	F	(1 week)	None IVH	Severe TC	WD 7–9 times Abdominal pain Fever (38.5°C)	Excellent	Excellent	18
10	CD	28	M	11.0	S 3.0 g/day IVH	Moderate EC	WD 3–5 times Abdominal pain	Excellent	Excellent	15
11	CD	18	F	4.5	None IVH	Moderate EC	WD 5–7 times Abdominal pain	Moderate	Moderate	17
12	CD	19	F	2.5	None IVH	Moderate EC	WD 2–4 times Abdominal pain	Moderate	No change	Drop out (Operation)
13	CD	37	M	8.0	ED	Moderate EC	WD 3–5 times Abdominal pain	Moderate	No change	Drop out (Operation)

PI, Prednisolone intravenously; *PO*, prednisolone orally; *S*, salazosulfapyridine; *IVH*, intravenous hyperalimentation; *ED*, elemental diet; *TC*, transmural colitis; *EC*, enterocolitis; *WD*, watery diarrhea; *LCAP*, lenkocytapheresis

a blood flow rate of 50 ml/min. Access and return lines were connected via cubital veins.[6] Nafamostat mesilate (Torii Pharmaceutical Co., Tokyo, Japan)[12] was used as an anticoagulant for the extracorphoreal circulation. LCAP was performed once weekly for 5 weeks as intensive therapy and approximately once every 4 weeks as maintenance therapy. Drug administration was gradually decreased and discontinued where possible, during or after the intensive or maintenance therapy period.

Clinical manifestations were recorded and conventional routine laboratory tests and endoscopic evaluation and/or barium enema X-ray examinations were conducted before and after the intensive therapy and after five sessions of the maintenance therapy. C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and the percentages of T, B, $OKT3^+$, $OKT4^+$, $OKT8^+$, $HLADR^+$, $HLADR^+CD3^+$, $HLADR^+CD4^+$, and $HLADR^+CD8^+$ cells on flow cytometry were also determined at the same times.

We classified the response to the LCAP therapy as: (1) excellent, if all clinical symptoms had subsided for both UC and CD, if objective examination by endoscopy or other image analysis showed complete remission of disease for UC and CD, and if the score, defined by the international organization for the study of inflammatory bowel disease (IOIBD), showed more than 50% improvement and the ESR and CRP levels were within normal limits for CD during the intensive or maintenance therapy; (2) moderately improved, if the clinical symptoms of both diseases had significantly subsided, if objective examination by endoscopy showed improvement but not remission for UC, and if the IOIBD score showed 25%–50% improvement for CD; (3) no change, if no clinical and no objective improvement, or deterioration, was found; and (4) deterioration, if subjective and/or objective deterioration was found during the therapy.

LCAP therapy was discontinued at the end of the intensive or maintenance therapy period for any patient showing no improvement at the end of that period, and was also continued after the end of the maintenance period for patients showing moderate to excellent improvement at that time.

Results

LCAP procedures were well tolerated by all patients. On average, 2.3×10^{10} white blood cells were removed per LCAP session. During the intensive therapy, clinical and objective improvement was found in 11 (84.6%) of the 13 patients; 6 had excellent improvement and 5 had moderate improvement. Both patients (nos 7 and 8; 15.4%) with UC of mild severity but of more than 10 years' duration prior to LCAP administration showed no change (Table 1). Clinical and objective improvement was maintained in 8 of the 13 patients (61.5%) during the maintenance therapy, 6 of whom maintained excellent improvement and 2 of whom maintained moderate improvement. One of the 2 UC patients with moderate improvement during the intensive therapy was returned to alternative therapies because endoscopic examination at the end of maintenance therapy showed no further improvement. Among the CD patients, LCAP maintenance therapy was discontinued for 2 (nos 12 and 13) of the 3 patients who had shown moderate improvement after intensive therapy since stenosis of the ileum had not been resolved and surgery was necessary to remove the stenotic area. One CD patient (no. 10) who had previously had stenotic areas removed surgically twice showed excellent improvement during both the intensive and the maintenance therapy. For all of the patients who showed moderate to excellent improvement during the intensive and the maintenance therapy, the administration of drugs that had already been given, such as prednisolone and salazosulfapyridine, before the initiation of LCAP was gradually decreased and then stopped altogether, if possible.

All four severe UC patients, who had a disease history of 1.5–3.5 years, responded dramatically to the LCAP therapy. The response of one of these (patient no. 1) is shown in some detail in Fig. 1. The patient, a 26-year-old male, was admitted to our department on March 22, 1993 because of severe watery diarrhea (more than ten times/day), bloody stool, weight loss, abdominal pain, and fever as a relapse of UC. The onset of symptoms had begun $1\frac{1}{2}$ years previously and he had been admitted to a hospital twice during that $1\frac{1}{2}$-year period. He had been receiving 60 mg/day of intravenous prednisolone, as well as intravenous hyperalimentation therapy, at an in-patient clinic for 1 month prior to his entry into the LCAP therapy, with no improvement having been seen. At the time of LCAP entry, he still had severe watery diarrhea, bloody stool, weight loss, abdominal pain, abdominal tenderness, and anemia. Recovery was rapid and dramatic (Fig. 1). The episodes of watery diarrhea decreased from seven to ten/day to three/day on the day after the first LCAP session and to zero on all subsequent days throughout the intensive and maintenance therapy, with no deterioration even after the discontinuation of prednisolone and the initiation of oral feeding. Endoscopic examination at the end of the intensive therapy period showed UC to be in remission. His WBC count and granulocyte/lymphocyte ratio had decreased to near normal by the time of the second LCAP session. Prior to the LCAP therapy, endoscopic examination had shown severe

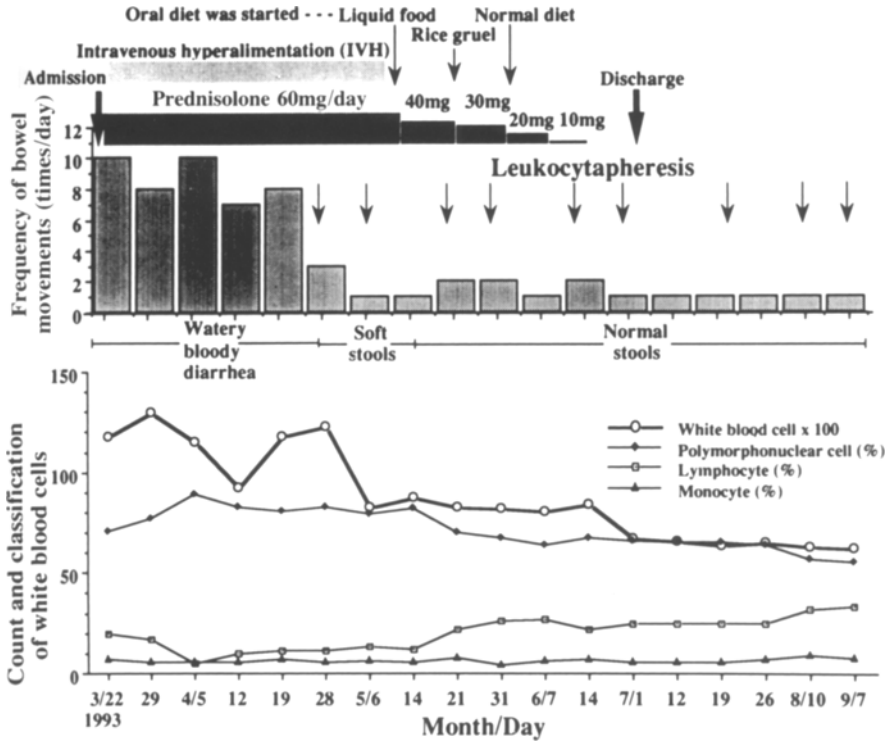

Fig. 1. Clinical course of patient no. 1, who had recurrent severe extensive ulcerative colitis. Watery diarrhea (seven–ten times/day) ceased on the day following the first leucocytapheresis (*LCAP*) session, and no deterioration was noted after the discontinuation of prednisolone and the initiation of oral feeding. The WBC count and granulocyte/lymphocyte ratio decreased to near normal values

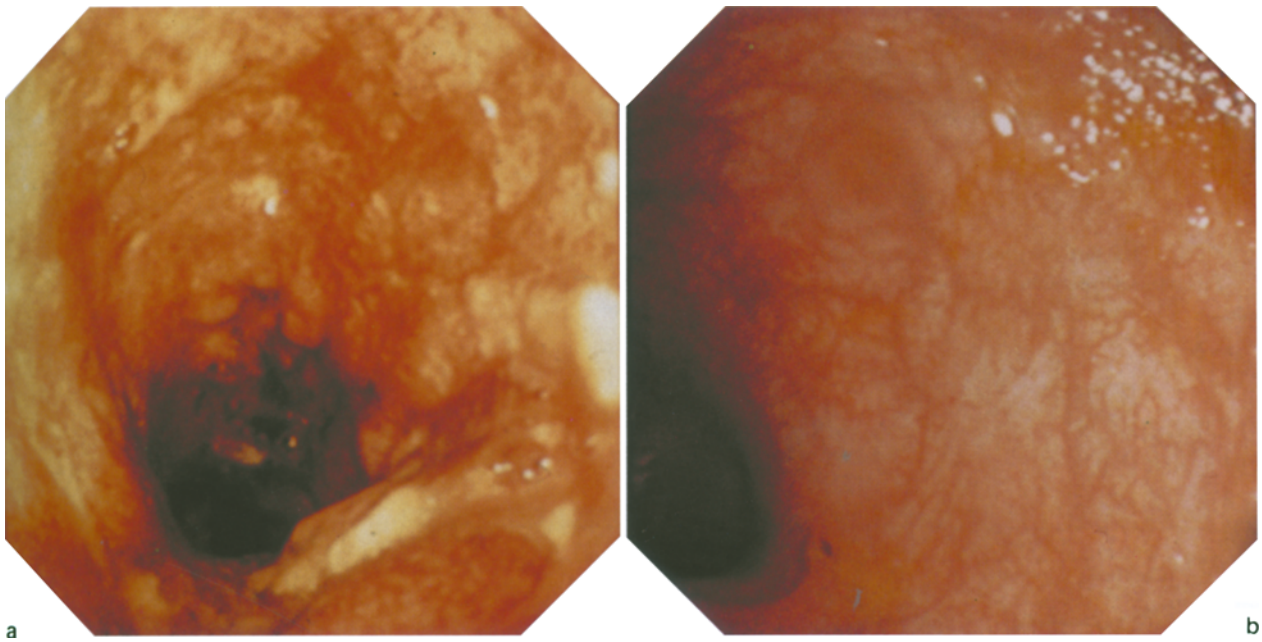

Fig. 2a,b. Endoscopic images of patient no. 1. **a** Before LCAP therapy, showing active-stage ulcerative colitis (*UC*) with very severe multiple ulcerations and erosions with bleeding. **b** After five LCAP sessions of the intensive therapy, showing remission of UC with no observable ulceration or erosion and apparently normal colonic mucosa

multiple erosions and ulcerations and easy contact bleeding on all colonic mucosa (Fig. 2a); the erosions and ulcers had disappeared after the five LCAP sessions of the intensive therapy (Fig. 2b), and the same state of remission was found at the end of the maintenance therapy period. The patient has subsequently continued with LCAP therapy once a month and is not receiving prednisolone or salazosulfapyridine; his

clinical condition and endoscopic findings have shown continued remission for a total of about 18 months (Table 1).

Another two of the five CD patients also showed dramatic improvement. Figure 3 shows the clinical course of one (patient no. 9) of the two patients, a 19-year-old female who had been admitted to our hospital suffering from her first episode of the colonic type of CD, with severe watery diarrhea (seven to nine times/day), abdominal pain, weight loss, high fever, abdominal tenderness, and anemia; an IOIBD score of 6; and a CRP level of 17.5 mg/dl. She was treated by intravenous hyperalimentation during her first 4 days in our hospital, but was enrolled for the LCAP intensive therapy for investigation of its effectiveness in IBD when used alone and without prior treatment by any of the conventional IBD therapies. The episodes of watery diarrhea ended on the day after her first LCAP session (Fig. 3), and these episodes did not recur even after the discontinuation of intravenous hyperalimentation therapy and the initiation of oral feeding. This patient's IOIBD score and CRP level decreased to 2 and 0.9 mg/dl, respectively, just before the second LCAP session, and remained at those levels thereafter. Her WBC count and granulocyte/lymphocyte ratio also decreased rapidly, to near the normal range. Radiological (Fig. 4a,b) and endoscopic examinations (Fig. 5a,b) at the end of the intensive therapy period revealed a greatly improved appearance, with no sign of the widespread multiple ulcers and aphthoid lesions that had been seen before her first LCAP session. The LCAP therapy has been continued subsequent to the maintenance therapy, and her clinical condition and objective findings now show that her remission has continued for a total of more than 16 months with no elemental diet and no treatment with prednisolone or other similar medication.

The results of the flow cytometry were classified according to the patients' clinical and objective (image analysis) responses. No comparison was made between UC and CD patients' flow cytometry findings, both because of the apparent similarity of these findings in both types of IBD and because of the small total number of patients.

As shown in Table 2, the responders (excellent and moderate improvement, $n = 8$) generally showed high initial values for the percentages of $HLADR^+$, $HLADR^+CD3^+$, and $HLADR^+CD8^+$ cells, which values decreased to near the normal range by the end of the intensive and maintenance therapies; together with a decrease in the percentage of $OKT8^+$ cells, an increase in the percentage of $OKT4^+$ cells and the OKT4/OKT8 ratio, and improvement in CRP and ESR. In the non-responders (no change in clinical or objective findings, $n = 5$), in contrast, the initial cell percentages were generally near or within the normal range, their initial CRP and ESR values were lower than those of the responders, and no significant changes were found in any of these values at the end of either the intensive or the maintenance therapy

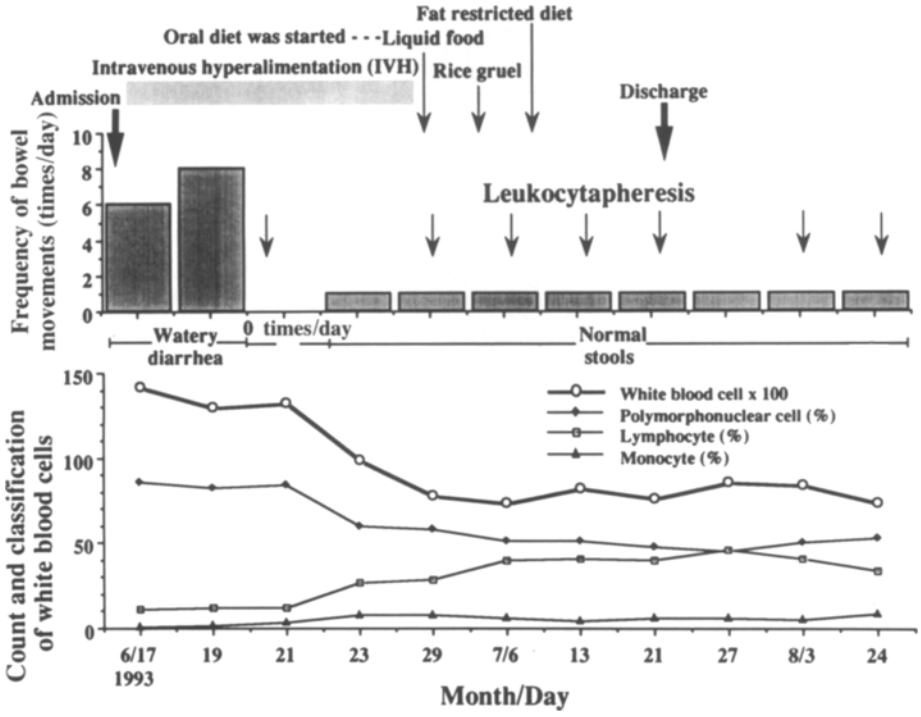

Fig. 3. Clinical course of patient no. 9, with the first episode of severe colonic type Crohn's disease: Watery diarrhea (eight times/day) ceased on the day after the first LCAP session, and no deterioration was noted after the initiation of oral feeding. WBC count and granulocyte/lymphocyte ratio decreased to near normal values

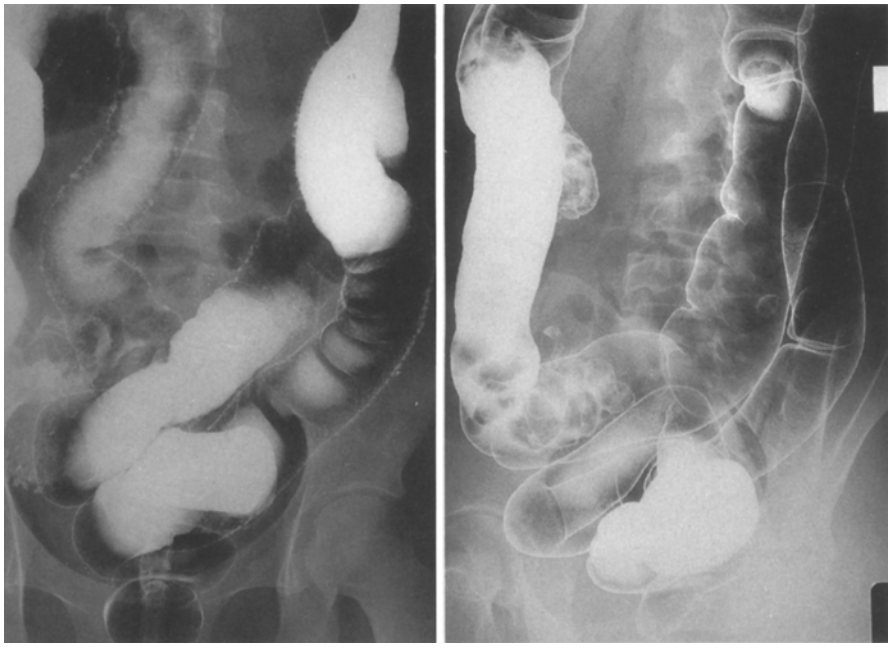

Fig. 4a,b. Barium enema X-ray images of patient no. 9 (colonic type Crohn's disease). **a** Before LCAP therapy, showing multiple spicular lesions, indicating small ulcers, throughout the entire colon. **b** After the five LCAP sessions of the intensive therapy; the small ulcers have disappeared

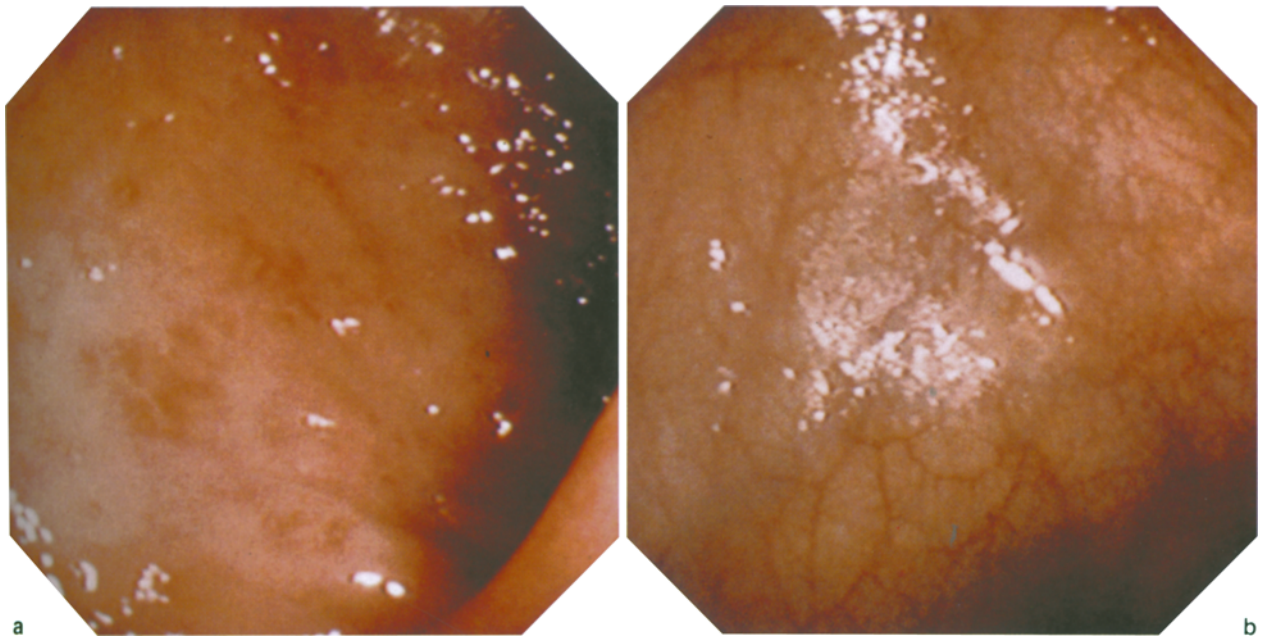

Fig. 5a,b. Endoscopic images of patient no. 9. **a** Before LCAP therapy, showing multiple aphthoid lesions throughout the entire colon. **b** After five LCAP sessions of the intensive therapy; the multiple aphthoid lesions have disappeared

period. No significant changes were found in the percentages of T, B, $OKT3^+$, or $HLADR^+CD4^+$ cells, or in the T/B ratio, in either the responders or the non-responders.

Discussion

The quantity of WBC removed per LCAP session was about the same as that reportedly removed by cen-

Table 2. C-reactive protein (*CRP*), erythrocyte sedimentation rate (*ESR*), and flow cytometry in responders and non-responders

		Responders Excellent on moderate improvement (n = 8)			Non-responders No change (n = 5)		
	Normal range	Pre-intensive	Post-intensive	Post-maintenance	Pre-intensive	Post-intensive	Post-maintenance
CRP (mg/dl)	0–0.3	5.91 ± 5.9*	0.28 ± 0.1**	0.18 ± 0.05**	2.0 ± 1.1*	1.25 ± 0.6	1.2 ± 0.6
ESR (1 h)	<10	48.5 ± 37*	13.5 ± 16.3**	11.3 ± 7.6*,**	13.6 ± 7.1*	12.5 ± 6.2	10.7 ± 10.6*
T cell (%)	66–89	84.2 ± 5.8	83.1 ± 5.1	80 ± 8	82.5 ± 5.1	81.8 ± 6.1	79.6 ± 11
B cell (%)	4–17	13.4 ± 2.4	16.1 ± 5.4	16.4 ± 5.7	15.9 ± 4.4	14.8 ± 4.9	14.6 ± 3.2
T/B cell (%)	4–7	6.4 ± 0.9	5.8 ± 2.4	6.0 ± 2.8	5.7 ± 1.5	5.6 ± 2.5	6.3 ± 1.6
$OKT3^+$ cells (%)	58–84	65.3 ± 12.3	66.8 ± 8.5	64.3 ± 8.2	63.2 ± 9.3	62.2 ± 8.6	64.3 ± 7.6
$OKT4^+$ cells (%)	25–56	37.6 ± 10.5	43.3 ± 5.9	47.4 ± 3.3*,**	42 ± 2.1	42.2 ± 3.6	37.4 ± 3.1*
$OKT8^+$ cells (%)	17–44	41.3 ± 8.1	34.1 ± 7.9**	31.6 ± 4.6**	37 ± 4.4	34.1 ± 4.4	37.9 ± 8.2
OKT4/8 (%)	0.6–2.9	1.0 ± 0.4	1.6 ± 0.4*,**	1.6 ± 0.1*,**	1.1 ± 0.2	1.1 ± 0.2*	1.0 ± 0.3*
$HLADR^+$ cells (%)	8–33	46.3 ± 14.3*	39.1 ± 9.6**	34.1 ± 7.6**	33 ± 12.1*	32.8 ± 12	30.2 ± 10
$HLADR^+CD3^+$ (%)	<8	26.4 ± 3.5*	16.2 ± 3*,**	16.8 ± 3.2*,**	6.8 ± 10.8*	7.2 ± 2.5*	7.3 ± 3.2*
$HLADR^+CD4^+$ (%)	<6	5.3 ± 2.3*	5.1 ± 1.7*	5.0 ± 2.1*	1.4 ± 2.3*	1.5 ± 3.2*	1.5 ± 2*
$HLADR^+CD8^+$ (%)	<10	19.1 ± 5.4*	14.3 ± 3.8*	11 ± 2.5*,**	8.9 ± 3.2*	7.2 ± 2.4*	6.4 ± 3.3*

P value <0.05 * Responders vs non-responders; ** pre vs post -intensive or post-maintenance

trifugation in the treatment of RA.[13] The LCAP therapy had no discernable side effects, and routine laboratory tests showed liver and renal functions to be normal during the period of this therapy. The results indicate that this therapy was effective for the treatment of UC patients with a relatively short history of the disease, regardless of the severity, but that it may be ineffective for those patients with a long history of the disease. The findings indicate, further, that although the LCAP therapy may be ineffective for CD patients with stenotic lesions, it appears to benefit patients who have previously had stenotic lesions removed surgically.

It has been reported that both UC and CD patients exhibit high numbers of active WBC.[14] Our flow cytometry analyses showed that, in those patients who responded to the LCAP therapy, the percentages of activated T cells (e.g., $HLADR^+$, $HLADR^+CD3^+$, $HLADR^+CD8^+$) were initially high but decreased to near the normal range with the therapy, whereas in the non-responders, these percentages were initially near the normal range and remained unchanged by the therapy (Table 2). The results for CRP, ESR, and flow cytometry, and the relatively mild disease severity in the non-responders suggest that inflammation of the intestinal mucosa may have already ceased in these patients prior to the initiation of LCAP therapy.

Excellent or moderate improvement was found in 84.6% and 61.5% of the IBD patients at the end of the intensive and maintenance therapy periods, respectively, and the clinical improvement, obtained as it was in the absence of any additional medical treatment, suggests that LCAP influences the causal mechanism(s) of IBD. Although the precise mechanisms responsible for IBD have yet to be elucidated, our results suggest that the occurrence of IBD is strongly associated with cell-mediated immune responses[15] and that LCAP exerts an immuno-suppressive or immuno-modulating effect in patients with IBD, as well as in those with RA.[16] In conclusion, our present results indicate that LCAP is a safe and effective therapy for patients with IBD and justify the performance of a larger scale controlled study of the efficacy of LCAP for IBD.

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