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Adsorptive granulocyte and monocyte apheresis for refractory Crohn's disease: an open multicenter prospective study

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Background. Active Crohn's disease (CD) is often associated with elevated levels of platelets, granulocytes, and monocytes that are activated and resistant to apoptosis. The level of neutrophils in the intestinal mucosa has been quantitatively related to the severity of intestinal inflammation in CD. We postulated that patients with CD that is refractory to conventional medications might respond to a reduction of granulocytes and monocytes by adsorptive apheresis. ***Methods.*** Twenty-one patients with a CD activity index (CDAI) of 200–399 and unresponsive to standard medication, which included nutritional intervention, received granulocyte and monocyte adsorptive apheresis (GCAP) as an adjunct to their ongoing medication. GCAP was performed with an Adacolumn, which adsorbs granulocytes, monocytes, and a small fraction of lymphocytes (FcγR and complement receptor-bearing leucocytes). Patients received one GCAP session/week for 5 consecutive weeks. CDAI, International Organization for the Study of Inflammatory Bowel Disease (IOIBD), and IBD questionnaire (IBDQ) scores were evaluated. ***Results.*** During the initial conventional/nutritional therapy, no significant improvement was seen in any patient. However, at week 7 of GCAP therapy, significant improvements in CDAI, IOIBD, and IBDQ scores were observed. The CDAI, IOIBD, and IBDQ scores before GCAP were 275.6 ± 54.2, 3.4 ± 1.4, and 152 ± 22, respectively. The corresponding values after GCAP were 214.8 ± 89.2 ($P = 0.0005$), 2.54 ± 1.5 ($P = 0.0224$), and 165 ± 29 ($P = 0.0327$), respectively. ***Conclusions.*** GCAP could be effective for inducing remission and improving quality of life in patients with active CD that is refractory to conventional therapy.

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Introduction

Crohn's disease (CD) is a chronic recurrent inflammatory bowel disorder with variable disease expressions, giving rise to a multitude of complications, including fever, abdominal discomfort, diarrhea, anemia, and weight loss. Although ulcerative colitis is primarily confined to the colon and the rectum, CD can affect any part of the gut, from the mouth to the perianal region; up to 65% of CD patients may have CD affecting the small intestine.[1] Such patients are likely to develop serious nutritional complications, with the need for nutritional support with elemental diets or total parenteral nutrition.[1–5]

Although genetic background may be associated with the onset of CD, and dietary antigens are thought to have an important role in the exacerbation of CD,[6–9] it is true to say that factors which initiate and perpetuate CD are not well characterized at present. However, because a relapse may be triggered by an inflammatory response to dietary antigens in the intestinal wall,[6–9] nutritional therapy is thought to minimize the contribution of the normal diet to disease activity, and to promote remission.[2–5] In line with this assertion, a response rate of up to 77% has been reported following a course of nutritional therapy.[2,5,10,11]

Alternative therapies for patients who do not respond to a salicylate (5-acetyl salicylic acid [ASA] sulfasalazine) plus elemental diets or total parenteral nutrition include high-dose corticosteroids[12] and, more recently, anti-tumor necrosis factor (TNF-α) antibod-

ies.[13] However, both corticosteroids and anti-TNF-α antibody are associated with frequent adverse side effects.[14,15] Indeed, in Japan, most of our patients are reluctant to receive corticosteroid therapy for fear of the steroid-related adverse effects.

A potential target for the treatment of inflammatory bowel disease could be granulocytes and monocytes/macrophages. We thought that patients with active CD who had not responded to standard conventional therapy, including nutrition therapy with an elemental diet (ED) or total parenteral nutrition (TPN), might respond to selective granulocyte and monocyte adsorptive apheresis as an adjunct to their ongoing medication. A major role for these leucocytes in the clinicopathological features of CD (tissue injury and symptoms) is indicated by several lines of evidence.[16–20] First, in both CD and ulcerative colitis, peripheral blood neutrophils and monocytes/macrophages are elevated, showing activation and increased survival time.[16–22] Second, biopsy specimens from CD lesions reveal a high density of neutrophils, macrophages, and other inflammatory leucocytes within the inflamed tissues.[1,23] Third, the level of neutrophils in the intestinal mucosa was quantitatively related to the severity of intestinal inflammation and clinical relapse in both CD and ulcerative colitis.[24,25] This indicates that, during clinical remission, neutrophils infiltrate the intestinal mucosa and have a major role in mucosal inflammation, tissue injury, and CD relapse.[24–26]

The device we used for the apheresis was an Adacolumn.[21] The volume of this apheresis column is 335 ml, and the column is filled with cellulose acetate beads (carriers), 2 mm in diameter, that are bathed in sterile saline. The carriers adsorb granulocytes and monocytes/macrophages that bear FcγR and complement receptors.[21,27] Blood cell counts from the column inflow and outflow points show that the carriers typically adsorb about 65% of granulocytes, 55% of monocytes, and a small fraction of lymphocytes from the blood in the column.[21,28] Preliminary studies in patients with rheumatoid arthritis,[21,29] ulcerative colitis,[21,22,26] and CD[30] showed that granulocyte and monocyte adsorptive apheresis (GCAP) was associated with the alleviation of clinical symptoms and marked reductions in the levels of various inflammatory cytokines which are produced by leucocytes.[21,22,29] These observations indicated that the clinical efficacy of GCAP might not be due to its effect on the level of peripheral blood granulocytes and monocytes per se. The present study was carried out to investigate the efficacy of GCAP in patients with CD who were refractory to conventional medication.

Methods

Study objectives

Our primary objective was to evaluate the efficacy of GCAP as an adjunct to therapy in patients with active CD that was refractory to standard Japanese conventional therapy for active CD (see "Conventional therapy" section below). The efficacy of the GCAP treatment was based on changes in the Crohn's disease activity index (CDAI) during or at the end of the therapy relative to the entry values, without a major focus on endoscopic or radiographic changes. Treatment safety and changes in the International Organization for the Study of Inflammatory Bowel Disease (IOIBD) score, according to de Dombal,[31] and changes in the inflammatory bowel disease (IBD) questionnaire (IBDQ) score were evaluated as our secondary objectives. The IBDQ has been reported to be a reliable indicator of quality of life in patients with IBD.[32]

Patients

The demography of the 21 patients recruited for this study is presented in Table 1. All patients had a previous diagnosis of Crohn's disease (colitis or ileocolitis). To qualify for entry to the study, patients had to have a CDAI score in the range of 200 to 399, and to have been classified as refractory to conventional therapy (including nutritional intervention) if no significant improvement in CDAI was observed (CDAI $>$ 200) during at least 2 weeks of therapy (see below).

Table 1. Demography of 21 patients with refractory Crohn's disease at entry who were recruited for granulocyte and monocyte adsorptive apheresis therapy with the Adacolumn

Demography	Number of patients
Male/Female	14/7
Age (years)	27.4 ± 9.0 (12–57)[a]
Duration of disease (months)	80.1 ± 53.5 (3–180)[a]
Location of lesions	
Ileocolitis	13
Colitis	8
Previous bowel resection	8
Nutritional therapy (30–40 kcal/kg per day)[c]	21
Stenosis/stricture	5
Anal lesions	13
Ongoing medications	
Prednisolone	6 (5–17 mg)[b]
5-ASA/Sulfasalazine	17 (2.25–4.0 g)[b]
Metronidazole	2 (500 mg)[b]
Crohn's disease activity index	275.6 ± 54.2
C-reactive protein (mg/dl)	2.7 ± 4.1 (0.14–17.59)[a]

[a] Values are means ± SD (range)
[b] Dose
[c] Nutritional therapy: elemental diet, 17; total parenteral nutrition, 4

Conventional therapy

In Japan, the Ministry of Health has approved standard guidelines of conventional therapy for active CD (first-line therapy) that comprises an aminosalicylate (5-ASA or sulfasalazine) plus nutritional therapy.[2] This regimen can alleviate the disease in the majority of patients within 2 weeks,[2,10] and those who do not respond are classified as having refractory CD.[30] In this study, nutritional therapy consisted of an elemental diet (ED; n = 17) or total parenteral nutrition (TPN; n = 4). ED treatment was done by using Elental (Ajinomoto, Tokyo, Japan), while TPN (30–40 kcal/kg per day) was done by using products from reputable suppliers in Japan. All products were brands approved by the Ministry of Health. Table 1 shows that 17 of the 21 patients were on aminosalicylates (2.25–4 g/day); 6 of these patients were on prednisolone (5–17 mg/day) as well, and 2 were receiving metronidazole (500 mg/day).

Adsorptive granulocyte and monocyte apheresis

The procedure for GCAP was essentially based on earlier studies in patients with rheumatoid arthritis and ulcerative colitis.[21,22,26,28,29] Briefly, the apheresis column (Adacolumn) and circuit lines were provided by Japan Immunoresearch Laboratories (Takasaki, Japan). The column has a capacity of 335 ml and is filled with cellulose acetate beads, 2 mm in diameter, as the column adsorptive carriers. Differential leucocyte counts have shown that the carriers adsorb granulocytes, monocytes/macrophages, and a small fraction of lymphocytes from the blood in the column.[21,27] This device has been CE marked (validated) by TUV (Notified Body) and approved by the Japan Ministry of Health for the treatment of active ulcerative colitis. The column was placed in an extracorporeal setting, with a perfusion rate of 30 ml/min; the duration of one GCAP treatment session was 60 min.

Study design and assessment of response to therapy

The study design is outlined in Fig. 1. This was a prospective open-label study, carried out at 16 centers in 13 cities throughout Japan. Therefore, with a target patient number of 21, the number of patients per center was very small. Further, centers recruited their own patients, but with strict adherence to the study protocol. During the first 2 weeks, patients were screened, and information about their disease course and drug therapy was compiled. As indicated above, the protocol specified that patients must have been on conventional therapy for at least 2 weeks prior to the initiation of GCAP therapy. Likewise, for patients who were on corticosteroid, 5-ASA or sulfasalazine therapy, the drug

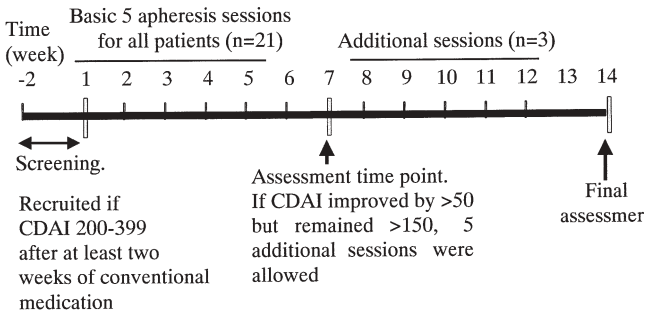

Fig. 1. Study design, patient screening, and treatment and efficacy assessment time points. *CDAI*, Crohn's disease activity index

therapy should have been continued at a stable dose during the previous 2 weeks for the corticosteroid and during the previous 8 weeks for salicylates. No change in conventional therapy was expected during the 5 weeks of GCAP treatment. Each patient was to receive one GCAP session per week for 5 consecutive weeks. Week 7 was the first efficacy assessment time point. Patients in whom the CDAI decreased by more than 50 points, but remained above 150, could have five further GCAP sessions, at the discretion of the attending physician. However, only 3 patients did have an additional GCAP session, and of these, just 1 patient reached week 14, which was the final assessment time point for patients with more than five sessions.

In accord with the principle of intention to treat, if a patient was withdrawn from the study, for whatever reason, assessment was done at that time point, and the data were included in the assessment at week 7. Clinical remission was assumed when the CDAI improved to below 150, while clinical response was defined as a decrease of the CDAI by more than 50 points. Likewise, if the CDAI had increased by more than 50 points, the patient's CD was considered to have worsened; otherwise, the patient's CD was considered unchanged. After the GCAP treatment course, patients who had active disease could continue with their conventional medication.

Exclusion criteria

Because leucocytapheresis is a non-drug therapy, that involves blood flow, its efficacy is likely to be affected by poor or intermittent blood flow. To minimize this possibility, patients who appeared to present difficulties in achieving blood access were excluded. Pregnancy, age less than 12 years or over 76 years, granulocyte count of less than 2000/μl, and hemoglobin less than 8 g/dl were other main exclusion criteria. Likewise, any patient who had an obvious need for surgery was not recruited.

Ethics

Leucocytapheresis with the Adacolumn is an approved therapy in Japan. Nonetheless, the final version of the study protocol was submitted to the Japan Ministry of Health and approval was obtained. Likewise, the institutional review board of each hospital approved the study protocol. Further, all patients provided written informed consent after they were informed of the purpose of the study and the nature of the procedures involved. In under age patients (less than 20 years), consent was obtained from the patient and one of the patient's parents. Patients were told that they could withdraw from the study at any time without jeopardizing their future treatment.

Statistical analysis

Where appropriate, data values are presented as means ± SEM, or as mean ± SD values, and comparisons were made by using the *t*-test, unless indicated otherwise. A significance level of 0.05 was used for all statistical tests, and two-tailed tests were applied when appropriate.

Results

Patient compliance

A total of six patients withdrew before week 7. Of these, one received only two sessions and was withdrawn due to a more than expected drop in the patient's leucocyte count. One patient withdrew after receiving three sessions, and another two patients withdrew after receiving four sessions, due to lack of efficacy. A further two patients withdrew after receiving five sessions. The outcome is presented in Fig. 2.

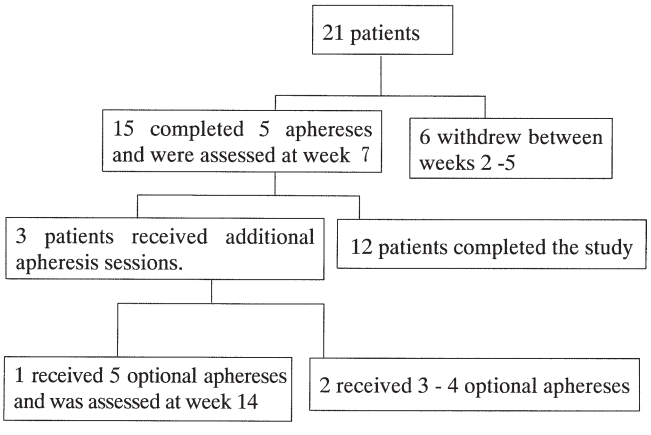

Fig. 2. Flow chart, showing the number of leucocytapheresis sessions dispensed during the 14 weeks of the study (design, shown in *Fig. 1*)

Changes in leucocyte counts during the apheresis procedure

Figure 3 shows changes in leucocyte counts at several time points during the 60-min apheresis therapy and then at 24 h. All counts, except for the values at 24 h, correspond to the column inflow and represent peripheral blood level. The total leucocyte count showed a drop of 49% at 30 min relative to the count at time 0. However, despite apheresis being continued, the inflow count at 60 min had markedly increased, showing only a 9% fall relative to the count at time 0. This reflects an influx of CD10-negative neutrophils from the bone marrow into the peripheral circulation.[24] Looking at the three main leucocyte populations, the inflow counts at the 15-min and 30-min time points relative to the counts at time 0 showed a significant drop for all three leucocyte populations. The fall in the inflow lymphocyte count during apheresis was unexpected, because the column carriers do not significantly adsorb lymphocytes.[21,27,28]

Changes in CDAI, IOIBD, and IBDQ following leucocytapheresis

Figure 4 shows significant improvements in the CDAI, IOIBD, and IBDQ scores after GCAP. The CDAI, IOIBD, and IBDQ scores (mean ± SD) at baseline were 275.6 ± 54.2, 3.4 ± 1.4, and 152 ± 22, respectively. The corresponding values after GCAP therapy were 214.8 ± 89.2 ($P = 0.0005$), 2.4 ± 1.5 ($P = 0.0224$) and 165 ± 29 ($P = 0.0327$), respectively, showing impressive alleviation of symptoms and improvement in quality of life. When we considered only the 11 responders, the

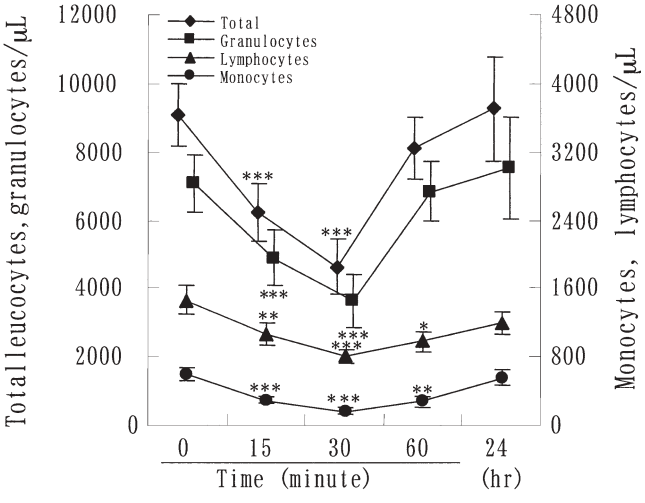

Fig. 3. Changes in peripheral blood leucocytes during and 24 h after Adacolumn leucocytapheresis therapy in 21 patients with refractory Crohn's disease. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, vs time 0

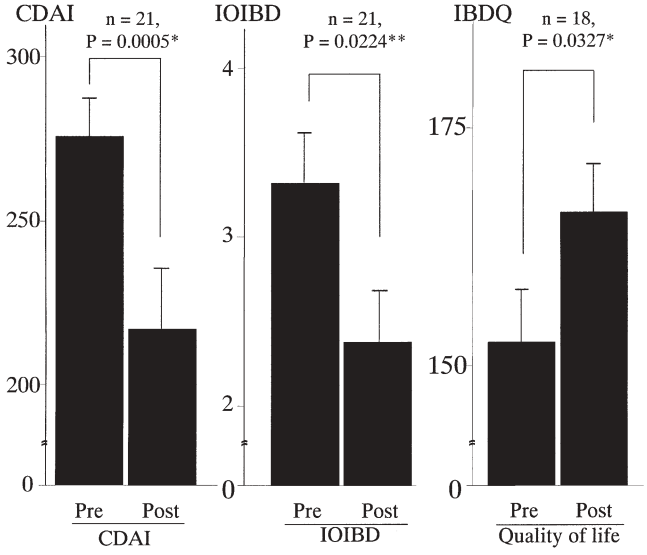

Fig. 4. Changes in the CDAI, International Organization for the Study of Inflammatory Bowel Disease (*IOIBD*), and IBD questionnaire (*IBDQ*) scores (mean ± SEM) during leucocytapheresis (GCAP) therapy in 21 patients with Crohn's disease unresponsive to conventional medications, including nutritional therapy. *By paired *t*-test; **by Wilcoxon signed rank test

above scores were as follows: 267 ± 38 ($P = 0.0001$), 3.6 ± 1.2, and 155 ± 25, and the corresponding values after GCAP therapy were 151 ± 49 ($P = 0.0001$), 1.6 ± 1.0 ($P = 0.002$), and 178 ± 26 ($P = 0.0212$), respectively.

Overall response to therapy

Eleven of the 21 patients (52.4%) responded to the therapy, 6 with clinical remission and 5 with significant improvement. The remaining 10 patients (non-responders) remained unchanged, and according to the protocol definitions, no patient worsened. It should be mentioned that 4 of the non-responders were not on 5-ASA prior to entry or beyond. There was no other significant difference in patient background between responders and non-responders to GCAP. Regarding the sustainability of the response, up to 6 months after the end of GCAP therapy, 2 of the 11 responders were hospitalized for alternative therapy due to the worsening of CD symptoms. One patient showed worsening of CD symptoms within 3 months after the last GCAP therapy, the other within 5 months.

Unexpectedly, there was no change in the mean C-reactive protein (CRP) level at week 7. The CRP value at entry was 2.7 ± 4.1 mg/dl (mean ± SD; range, 0.14–17.6 mg/dl). The values at week 5 and post GCAP were 1.9 ± 2.6 mg/dl (range, 0.11–11.6 mg/dl) and 2.7 ± 2.8 mg/dl (range, 0.33–11.6 mg/dl), respectively. The corresponding values for the 11 responders were 1.9 ± 2.3 mg/dl (range, 0.14–7.44 mg/dl), 0.9 ± 0.6 mg/dl (range, 0.11–2.01 mg/dl), and 1.9 ± 1.4 mg/dl (range, 0.33–4.66 mg/dl), respectively, showing no statistically significant improvement. However, in patients who had anal lesions and in whom the mean entry CRP was 2.7 mg/dl or more the CRP level decreased significantly, from 7.7 ± 5.8 mg/dl to 5.1 ± 4.0 mg/dl ($P = 0.0483$; $n = 5$).

Study safety

During GCAP therapy, a total of ten non-severe side effects in six patients were reported. These were: palpitation ($n = 1$), headache ($n = 3$), congested nostrils ($n = 1$), dizziness ($n = 1$), feeling of weariness in one patient ($n = 2$ occasions), rash on the legs ($n = 1$), and pelvic pain ($n = 1$). However, no patient discontinued GCAP therapy due to these side effects, except for the patient with the pelvic pain, which resolved within 24 h. Further, there was no evidence of opportunistic infection in any patient during or after GCAP therapy.

Discussion

In earlier studies, we found that the majority of patients with active CD responded to conventional medication that included nutritional therapy, without corticosteroids,[2,10] but a minority of them remained refractory.[30] The present study was initiated in June 2001 and was completed in April 2003. The major time-consuming factor was the difficulty in finding patients who did not readily respond to conventional therapy (a salicylate plus nutritional therapy) and who could therefore be classified as refractory and meet our protocol inclusion criteria. Given that all 21 patients in the present study were found to be unresponsive to conventional medications, GCAP was considered to be a rescue therapy for these patients.

In Japan, the initial first-line medication for active CD is 5-ASA or sulfasalazine, together with nutritional therapy with elemental diets (EDs) or TPN.[2,5,10,11,33] Nutritional therapy, in addition to providing energy, protein, and essential nutrients, has two other major benefits: (a) it ameliorates the diarrhea and abdominal discomfort associated with a normal diet; and (b) it appears to minimize intestinal inflammation provoked by dietary antigens.[6–9] Based on the experience in Japan, nutritional therapy can induce remission in up to 77% of patients during 1 month of therapy.[11] Nonetheless, it may be argued that, although all of the 21 patients we recruited were refractory to nutritional therapy, some of them might have responded to corticosteroids. While this argument should not be denied, most of the 15 patients who were not on corticosteroids

at entry to GCAP therapy had received corticosteroid treatment at some stage in the past and either had become refractory to steroids or were reluctant to receive steroids.

The most significant outcome of the leucocyte reduction therapy in this study was the marked improvement in CDAI, IOIBD, and IBDQ scores, with an overall response rate of 52.4%, in patients who had failed to respond to conventional therapy. The therapy was well tolerated and no severe adverse effects were observed. This is similar to the experience during GCAP therapy in patients with rheumatoid arthritis,[21,29] and those with ulcerative colitis,[26] in trials that reported adverse events such as mild headache or light-headedness in a small number of patients.[26] The safety, tolerability, and efficacy of leucocytapheresis with the Adacolumn suggest that, in contrast to steroids, with this therapy, unpleasant side effects are unlikely. Further, the outcome of this study should stimulate further initiatives to directly target granulocytes and monocytes/macrophages in the therapy of CD. However, a major factor that could strengthen the impact of our results would be the inclusion of a control group, which was missing in this study. With this in mind, we believe that a future study, using a larger cohort of patients together with the inclusion of a control group, could enhance our understanding of the full efficacy of adsorptive granulocyte and monocyte apheresis therapy in CD.

Although the column carriers adsorbed a large fraction of granulocytes and monocytes from the blood in the column, the number of these leucocytes in the patients' systemic circulation did not fall below the normal range under the conditions used in this study. Instead, flow cytometry indicated a net influx of immature neutrophils (CD10-negative neutrophils) into the circulation from marginal pools, including the bone marrow, and these cells would be less inflammatory than those removed.[24] Furthermore, in patients with rheumatoid arthritis and those with ulcerative colitis, after adsorptive leucocytapheresis therapy, the production of proinflammatory cytokines (TNF-α, interleukin [IL]-1β, IL-6, and IL-8) by peripheral blood leucocytes was markedly suppressed, together with the downmodulation of L selectin, which has a key role in the initiation of leucocyte extravasation.[21,22,29,34] Hence, the overall effect of granulocyte and monocyte reduction therapy should be reduced levels of activated leucocytes and inflammatory cytokines, and diminished leucocyte infiltration of the mucosa.

In conclusion, it appears that the reduction of monocytes/macrophages and neutrophils is associated with improvements and remission in some patients with active refractory CD. Alternatively, it could be said that granulocytes and monocytes have an active role in mucosal inflammation and the exacerbation of CD. Granulocyte and monocyte adsorptive apheresis should serve as a non-pharmacological adjunct to therapy in CD when conventional medications fail.

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