Cytokine Profiling Demonstrated Unaltered Serum Cytokine Levels in a Cytomegalovirus-infected Patient Treated by Leukocytapheresis

Y KASHIWAGI, S GO, S NISHIMATA, H KAWASHIMA

Abstract
Leukocytapheresis (LCAP) therapy was administered to 2 patients with steroid-resistant ulcerative colitis (UC). In one patient, serum levels of cytokines decreased and clinical improvement was observed after each LCAP therapy session. In the other patient who was infected with cytomegalovirus (CMV), serum levels of cytokines did not decrease progressively and minimal clinical improvement was observed. CMV infection is considered to be an important exacerbating factor in patients with UC. Therefore, unaltered cytokine levels in patients receiving LCAP therapy may be associated with refractory UC.

Key words
Cytokine; Cytomegalovirus; Leukocytapheresis; Ulcerative colitis

Introduction
Leukocytapheresis (LCAP) has recently been developed in Japan as a possible treatment for active ulcerative colitis (UC). Its aim is to correct imbalances in immunological mechanisms by removing activated lymphocytes and monocytes/macrophages from the peripheral blood.1 The Japanese study group for pediatric UC demonstrated that LCAP was as effective as that in adults.2 However, the types of pediatric patients who are best suited for LCAP therapy and the specific LCAP methods for efficient treatment have not been determined for pediatric UC. Here we report 2 steroid-resistant UC patients who received LCAP therapy. Because fluctuations in cytokine levels during LCAP remain poorly understood, we measured circulating cytokine levels in these patients before and after LCAP therapy after we took the informed consent from the parents and assent from the children. We used Pediatric Ulcerative Colitis Activity Index (PUCAI), remission was defined below 10 scores. The immunologic testing in both cases was done in the same laboratory.

Case 1
The patient was a 14-year-old adolescent boy who presented with a 5-month history of approximately 10 haematochezia episodes per day and abdominal pain. He was diagnosed as having left-sided UC by colonoscopy. He was treated with intravenous predonisolone and mesalazine, which resulted in minimal improvement, however, PUCAI was more than 10 scores. Other infections (bacterial culture, Helicobacter pylori, Cytomegalovirus (CMV), Epstein-Barr virus, et al) were all negative. This was regarded as a steroid-resistant case. LCAP therapy was chosen as the next line of treatment after informed parental consent.

Ten sessions of LCAP was performed (3 sessions per week for 4 consecutive weeks) without complications. Serum samples were collected on the first, fifth, and tenth sessions, both before LCAP and after LCAP (at 0, 2, 24, and 48 hours after the first session, and at 0, 2, 6, 24, and 48 hours after the fifth and last sessions). The levels of 4
cytokines (interleukin [IL]-6, 7, 8, and tumour necrosis factor-α [TNF-α]) were measured using a Bio-Plex suspension array (Bio-Rad Laboratories, Tokyo, Japan). After the fifth LCAP therapy session, the patient’s abdominal pain gradually resolved and the frequency of haematochezia decreased. PUCAI was <10 scores.

Case 2

The patient was a 10-year-old girl who presented with a 1-week history of approximately 10 haematochezia episodes per day, fever, and abdominal pain. She was diagnosed as having pancolitis-type UC by colonoscopy. CMV pp-65 antigen was detected in the peripheral blood, and CMV was detected in colon specimens using monoclonal antibodies. She was treated with antiviral therapy. After her peripheral blood level of CMV pp-65 antigen became undetectable, she was treated with intravenous prednisolone and mesalazine. However, haematochezia, fever, and abdominal pain continued, and hence this was regarded as a steroid-resistant case. We tested other infections (bacterial culture, Helicobacter pylori, Epstein-Barr virus, et al.), results were all negative. LCAP therapy was chosen as the next line of treatment after informed parental consent.

Eleven sessions of LCAP was performed (3 sessions per week for 4 consecutive weeks) without complications. Serum samples were collected on the first, fifth, and eleventh sessions, both before LCAP and after LCAP (at after 0, 2, 6, 24, and 48 hours). The levels of 4 cytokines were measured using a Bio-Plex suspension array (same as in case 1). After LCAP therapy, the patient’s abdominal pain resolved and the frequency of haematochezia decreased temporarily. However, the patient’s abdominal pain and the frequency of haematochezia subsequently increased again, the patient did not reach the remission. The CMV pp-65 antigen and CMV were again detected in the peripheral blood and colon specimens, respectively. After another round of antiviral therapy, the patient’s abdominal pain resolved and the frequency of haematochezia decreased, the patient reached the remission.

IL-6 levels decreased gradually whenever LCAP was performed in both patients (Figure 1A-1 and 1A-2). In case 1, IL-7 (Figure 1A-3), IL-8 (Figure 1B-1), and TNF-α (Figure 1B-3) decreased gradually whenever LCAP was performed; however, the levels of IL-7 and IL-8 were unaltered in case 2 infected with CMV (Figure 1A-4 and 1B-2). The levels of TNF-α in case 2 were low overall (Figure 1B-4).

CMV infection is considered to be an important exacerbating factor in patients with UC. However, virological criteria for diagnosing CMV infection are not standardised. PP-65 antigenemia assay and histological examination can be negative even if the CMV DNA load is high at the tissue level. Yoshino et al reported the usefulness of a mucosal PCR methods. In our case, CMV infection was suspected to be latent and reactivation.

Circulating CMV-infected monocytes migrate into organ tissues, where they differentiate into resident macrophages and dendritic cells. Smith et al demonstrated that CMV infection of macrophages significantly enhanced TNF-α and IL-8 gene expression and protein production in response to toll-like receptor 4 and 5 stimulation with lipopolysaccharide. In case 2 who was infected with CMV, IL-8 did not progressively decrease. It is suspected that IL-8 might be produced from the CMV-infected macrophages that differentiated into organ tissues though activated lymphocytes and monocytes/macrophages from peripheral blood were removed by LCAP therapy.

Nemoto et al demonstrated that IL-7 acted to sustain colitis by forming a niche for colitogenic CD4 memory cells. In case 2 who was infected with CMV, the levels of IL-7 were not decreased by LCAP therapy. IL-7 may therefore become a treatment target as a factor contributing to prolonged inflammation.

CMV is frequently detected in severe cases of UC. However its clinical significance is still controversial, and how and when to start antiviral therapy for UC patients with concomitant CMV infection are not determined. Fukuchi et al demonstrated that the clinical remission rates following apheresis did not differ between UC patients positive and negative for CMV. In our report, unaltered levels of cytokines in patients undergoing LCAP therapy may be associated with refractory UC (case 2), however, this is based on only 1 UC patient positive for CMV. Considering this issue, it is very hard to draw any conclusion. Large-scale studies including multivariate statistical analysis are needed to prove that CMV is a causative agent and establish therapy in UC patients with concomitant CMV infection.

Declaration of Interest

All the authors report no conflicts of interest.
Figure 1A  Levels of IL-6 and IL-7 after the first, fifth, and last LCAP sessions. (1) IL-6 levels in patient 1. (2) IL-6 levels in patient 2 (CMV+). (3) IL-7 levels in patient 1. (4) IL-7 levels in patient 2 (CMV+).

Figure 1B  Levels of IL-8 and TNF-α after the first, fifth, and last LCAP sessions. (1) IL-8 levels in patient 1. (2) IL-8 levels in patient 2 (CMV+). (3) TNF-α levels in patient 1. (4) TNF-α levels in patient 2 (CMV+).
References


