

## Cytapheresis as a Non-Pharmacological Therapy for Inflammatory Bowel Disease

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### Key Words

Inflammatory bowel disease · Ulcerative colitis · Apheresis · Leukocytapheresis · Granulocytapheresis

### Summary

Although inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is a chronic recurrent disease with unknown etiology. Recent immunological studies suggest close relation to autoimmune status featured by antibodies against colonic epithelial cells. For patients with IBD, 5-aminosalicylates are often used in case of mild disease, and corticosteroids are standard therapy for moderate-to-severe disease. However, we often encounter patients who are resistant to or dependent of conventional therapy, which are likely to lead to future problems in quality of life due to adverse effects of drugs used, especially corticosteroids. Extracorporeal leukocyte removal therapy (cytapheresis) is one of the adjunctive therapies for IBD patients refractory to steroids. By removing circulating activated leukocytes, especially granulocytes and lymphocytes, impaired immune response is suppressed. In the present article recently published studies are reviewed in order to reflect the current state of the art in the use of cytapheresis for treating IBD, especially UC and CD. Although there are only few randomized controlled trials, clinical experience so far suggests that cytapheresis has superior efficiency than conventional therapies in steroid-resistant moderate-to-severe UC. Moreover, cytapheresis features its safety characteristic compared with other conventional medications for severe UC, cytapheresis is regarded as safe treatment regimen.

### Schlüsselwörter

Inflammatorische Darmerkrankung · Colitis ulcerosa · Apherese · Leukozytapherese · Granulozytapherese

### Zusammenfassung

Entzündliche Darmerkrankungen wie Colitis ulcerosa und Morbus Crohn sind chronische wiederkehrende Erkrankungen mit unklarer Ätiologie. Neue immunologische Untersuchungen lassen einen durch Antikörper gegen Kolonepithelzellen vermittelten Autoimmunstatus vermuten. Patienten mit leichter inflammatorischer Darmerkrankung werden häufig mit Aminosalicylaten behandelt; Kortikoide sind die Standardtherapie bei mittlerer bis schwerer Erkrankung. Non-Responder dieser Therapieformen sind erheblich in ihrer Lebensqualität eingeschränkt. Die extrakorporale Leukozytenbehandlung durch Zytapherese ist eine Therapieoption für Patienten mit inflammatorischer Darmerkrankung, die steroidrefraktär sind. Durch Zytapherese werden zirkulierende aktivierte Leukozyten, insbesondere Granulozyten und Lymphozyten, entfernt, was mit einer Unterdrückung der Immunantwort einhergeht. Die vorliegende Übersichtsarbeit beschreibt die neuesten Studien zum aktuellen Status der therapeutischen Apherese bei Patienten mit inflammatorischer Darmerkrankung, insbesondere Colitis ulcerosa und Morbus Crohn. Obwohl die Anzahl der randomisierten kontrollierten Studien gering ist, zeigt die bisherige klinische Erfahrung, dass die therapeutische Zytapherese als nichtpharmakologische, immunmodulative Therapie bei steroidrefraktärer mittlerer bis schwerer Colitis ulcerosa effektiv ist. Im Vergleich zur konventionellen Pharmakotherapie bei schwerer Colitis ulcerosa stellt die Zytapherese ein sicheres Behandlungsregime dar.

## Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are chronic intestinal inflammatory diseases of yet unknown origin, which may lead to disabled quality of life (QOL) through long-lasting symptoms such as diarrhea, bloody stool, and abdominal pain [1]. They are subsumed by the term idiopathic inflammatory bowel disease (IBD). In Japan, the number of patients with UC and CD has been increased by a factor of 10 since the 1980s. The etiology of IBD however is unclear. An autoimmune disturbance is thought to play an important role in this incurable disease.

Currently, systemic administration of corticosteroids is the gold standard in the therapy of moderately-to-severe UC, but is likely to cause dose-dependent adverse effects such as moon face, infections, diabetic disease, and osteoporosis. In such steroid-resistant or steroid-dependent patients, immunomodulators such as cyclosporine, tacrolimus, 6-MP, or azathiopurine have been widely used.

Cytapheresis therapy which removes leukocytes from peripheral blood has first been applied in the treatment of UC in 1995 [2]. The primary aim of cytapheresis is to suppress and reduce impaired immune responses in the diseased intestine by removing circulating activated leukocytes, especially granulocytes, which have been shown to cause intestinal crypt abscess. Currently available cytapheresis techniques for active IBD patients are filtration leukocytapheresis (LCA), adsorption granulocyte/monocyte apheresis (GMA), and centrifugal lymphocytapheresis (CLA). GMA and LCA have been approved by the Japanese national health insurance policy for treating active UC since 2000 and 2001, respectively, and have been widely used as non-pharmacological and non-surgical therapeutic option for intractable UC patients. On the other hand, cytapheresis, although GMA is in the final stage for getting the government approval, has never been approved for CD in Japan.

There are only a few data addressing which patients are more likely to respond to cytapheresis therapy and on which physiological mechanism the curative effect of cytapheresis in IBD is based.

### **Cytapheresis Techniques Currently Available for Patients with Active Inflammatory Bowel Disease**

If cytapheresis techniques were applied in steroid-resistant and/or steroid-dependent patients with active UC, mainly LCA and GMA, but only rarely CLA, are used. In the following, the standard procedures of these techniques are summarized.

### *Filtration Leukocytapheresis*

LCA is carried out using a CellSORBA EX™ (Asahi Kasei Kuraray Medical Co., Ltd., Tokyo, Japan) column which is filled with polyester unwoven filter. This polyester unwoven leukocyte removal filter installed in polycarbonate outer shell is designed to remove almost 100% of granulocytes and monocytes and 64% of lymphocytes [3]. Furthermore, approximately 35% of platelets can be trapped to the filter from processed peripheral blood [3]. LCA is usually performed weekly using a CellSORBA EX set in a simple one-way hemofiltration circuit [2–4]. A roller pump drained peripheral blood of the patient from an antecubital vein under constant flow rate of 30–50 ml/min. The anticoagulant nafamostat mesilate (Futhan®; Torii Pharmacol., Co, Ltd., Tokyo, Japan) or heparin was mixed with saline and was added to the patients' drained peripheral whole blood as anticoagulant before infusion into the column. Although use of nafamostat mesilate is sometimes associated with allergic adverse effects, the half-life of the drug is too short to affect bleeding accompanying ulcerative lesions in UC. Anti-nafamostat mesilate IgE was present in 12% of the symptomatic UC patients whose adverse effects were highly suspected to be caused by nafamostat [5]. However, in another 43% of patients anti-nafamostat mesilate IgE could not be detected in spite of the fact that their adverse effects were also highly suspected to be induced by nafamostat. If the patient is in severely active condition, two sessions are allowed in the first week of the treatment followed by 4 consecutive weekly sessions.

### *Adsorption Granulocyte/Monocyte Apheresis*

GMA is carried out using the Adacolumn (JIMRO Co. Ltd., Takasaki, Japan) which is filled with cellulose acetate beads. The beads are designed to adsorb about 65% of granulocytes, 55% monocytes/macrophages and a smaller fraction of lymphocytes from peripheral blood. One of the mechanisms which adsorb leukocytes to the beads is through so-called Fc-R and complement receptors [6–8]. The circuit diagram for GMA is almost the same as that of LCA. The duration of one GMA session is 60 min, at 30 ml/min with optimal amount of nafamostat mesilate or heparin as an anticoagulant. As GMA does not remove platelets, this method is preferably used in patients with moderate-to-severe bleeding.

### *Centrifugal Lymphocytapheresis*

CLA is performed using a centrifugal cell-separator (Component Correction System: CCS, Haemonetics Japan, Tokyo, Japan). Peripheral whole blood drained from patient is collected into a polycarbonate disposable bowl. CCS gives the 125 ml bowl spin to generate centrifugal force, and the refined

lymphocyte-rich layer (buffy coat) is then removed selectively. After the lymphocyte removal, the separated blood is re-transfused to the patient via the same catheter. CLA processes approximately 2,400 ml whole blood. In LCA for patients with UC, the relatively heavy layer of the leukocytes is removed to increase efficacy.

### Cytapheresis for Patients with Ulcerative Colitis

LCA and GMA have been widely used in Japan as an effective therapeutic option for patients with active UC; however, our current level of knowledge for this unique therapy is still fragmentary and based on empiric recommendations.

#### *LCA for UC*

Although the mechanisms underlying the therapeutic efficacy of LCA treatment have not been fully elucidated, immune modulations such as cytokine production and immune regulation induced during LCA were reported previously. It was demonstrated that LCA enhances the ability of the peripheral lymphocytes to produce the anti-inflammatory cytokine IL-4 [12]. Furthermore, LCA was shown to decrease IL-6 release (a pro-inflammatory cytokine) from peripheral blood lymphocytes, accompanying a concomitant increase of IL-10 production. These modifications in inflammatory and anti-inflammatory cytokine production may induce inhibition of IL-1 at the protein and mRNA expression level during the cytapheresis procedure [13].

On the other hand, approximately 35% of peripheral blood platelets adhere onto the surface of polyester filter of Cellsorb and thus are removed by LCA [4]. Recently, it has been reported that circulating platelets are important cells not only in hemostasis, but also in a variety of inflammatory responses [14]. An increase of peripheral platelet count has often been recognized as a common feature during chronically active IBD [15]. As reported previously, the high platelet number correlates well with disease severity [16]. We therefore hypothesized that the significant platelet removal achieved during LCA might play an active role in down-regulating severe immunological reactions in UC patients with an acute flare. We have proven that the clinical efficacy of LCA in severe UC patients can be predicted by the reduction of activated platelets achieved during the first LCA session [17].

Sawada et al. [3] conducted a randomized multicenter trial on the effects of LCA in patients with active UC. UC patients who were resistant to conventional steroid therapy with at least 30 mg/day were randomized to either receive LCA (adding LCA without increasing the corticosteroid dose) or high-dose steroid therapy (h-PSL; increasing the corticosteroid dose to 60 mg/day). LCA showed a significantly higher efficacy compared with conventional h-PSL (74.1 vs. 31.8%;

$p < 0.05$ ). However, no significant difference in clinical efficiency between LCA and h-PSL was found in steroid-naïve UC patients. The major advantage of LCA was the better safety profile, showing no serious adverse side effect, while a substantial number of patients in the h-PSL group suffered from severe adverse effects such as infection.

#### *GMA for UC*

Several clinical studies [18–22] showed remission induction by GMA in patients with UC. As stated above, GMA utilizes cellulose acetate beads, which adhere granulocytes through Fc receptors. Cellular contact to the beads may exert other immunological effects to other types of cells such as lymphocytes. We have demonstrated that at the first session of GMA, the proportion of regulatory T cells (CD25<sup>high</sup> CD4<sup>+</sup> T cells) in the peripheral blood of patients with UC increases; these cells may suppress impaired immune responses in UC. In patients with active IBD, peripheral blood granulocytes and monocytes/macrophages are elevated and show activation behavior and increased survival time [23–28]. As these leukocytes are the major sources of inflammatory cytokines [29, 30], they may contribute to the exacerbation and perpetuation of IBD [31, 32]. Furthermore, the level of neutrophil infiltration into the mucosal tissue in patients with active IBD is related to the severity of intestinal inflammation and clinical relapse [33–35].

We showed that peripheral regulatory T cell expression, which is suppressed in active UC, was significantly increased after a single GMA session [36]. Impaired activity and/or proportion of regulatory T cells results in over-activation of immune responses, including polyclonal antibody production, and leads to autoimmunity-mediated tissue destruction. The increase in CD25<sup>high</sup> CD4<sup>+</sup> regulatory T cells after GMA should contribute to improved immune function of the patient. This rise could reflect depletion of non-CD25<sup>high</sup> CD4<sup>+</sup> T cells. Likewise, several other investigators reported favorable immunological observations associated with GMA [37, 38]. Andoh et al. [38] reported a significant decrease of IL-1 $\beta$ - and TNF- $\alpha$ -induced IL-8 and IL-6 release from peripheral leukocytes following GMA. In conclusion, GMA may correct a part of impaired immune responses through regulatory T cells and cytokine production from lymphocytes in the peripheral blood. The mechanisms how the changes in peripheral blood immune cells cause changes in intestinal immune cells should be further examined. A multicenter trial of GMA for active UC patients [39] showed that GMA had a significantly higher efficiency for relapsing UC patients than conventional h-PSL therapy (GMA vs. h-PSL = 54.8 vs. 39.5%;  $p < 0.05$ ), and GMA had a significantly lower ratio of adverse events compared with h-PSL (GMA vs. h-PSL = 89.9 vs. 58.9%;  $p < 0.001$ ).

## Cytapheresis for Patients with Crohn's Disease

In order to learn more about the efficacy of cytaphe-  
resis in patients with active CD, we have aimed to conduct a multi-  
center open label study on GMA in Japan for patients with ac-  
tive CD refractory to more than 1,200 kcal/day enteral nutri-  
tion with elemental diet [9]. Prior to this multicenter study, we  
originally checked the efficacy of cytaphe-  
resis in active CD in a preliminary clinical trial [10]. According to these two studies,  
GMA showed preferable effects in patients with active CD  
with colonic disease as a major involvement. As shown by  
Fukuda et al. [7], significant improvements in CDAI, IOIBD,  
and IBDQ scores were observed at week 7 of GMA therapy.  
Before CMA, the mean values of the CDAI, IOIBD and  
IBDQ score were 275.6, 3.4, and 152, respectively. The corre-  
sponding values after GMA were 214.8 ( $p = 0.0005$ ), 2.54  
( $p = 0.0224$ ), and 165 ( $p = 0.0327$ ). Currently, GMA for CD is  
considered as an option of medical therapy, and it is on the  
final stage for getting the government approval. Although  
LCA has been reported to have superior efficiency in patients  
with active CD [11], there is no sufficient data eligible for get-  
ting approval from the government.

## Cytapheresis for Inflammatory Bowel Disease in the USA

In the USA, S.B. Hanauer (The University of Chicago) and  
L.F. Mayer (Mount Sinai School of Medicine) have been con-  
ducted a pilot study of LCA for patients with active UC re-  
fractory to steroid and 5-aminosalicylic acid (5-ASA). However,  
their treatment schedule and column were different from  
those of the Japanese trial. Their regimen was designed to per-  
form LCA twice a week for 3 weeks (in total 6 sessions), and  
the column (Cellsorba FX) used in the study was modified to  
be usable under acid-citrate dextrose sodium (ACD) instead  
of nafamostat mesilate or heparin as an anticoagulant. In total,  
12 steroid-refractory and 24 5-ASA-refractory UC patients  
have been set as their goal for this pilot study.

In the USA, GMA has also been evaluated for patients with  
moderate-to-severe active UC in a multicenter double blind,  
randomized, sham-controlled trial. This trial has been also  
conducted by Hanauer et al. and scheduled to perform GMA  
twice a week for 3 weeks as in the US trial for LCA. The num-  
ber of the patients enrolled currently is 168 cases (September  
2006).

## Cytapheresis for Inflammatory Bowel Disease in Europe

LCA got a CE mark and has been approved as medical device  
in Europe. The column used in Europe is Cellsorba FX which  
is modified to use with ACD as in the USA. A clinical trial has  
been started for active UC refractory to conventional medica-

tion therapy in Germany. I. Emmrich (University of Rostock)  
has been conducted the trial which consisted of two phases. In  
the first phase, patients have been enrolled to 5 weekly GMA  
series. The responder, who could be induced to their clinical  
remission after the first phase, has been enrolled into the sec-  
ond phase, a randomized controlled trial. Patients enrolled to  
the second phase have been randomly divided into two groups  
of either continuing 5 monthly GMA sessions or receiving  
conventional medication alone. The number of the patients  
enrolled currently is total 20 (September 2006). Moreover, in  
Israel, Italy, Spain, Sweden, and the UK other clinical trials  
have been carried out.

A multicenter randomized, double blind, sham-controlled trial  
of GMA for patients with acute UC has been carried out in  
EU countries of Austria, Belgium, Denmark, France, Ger-  
many, Italy, Norway, Spain, Sweden, and the UK. The protocol  
in this European trial has been designed to be same as in the  
US trial mentioned above. The total number patients enrolled  
currently is 51 (September 2006).

## Future Development in Cytapheresis for the Management of Inflammatory Bowel Disease

Factors which may influence clinical effectiveness of LCA and  
GMA include blood flow speed ( $Q_b$ ), proceeding time, and  
proceeding frequency ( $Q_f$ ). Basically, slower  $Q_b$  increase the  
leukocyte removal rate of the column. However, with Cellsor-  
ba, the column used in LCA, coagulation problems in the col-  
umn may arise at  $Q_b$  lower than 20 ml/min since the platelets  
in the column cause foamy thromboses. On the other hand,  
Adacolumn, the column used in GMA, adsorbs granulocytes,  
monocytes/macrophages and a smaller fraction of lympho-  
cytes from patient's peripheral whole blood on cellulose ac-  
etate beads filled with the device. As peripheral blood leuko-  
cytes bear a Fc  $\gamma$  R and complement receptors [8, 40] and the  
column is not likely to adhere platelets, GMA is suitable for  
processing under slow  $Q_b$  conditions. We, then, focused on  
platelet removal performances of LCA and GMA as a possi-  
ble factor to understand their therapeutic mechanisms. GMA  
may be better if the patient is in severe inflammatory condi-  
tion and/or dehydrated status because of blood hyperviscosity.  
GMA is also recommended for patients bearing high risks  
such as younger age, older age, and small body weight. Con-  
versely, LCA can be primarily used for patient with severe in-  
flammatory condition of the colonic mucosa, such as grade 4  
in the Matts' classification [41] which is often seen in in-  
tractable patients. We hypothesized that the platelet reduction  
achieved during LCA might amplify its therapeutic efficiency  
for UC patients with severe mucosal damage by restoring mi-  
croblood circulation in the colonic mucosa.

The clinical response to cytaphe-  
resis (clinical and endoscopic  
indices) is usually seen at week 5. During this period, there  
could be serious deteriorations together with debilitating im-



pact on the QOL for patients who become non-responder. Since established evidence suggest that both functional suppression of the circulating leukocytes and the quantitative removal of activated leukocytes contribute to the efficacy of this non-pharmacological therapy, it has been hypothesized that there might be an inverse proportion between Qf and immunological effect of cytapheresis. A recent unpublished randomized controlled study comparing intensive and once a week regimen showed that intensive treatment, which consists of two aphereses in a week for 5 consecutive weeks, results in higher remission rates according to both clinical and endoscopic criteria and shorter duration before getting clinical response.

Although, for steroid-resistant severe UC, intravenous infusion therapy of cyclosporine has been widely accepted as an

adjunct therapy [42], a large proportion of patients may have surgery in future years. Clinical experience so far suggests that cytapheresis has superior efficiency as a non-pharmacological immunomodulative therapy for steroid-resistant UC patients before colectomy [1–4].

## Conclusion

Cytapheresis is an effective non-pharmacological therapy for steroid-resistant moderate-to-severe UC. We have to clarify which patients are good candidates for cytapheresis and what are the mechanisms of action in UC. Clinical efficacy and indication based on disease activity for patients with steroid-naïve UC should be determined to improve QOL of the patients.

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