Peripheral Blood CD64 Levels Decrease in Crohn’s Disease following Granulocyte and Monocyte Adsorptive Apheresis

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Key Words
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Abstract
Granulocyte and monocyte adsorptive apheresis (GMA) is reportedly useful as induction therapy for Crohn’s disease (CD). However, the effects of GMA on CD64 have not been well characterized. We report here our assessment of CD64 expression on neutrophils before and after treatment with GMA in two patients with CD. The severity of CD was assessed with the CD activity index (CDAI). The duration of each GMA session was 60 min at a flow rate of 30 ml/min as per protocol. CD64 expression on neutrophils was measured by analyzing whole blood with a FACScan flow cytometer. In case 1, CD64 levels after each session of GMA tended to decrease compared to pretreatment levels, whereas in case 2, CD64 levels dropped significantly after treatment. The CDAI decreased after GMA in both cases 1 and 2. A significant correlation was noted between CDAI scores and CD64 levels in both cases. In conclusion, GMA reduced blood CD64 levels, which would be an important factor for the decrease of CDAI scores.
Introduction

Crohn’s disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract that is characterized by recurrent inflammation at any location along its length [1]. Despite the advent of immunomodulatory therapies such as azathioprine and 6-mercaptopurine, approximately 75% of CD patients require intestinal resection for complications related to stricturing or penetrating disease [2]. CD is associated with a Th1 immune response in which Th1 cells secrete tumor necrosis factor α (TNF-α) [3]. TNF-α, a proinflammatory cytokine that plays an important role in CD pathogenesis, can be upregulated by interleukin 1 (IL-1) and granulocyte macrophage colony-stimulating factor [4]. Infliximab (IFX) treatment has been shown to significantly reduce plasma CD40 levels and to reduce the number of activated Th1 cells in the peripheral blood and mucosa [5].

In studies of blood returned to patients from the outflow of a granulocyte and monocyte adsorptive apheresis (GMA) column, it was found that T-regulated cytokines such as IL-10 and TGF-β1 were elevated while proinflammatory cytokines such as interferon-γ (IFN-γ) were not. This action of GMA is potentially very interesting in patients with immune disorders, such as CD patients [6].

The clinical efficacy of GMA on inflammatory bowel disease (IBD) and related extraintestinal manifestations has been associated with an expansion of circulating CD4+ CD25+ Tregs and higher expression of FoxP3 in CD4+ T cells [7]. Accordingly, elevated CD4+ CD25+ FoxP3 may be a valuable index of remission in patients with IBD and other chronic relapsing-remitting inflammatory conditions during treatment with GMA [8]. Thus, GMA could be useful for CD patients as it improves the CD activity index (CDAI) [9]. However, the influence of GMA on CD64 levels has not been well characterized. In the present study, we examined CD64 levels in CD patients before and after GMA.

Case Reports

Case 1
A 27-year-old woman had been diagnosed with CD. She was steroid-refractory or -dependent for 3 years, and IFX and adalimumab were administered. However, she did not achieve remission after anti-TNF-α treatment. Thus, GMA was selected for therapy for her CD. After 5 sessions of GMA once a week, her abdominal symptoms and defecation were improved. Blood CD64 was examined before and after GMA, and CDAI was assessed before each GMA session. No adverse effects of GMA were observed.

Case 2
A 37-year-old woman was diagnosed with CD. She was administrated IFX every 8 weeks. However, she did not also achieve remission after IFX treatment. Then GMA was selected for therapy for her CD. After 6 total sessions of GMA, her abdominal symptoms and defecation were improved. Blood CD64 was examined before and after GMA, and CDAI was also assessed before each GMA session. No adverse effects of GMA were observed.

Methods

Two CD patients (both women, 27 and 37 years old) were enrolled, and CDAI was assessed as previously described [10]. GMA treatment was performed as a previously described [11]. Briefly,
Adacolumns and blood circuit lines were primed with sterile saline to remove air bubbles from the column and flow lines, and a second priming of the system was carried out with heparinized saline. Blood access was through the antecubital vein in one arm. The outflow blood was returned to the patient through the antecubital vein in the contralateral arm. The duration of each GMA session was 60 min at a flow rate of 30 ml/min as per protocol. Each patient was treated with a GMA session once per week, and this continued for 5 or 6 weeks. With respect to CD status, each patient was evaluated by CDAI at screening, baseline, and then before each GMA session.

Peripheral venous blood was obtained within 5 min of starting GMA and 5 min after completing each GMA session. CD64 expression on neutrophils was measured by staining whole blood with QuantiBrite CD64PE/CD45PerCP (Beckton-Dickinson, San Jose, Calif., USA) according to the manufacturer’s instructions. Briefly, 20 µl of QuantiBrite CD64PE/CD45PerCP was added to 50 µl of whole blood and incubated for 60 min in the dark at room temperature. This was followed by lysis of red blood cells with 2 ml of 1× FACS™ lysing solution without washing, followed by an additional 60 min incubation to reduce nonspecific background staining [12]. These specimens were analyzed using a FACSscan flow cytometer (Beckton-Dickinson) calibrated with QuantiBrite PE beads (Bedton-Dickinson). The QuantiBrite PE beads contain four different beads with known numbers of phycoerythrin (PE) molecules that make it possible to create a standard curve for determining the mean number of PE molecules present on a cell. As the CD64-PE antibody has been designed to bind one PE molecule per antibody, the mean number of CD64 molecules expressed on cells can be calculated using the PE fluorescence quantification kit with QuantiBrite PE beads. The three different cell populations (lymphocytes, monocytes and granulocytes) were identified and gated by CD45 vs. side scatter profile.

CD64 and CDAI data are expressed as means ± standard deviation. Statistical analyses comparing the CD64 data of the two groups before and after GMA treatment were performed using paired Student’s t test. Correlation coefficients were assessed by linear regression analysis. A p value <0.05 was considered to be statistically significant.

**Results**

In case 1, CD64 levels before GMA averaged 12,156.2 ± 2,301.1 molecules/cell, and the average value decreased to 11,855.8 ± 2,616.8 molecules/cell after GMA (p = 0.31). In case 2, CD64 levels before GMA were 5,080.5 ± 816.3 molecules/cell, and this decreased to 4,744.0 ± 815.7 molecules/cell after GMA (p = 0.02). Thus, CD64 levels were significantly decreased after GMA treatment compared to pretreatment levels in case 2 but not in case 1 (fig. 1). CDAI scores were 606 at baseline and 408 after 5 sessions of GMA in case 1, and 392 at baseline and 203 after 6 sessions of GMA in case 2.

**Discussion**

CD64, one of the Fc receptors for IgG, plays a role in antibody-dependent cytotoxicity, clearance of immune complexes, and phagocytosis of targets opsonized with IgG. It also mediates release of cytokines including IL-1, IL-6 and TNF-α. CD64 is constitutively expressed on macrophages and monocytes and is upregulated on neutrophils as a physiological response to microbial wall components such as lipopolysaccharide (LPS), complement split products, and some cytokines such as IFN-γ and granulocyte colony-stimulating factor (G-CSF). Upregulation of CD64 expression on neutrophils occurs 4–6 h after stimulation with IFN-γ, G-CSF or LPS [7, 13].
The expression of CD64 is elevated in patients with IBD compared to healthy subjects and correlates with clinical and biochemical disease variables. As reported earlier, clinical improvement after steroid therapy is reflected by downregulated CD64 expression by polymorphonuclear leukocytes [14]. Therefore, CD64 expression is responsive to therapeutic interventions [15]. We investigated blood CD64 levels before and after GMA in CD patients, which was reduced after GMA. Further study will be needed to determine the effects of GMA on CD64 in ulcerative colitis.

In conclusion, GMA reduced blood CD64 levels, which would be an important factor for the decrease of CDAI scores.

![CD64 in case 2](image)

**Fig. 1.** CD64 levels before and after GMA in case 2. CD64 levels were significantly decreased after GMA treatment compared to those before GMA.

**References**


