

High-dose intravenous glucocorticoid therapy abrogates circulating dendritic cells

To the Editor:

Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs), and they play a central role in initiating the primary immune response. In human beings, circulating DCs have recently been identified in the peripheral blood.¹ These cells comprise two distinct subsets, myeloid (mDCs) and plasmacytoid DCs (pDCs), which primarily migrate to peripheral tissues and lymph nodes, respectively.¹⁻³ Our previous study and others showed an alteration in the number of circulating DCs as well as the mDC/pDC balance under pathologic conditions,^{4,5} but the effect of certain treatments, such as glucocorticoid (GC) therapy, on circulating DCs is largely unknown. There has been only one recent study on the effect of orally short GC therapy on blood pDCs showing that this therapy moderately decreased its number.⁶

High-dose intravenous glucocorticoid therapy (HD-IVGCT) has been widely used in the treatment of autoimmune or inflammatory disorders.⁷ This therapy is believed to achieve a more rapid and potent anti-inflammatory action. Considering the extremely high doses of GC used in HD-IVGCT, this therapy may profoundly affect immune competent cells in vivo. However, little is known about the effect of HD-IVGCT on these cells, particularly on APCs. In the current study, we attempted to elucidate the effect of HD-IVGCT on circulating DCs.

The subjects consisted of 20 patients (14 men and 6 women, with a mean age of 61.8 years), including 6 with severe Graves ophthalmopathy, 13 with rapidly progressive interstitial pneumonia, and 1 with microscopic polyangitis (MPA). Methylprednisolone (1 g/d) was administered intravenously (HD-IVGCT) over a period of 30 minutes on 3 consecutive days.

Peripheral blood cells were obtained from the patients before HD-IVGCT (day 0) and again the day after the therapy (day 4) and analyzed in a FACS caliber flow cytometer (Becton Dickinson) by 3-color flow cytometry as described previously.⁴ The monoclonal antibodies, purchased from Becton Dickinson (San Jose, Calif), included phycoerythrin (PE)-conjugated anti-IL-3 receptor α -chain (CD123), PE-conjugated anti-CD11c, peridin chlorophyll protein (PerCP)-conjugated anti-HLA-DR, and fluorescein isothiocyanate (FITC)-conjugated lin 1 (anti-CD3, -CD14, -CD16, -CD19, -CD19, and -CD56). We defined mDCs and pDCs as lin⁻/HLA-DR⁺/CD11c⁺ and lin⁻/HLA-DR⁺/CD123⁺ cells, respectively. The absolute number of mDCs and pDCs was calculated from the white blood cell (WBC) count multiplied by the proportion of each subset within the WBC. For statistical analysis, the Wilcoxon signed rank test was used. A *P* value < .05 was considered significant.

Strikingly, after HD-IVGCT, the two DC subsets disappeared in the peripheral blood (Fig 1). Among all

the subjects studied, HD-IVGCT completely abrogated blood DCs, mDCs and pDCs, whereas it increased WBC and neutrophils (Fig 1 and data not shown, respectively). To examine how long the effect of HD-IVGCT lasts, we measured the number of circulating DCs on days 0, 4, 6, and 8 during the first cycle of HD-IVGCT in 4 patients with Graves ophthalmopathy. As shown in Fig 2, mDCs or pDCs were not detected on day 4. Then, both DC populations gradually increased over time, although on day 6 the numbers of circulating DCs were still significantly lower than those on day 0. On day 8, the number of mDCs almost returned to the pretreatment levels. By contrast, the number of pDCs persisted at a significantly lower level on day 8 compared with that of day 0.

The current study is the first to document the effect of HD-IVGCT on circulating DCs. Our data showed that HD-IVGCT completely abolished circulating DCs, including both mDCs and pDCs. pDCs have been shown to be the primary cells producing interferon- α in vivo in response to certain infections.^{1,3} They are thought to recognize blood-borne pathogens, such as viruses, in the circulation and augment the protective immunity against them.³ Thus, the depletion of pDCs by HD-IVGCT would lead to an increased susceptibility to infections with these pathogens. On the other hand, circulating mDCs are a major source of resident DCs in the peripheral tissues. Therefore, the loss of blood mDCs may be attributable to a decrease in the number of the resident DCs in the body. Indeed, our previous study showed that an administration of high-dose dexamethasone into mice markedly decreased the resident DCs in the lung and liver as well as the blood.⁸ Taken together, the potential consequence of complete abrogation of the two DC populations by HD-IVGCT may be related to the impairment of the ability to initiate an effective immune response.

The exact mechanism by which IV-HDGCT abrogates circulating DCs is currently unknown but may be accounted for in several ways. First, the redistribution of circulating DCs, such as homing to lymphoid organs or peripheral tissues, may cause the loss of blood DCs. However, our previous study documented that an administration of high-dose dexamethasone resulted in a marked decrease in DC numbers in any compartment of the body, including the peripheral tissues, lymphoid organs, and blood.⁸ Thus, redistribution is unlikely to be the cause of abrogating blood DCs. Second, a decreased production or differentiation of DCs from bone marrow CD34⁺ progenitors or blood DC precursors may be responsible for the depletion of circulating DCs. Indeed, GC was shown to inhibit the generation of DCs from CD14⁺ precursors.⁹ Finally, DCs may be eradicated from the blood by apoptosis. In vitro studies demonstrated that GC directly induces apoptosis of CD34⁺ pre-

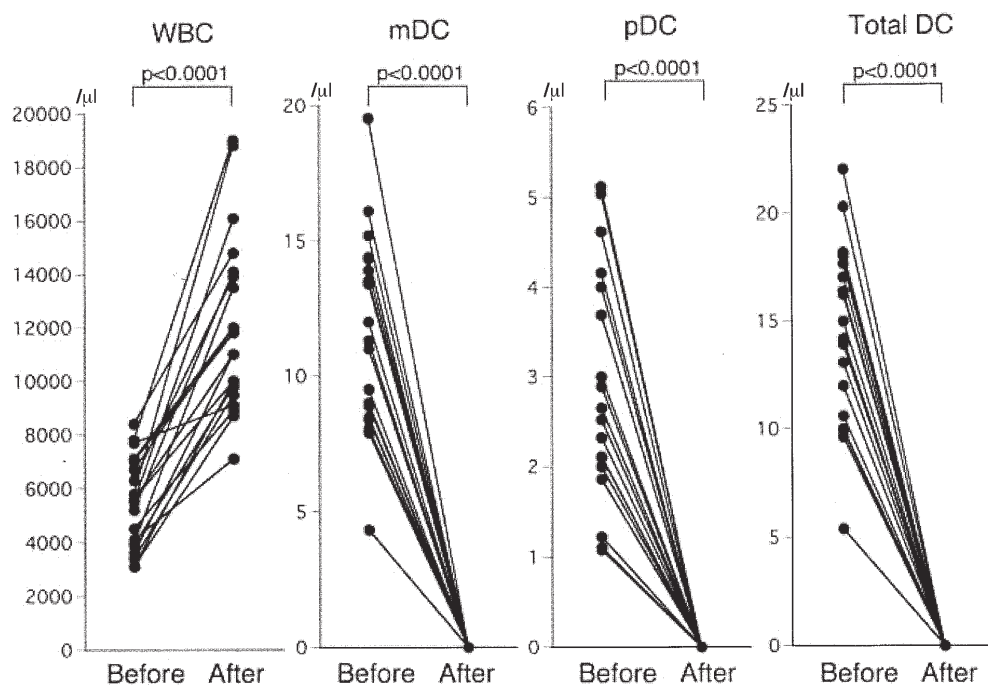


FIG 1. Effect of high-dose intravenous glucocorticoid therapy on the numbers of WBC and circulating DCs. Peripheral blood samples were collected from the same subject before and on the day after HD-IVGCT. mDCs and pDCs were defined as $\text{lin}^+/\text{HLA-DR}^+/\text{CD11c}^+$ and $\text{lin}^+/\text{HLA-DR}^+/\text{CD123}^+$ cells, respectively. Each symbol represents a single sample. *P* values were determined by means of the Wilcoxon signed rank test.

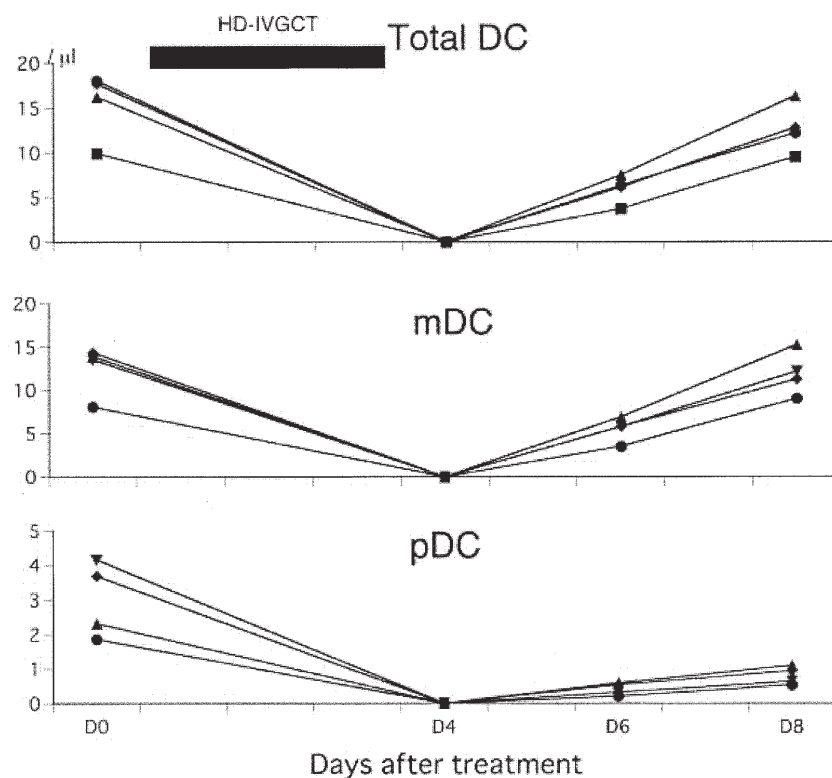


FIG 2. Kinetics of the number of circulating DCs after high-dose intravenous glucocorticoid therapy. Peripheral blood samples were collected from 4 patients with Graves disease on days 0 (before therapy), 4 (the day after HD-IVGCT), 6, and 8. mDCs and pDCs are characterized as shown in Fig 1. Each symbol represents a single sample.

cursor-derived DCs.¹⁰ Interestingly, there was a difference in recovery after HD-IVGCT between mDCs and pDCs. The number of mDCs returned to the pretreatment levels by day 8, whereas the number of pDCs was still lower on day 8. Because both DC subsets completely disappeared on day 4, this discrepancy of recovery may reflect a difference in the turnover rate between mDCs and pDCs.

In conclusion, the present study provides new and important information on the effect of HD-IVGCT, namely, the complete abrogation of circulating DCs. These findings raise important questions with regard to the potential effects of HD-IVGCT on the initiation of effective immunity against newly encountered pathogens.

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