Anti-interleukin 6 receptor antibody tocilizumab reduces the level of serum hepcidin in patients with multicentric Castleman's disease

We report two cases of multicentric Castleman's disease (MCD) whose serum hepcidin levels were rapidly down-regulated by administration of tocilizumab, an anti- interleukin 6 (IL-6) receptor antibody. Our results indicate that IL-6-induced hepcidin over-production may be involved in the pathophysiology of microcytic anemia commonly observed in this disease.

Haematologica 2007; 92:857-858

MCD is a rare lymphoproliferative disorder with systemic manifestations, and over-production of IL-6 has been suggested to be a key event in its pathogenesis.[1-3] Recently, tocilizumab (Chugai Pharmaceutical, Tokyo, Japan), which competitively blocks IL-6 binding to its receptor, has been successfully used to alleviate MCD symptoms.[4] IL-6 up-regulates hepatic expression of hepcidin, a key regulator of iron metabolism[5-7] by blocking the release of iron from macrophages and down-regulating iron uptake from the intestine.[8] We monitored the level of serum hepcidin-25, the major active form of hepcidin, in two MCD patients receiving their initial dose of tocilizumab at Kyoto University Hospital. Tocilizumab (8 mg/kg body weight) was administered intravenously at 2-week intervals. Serum hepcidin-25 was semi-quantitatively analyzed using SELDI-TOF mass-spectrometry as described previously.[9] To compensate for variations in sample concentrations, serum profilings were normalized by total ion current using Biomarker Wizard (Ciphergen ProteinChip Software 3.1.1), and the peak intensity at 2,789 was shown as arbitrary unit (AU; the range in healthy volunteers was 0-25 AU). This study was approved by the Ethics Committee of Kyoto University Graduate School and the Faculty of Medicine. Written informed consent was obtained from each patient.

Case #1 was a 24 years old woman previously treated with corticosteroids for three years. On admission, her Hb was 4.5 g/dL, mean corpuscular volume (MCV) was 69 fL, CRP was 28.9 mg/dL (normal range <0.2 mg/dL), serum iron was 9 µg/dL (40-148 µg/dL) and ferritin was 151.4 ng/ml (14-150 ng/mL). On admission, she was taking 15 mg oral prednisolone daily, and this was continued after administration of tocilizumab. No blood transfusions were given after this administration.

Case #2 was a 32 years old woman who had been receiving a series of corticosteroid treatments as well as combination chemotherapy since she was diagnosed with MCD at the age of 19. On admission, her Hb was 9.8 g/dL, MCV was 82 fL, CRP was 14.6 mg/dL, serum iron was 39 µg/dL and ferritin was 327 ng/mL. She was taking 10 mg oral prednisolone daily and 50 mg cyclophosphamide every alternate day and these medications were continued after administration of tocilizumab.

In both cases, serum IL-6 levels (normal range <4 pg/mL) were highly elevated on admission, and levels increased further after administration of tocilizumab (215 pg/ml before administration and 1,390 pg/mL on day 14 in Case #1; 15.2 pg/mLbefore and 411 pg/mL on day 14 in Case #2). In a previous report,[10] tocilizumab competitively inhibited IL-6 binding to its receptor, resulting in IL-6 accumulation in serum. In both cases, the level of serum hepcidin-25 dramatically dropped within 24 h after the first dose of tocilizumab (from 18 to 3 AU in Case #1; from 35 to 19 AU in Case #2), followed by gradual decreases in CRP and serum ferritin and gradual increases of Hb and serum albumin (Figure 1).

These results indicate that if the IL-6 pathway is properly blocked, the serum hepcidin-25 level decreases rapidly despite very high serum IL-6 levels. In Case #1, urine samples were also applied to the hepcidin assay. Clear peaks corresponding to hepcidin-20 and -25 detected before tocilizumab administration disappeared after treatment (Figure 2).

Interestingly, the serum hepcidin level in Case #1 was lower than that in Case #2 despite the much higher serum IL-6 level in Case #1. This may reflect the complexity of the mechanisms regulating serum levels of hepcidin. IL-6 and other factors, such as body iron status and erythropoietic activity, could influence hepcidin expression.[8]

Down-regulation of hepcidin by very severe anemia may have counteracted the effect of IL-6 on hepcidin pro-

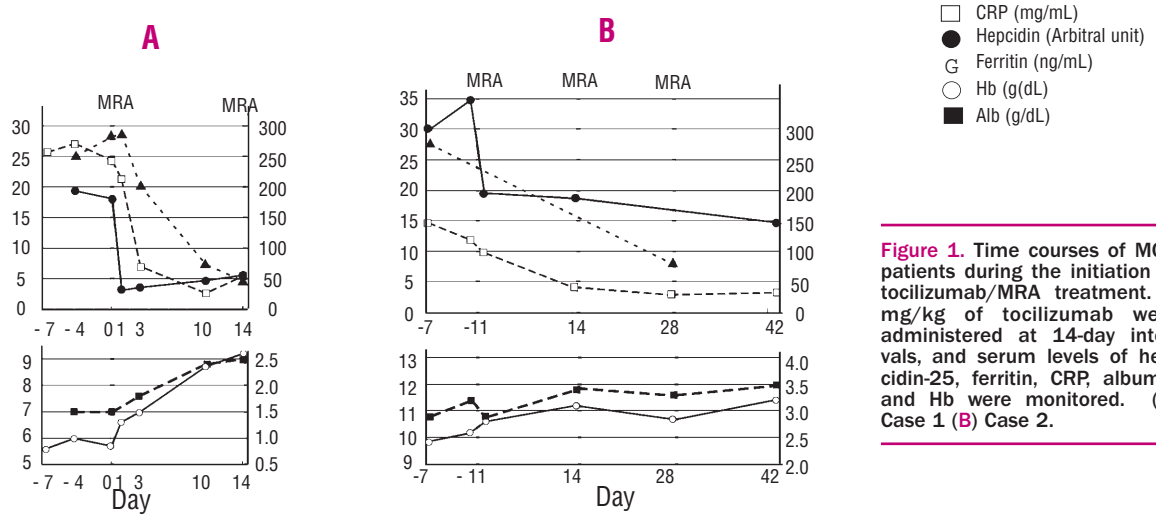

Figure 1. Time courses of MCD patients during the initiation of tocilizumab/MRA treatment. 8 mg/kg of tocilizumab were administered at 14-day intervals, and serum levels of hepcidin-25, ferritin, CRP, albumin and Hb were monitored. (A) Case 1 (B) Case 2.

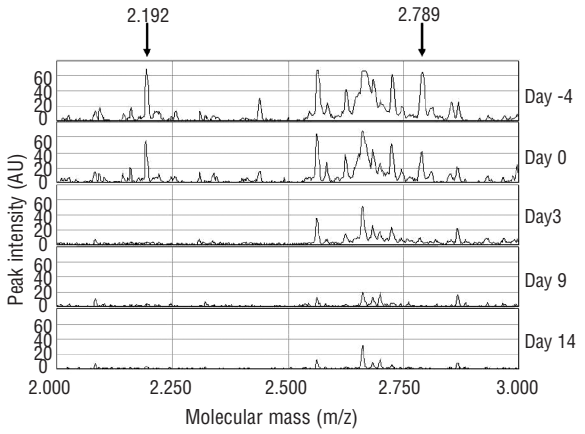

Figure 2. Urinary profile of case 1 showed a range of 2,000 to 3,000 m/z before and after the initial dose of tocilizumab. The peaks at 2,192 and 2,789 m/z corresponding to hepcidin-20 and hepcidin-25 respectively disappeared after day 3.

duction in Case #1. After 3 months of tocilizumab treatment, Hb increased to 12.3 and 13.0 g/dL in cases #1 and #2 respectively. Our results indicate that inappropriate production of hepcidin, possibly related to the pathophysiology of microcytic anemia in MCD, can be reversed quickly by blocking the IL-6 pathway with tocilizumab.

Hiroshi Kawabata, Naohisa Tomosugi,° Junya Kanda,* Yasuhiro Tanaka,* Kazuyuki Yoshizaki,# Takashi Uchiyama**

**Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto, Japan 606-8507; °Proteomics Research Unit, Division of Advanced Medicine, Medical Research Institute, Kanazawa Medical University, Ishikawa, Japan 920-0293; #Department of Clinical Immunology, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan 565-0871*

Funding: this work was supported in part by a grant-in aid for scientific research from the Ministry of Education, Science, Sports and Culture of Japan and a grant from Takeda Science Foundation.

Key words: interleukin-6, Castleman's disease, tocilizumab, iron metabolism, hepcidin.

Correspondence: Hiroshi Kawabata, Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto, Japan 606-8507. Fax: international +81.75.7514963. E-mail: hkawabat@kuhp.kyoto-u.ac.jp

References

1. Hirano T, Kishimoto T. Interleukin 6 and plasma cell neoplasias. Prog Growth Factor Res 1989;1:133-42.
2. Yoshizaki K, Matsuda T, Nishimoto N, Kuritani T, Taeho L, Aozasa K, et al. Pathogenic significance of interleukin-6 (IL-6/BSF-2) in Castleman's disease. Blood 1989;74:1360-7.
3. Waterston A, Bower M. Fifty years of multicentric Castleman's disease. Acta Oncol 2004;43:698-704.
4. Nishimoto N, Kanakura Y, Aozasa K, Johkoh T, Nakamura M, Nakano S, et al. Humanized anti-interleukin-6 receptor antibody treatment of multicentric Castleman disease. Blood 2005;106:2627-32.
5. Nemeth E, Valore EV, Territo M, Schiller G, Lichtenstein A, Ganz T. Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. Blood 2003; 101:2461-3.
6. Kemna E, Pickkers P, Nemeth E, van der Hoeven H, Swinkels D. Time-course analysis of hepcidin, serum iron, and plasma cytokine levels in humans injected with LPS. Blood 2005;106:1864-6.
7. Lee P, Peng H, Gelbart T, Wang L, Beutler E. Regulation of hepcidin transcription by interleukin-1 and interleukin-6. Proc Natl Acad Sci USA 2005;102:1906-10.
8. Ganz T. Hepcidin - a regulator of intestinal iron absorption and iron recycling by macrophages. Best Pract Res Clin Haematol 2005;18:171-82.
9. Tomosugi N, Kawabata H, Wakatabe R, Higuchi M, Yamaya H, Umehara H, et al. Detection of serum hepcidin in renal failure and inflammation by using ProteinChip System(R). Blood 2006;108:1381-7.
10. Ito H, Takazoe M, Fukuda Y, Hibi T, Kusugami K, Andoh A, et al. A pilot randomized trial of a human anti-interleukin-6 receptor monoclonal antibody in active Crohn's disease. Gastroenterology 2004;126:989-96.