Mod Rheumatol (2006) 16:101–104
DOI 10.1007/s10165-006-0462-x

CASE REPORT

Rie Tabata · Chiharu Tabata · Hiromi Fukuda
Hiroyuki Kotani

Expansion of large granular lymphocytes following *Pseudomonas* infection in a patient with adult-onset Still's disease

Received: November 21, 2005 / Accepted: February 14, 2006

Abstract We report a patient who had a 4-year history of adult-onset Still's disease (AOSD) and showed a prominent increase in large granular lymphocytes (LGL) when she developed severe *Pseudomonas* conjunctivitis due to *Pseudomonas aeruginosa*, skin eruptions, liver damage, and abnormal findings in coagulation studies, without any evidence of active viral activation, hemophagocytosis, or malignancies. The increased LGL cells were CD3(+)CD8(+), and disappeared promptly after the administration of antibiotics combined with prednisolone, with subsequent stabilization of her general condition.

Key words Adult-onset Still's disease (AOSD) · Large granular lymphocytes (LGL)

Introduction

Large granular lymphocytes (LGL) are identified as cells larger than normal lymphocytes, and with abundant cytoplasm containing prominent azurophilic granules. They are divided into two major lineages; CD3(–) natural killer (NK)-cell and CD3(+) T-cell types. Clonal expansion of peripheral CD3(+)CD8(+) LGL is observed in approximately one third of patients with Felty's syndrome.[1–3] On the other hand, patients with T-cell lineage large granular lymphocytic lymphoma are known to have a high incidence of autoimmune disorders.[4]

We report here the unique case of a female patient with a 4-year-history of adult-onset Still's disease (AOSD), complicated by a markedly elevated number of CD3(+)CD8(+) LGL, in both the peripheral blood and bone marrow, when she developed severe conjunctivitis, skin eruptions, liver damage, and abnormal findings in coagulation studies. Although transient CD3(+) LGL expansion can be observed in cases of viral infections such as Epstein–Barr (EB) virus, none of the viruses examined was shown to be active, and localized orbital cellulitis caused by *Pseudomonas* infection was the only sign of significant infection.

Case report

A 28-year-old woman was admitted to our hospital for occasional fever of 3 days duration, skin eruption, conjunctivitis of the left eye, and liver dysfunction. Previously, she had been treated with a gradually tapered dosage of prednisolone for 4 years since the initial diagnosis of adult-onset Still's disease (AOSD): at onset, she developed antibiotic-refractory persistent fever for 8 weeks, arthralgia with multiple joint swelling, swelling of several lymph nodes, and hepatosplenomegaly. A transient "salmon-pink" skin rash was observed during the febrile state. Laboratory findings showed liver damage and elevation of the white blood cell count with neutrophilia. The serum ferritin level was highly elevated, but neither rheumatoid factors nor antinuclear antibodies were detected. No firm evidence to support the presence of active infections or malignancies was obtained, and a diagnosis of AOSD was made according to the reported criteria,[5] with major criteria 1–4 and minor criteria 2–4. The administration of prednisolone (30 mg/day) rapidly induced clinical and laboratory improvement, and she was then placed on a daily maintenance dose of 8 mg.

At the current admission, she had small lymph nodes in the bilateral cervical and right axillary regions, but

R. Tabata (✉)
Department of Internal Medicine, Hyogo Prefectural Tsukaguchi Hospital, 6-8-17 Minamitsukaguchi, Amagasaki 661-0012, Japan
Tel. +81-6-6429-5321; Fax +81-6-6422-7405
e-mail: fumitabata@oct.zaq.ne.jp

C. Tabata
Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan

H. Fukuda
Department of Ophthalmology, Osaka Kita Japan Post Hospital, Osaka, Japan

H. Kotani
Department of Internal Medicine, Osaka Kita Japan Post Hospital, Osaka, Japan

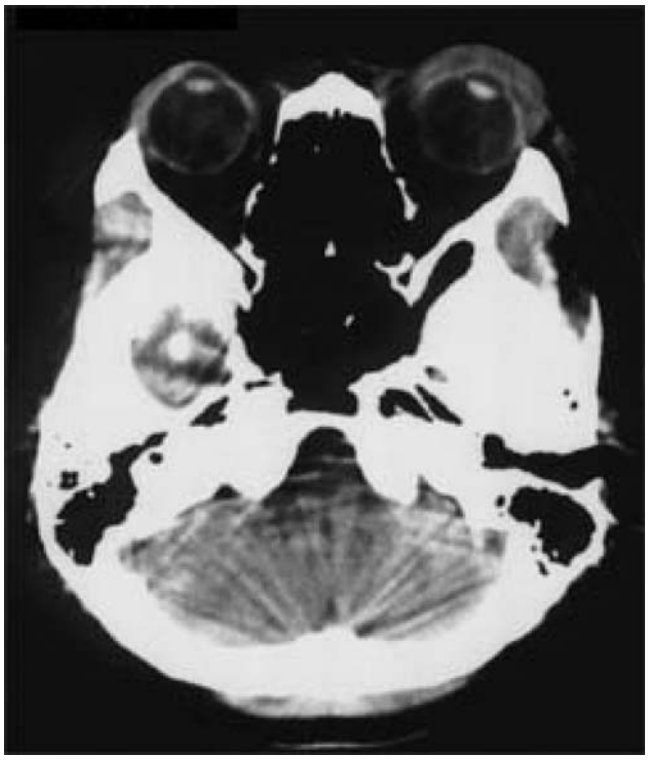

Fig. 1. Computed tomographic image of the head showing swelling of the left eyelid. The axis of the left eyeball is slightly shifted toward the ear, suggesting the paralysis of cranial nerves. No mass lesions oppressing the nerves are seen in the brain

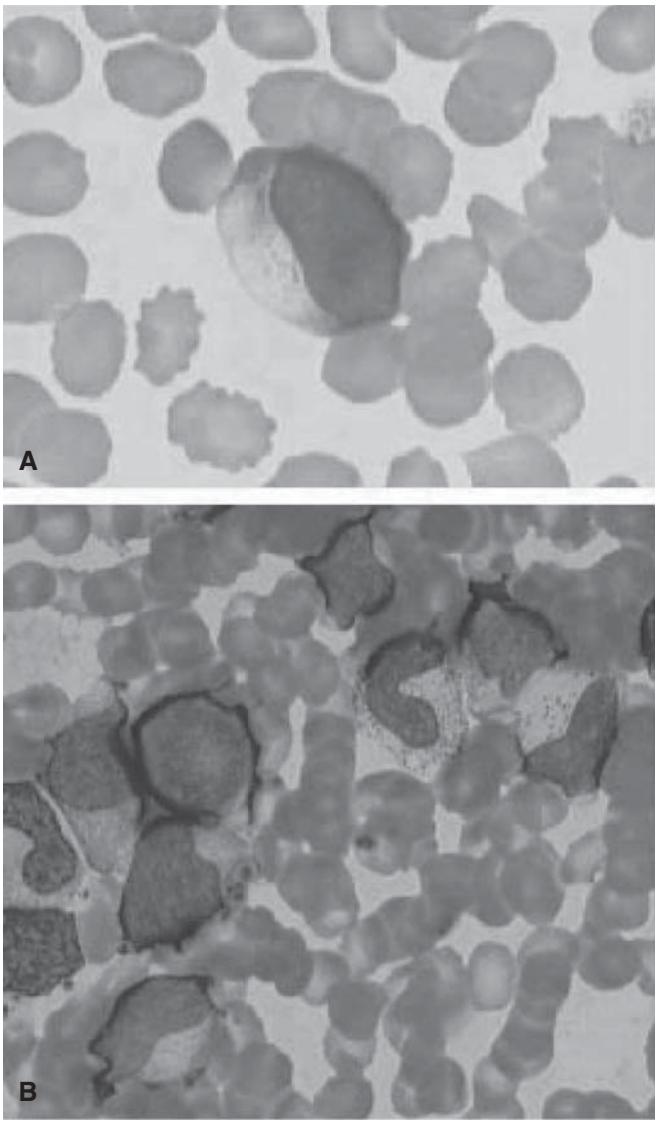

Fig. 2. **A** Atypical lymphocytes are increased in the peripheral blood on the day of admission. The cells are larger than normal lymphocytes and have abundant cytoplasm containing fine granules (May–Giemsa stain, ×1000). **B** Atypical lymphocytes are also increased in the bone marrow. Dysplasia is not observed in erythroid cells, myeloid cells, or megakaryocytes (May–Giemsa stain, ×1000)

hepatosplenomegaly was not present. Skin eruptions of 1–2 mm in diameter were observed all over her body. She also suffered from severe edema and ptosis of the left eyelid accompanied by chemosis, with disturbed motility and visual acuity of the left eye, which led to a suspicion of paralysis of the oculomotor, abducens, and trochlear nerves. Computed tomography of the head showed severe swelling of the left eyelid (Fig. 1), with no intracranial lesions. Subsequently, a diagnosis of serious orbital cellulitis was made when *Pseudomonas aeruginosa* was isolated from the discharge, but not from blood culture. No evidence of other infections or malignancies was observed by chest X-ray, and cardiac or abdominal ultrasonography. Laboratory findings revealed glutamyl oxaloacetic transaminase 347 IU/ml, glutamyl pyruvic transaminase 187 IU/ml, alkaline phosphatase 612 IU/ml, total bilirubin 2.4 mg/dl, lactate dehydrogenase (LDH) 1800 IU/ml, and C-reactive protein 2.2 mg/dl. Other laboratory data supported the diagnosis of disseminated intravascular coagulation (DIC) according to the criteria of the Ministry of Health, Labour and Welfare, 1998 version: platelet count $11.8 \times 10^4/\mu l$ (score 1), fibrinogen 147 mg/dl (score 1), fibrinogen/fibrin degradation products 80 μg/dl (score 3) and AOSD as a basal disease (score 1), D dimer >30 μg/ml (normal, <1.0), α_2-plasmin inhibitor-plasmin complex 6.2 μg/ml (normal, <0.8), and thrombin antithrombin III complex 24.2 ng/ml (normal, <3.0). The white blood cell count was 7700 /ml, and included 64% atypical lymphocytes (Fig. 2A) with reduced neutrophils (17%). They were large with abundant cytoplasm containing fine granules (LGL). The percentages of CD4+ and CD8+ cells in peripheral blood mononuclear cells were 15.9% and 82.9%, respectively. Bone marrow aspiration revealed an increase (29%) in such atypical lymphocytes (Fig. 2B). The percentage of macrophages was 0.4%, with no hemophagocytosis. Flow cytometric analysis showed that more than 90% of these cells were positive for CD2, CD3, CD5, CD7, and CD8. HLA-DR was positive in 67.9% of the cells, while CD56 was partially positive (13.6%). Chromosomal analysis showed a normal karyotype (46, XX). Both anti-nuclear antibodies and rheumatoid factors were negative. Serum ferritin (>3000 ng/ml; normal range, 3.4–89) and soluble IL-2 receptor (sIL-2R, 7430 U/ml; normal, 220–530) were highly elevated. None of the viruses

examined was demonstrated to be activated, as follows; EB-VCA IgG, 1:160; EB-VCA IgM, less than 1:10; EBNA, 1:40; herpes simplex virus (HSV) IgM (EIA), 0.28 (normal, <0.80); human herpes virus type 6 (HHV-6) DNA (serum), $<1.0 \times 10^2$ copies/ml; and cytomegalovirus (CMV) antigen C7-HRP, negative. The activity of hepatitis B included HBe antigen (RIA cutoff index) of 0.4 (negative, <0.9), HBe antibody (RIA, inhibition%) of 100 (positive, >70), and HBV DNA (PCR) of 3.4 log copies/ml (normal, <2.6). The serum cytokine levels upon admission were as follows; interferon (IFN)-γ (EIA), 2.8 IU/ml (normal, <0.1); interleukin (IL)-6, 7.8 pg/ml (normal, <4.0), and no detectable tumor necrosis factor (TNF)-α.

Antibiotics (meropenem trihydrate 1 g/day) and prednisolone (30 mg per day) were administered on day 2 of admission in combination with additional canthotomy performed on day 3 for decompression of the optic nerve, but no specific therapy was given for DIC. Atypical lymphocytes disappeared from the peripheral blood on the 5th day. The patient's ocular symptoms gradually disappeared and her visual acuity, eye movement, and visual field returned to normal on the 14th hospital day. Abnormal laboratory findings of liver functions and coagulation studies returned to the normal range 1 week after the initiation of treatment. Serum levels of ferritin and sIL-2R also gradually improved, and 3 weeks later they were, respectively, 21 ng/ml and 301 U/ml. The patient was thereafter treated with a tapered dosage of corticosteroid without recurrent episodes of LGL expansion or hepatitis B viral activation, based on a liver function test and HBV DNA (PCR).

Discussion

Adult-onset Still's disease is a rare inflammatory rheumatic disease, characterized by a sudden onset of high spike fever, arthralgia, and transient skin rash,[6] with variable clinical courses ranging from spontaneous remission to a fatal course when complicated by severe liver damage or DIC. In some reported life-threatening cases, associated hemophagocytosis suggested a relationship with macrophage activation syndrome (MAS).[7–9] Additional findings in patients with AOSD or juvenile rheumatoid arthritis include increased levels of cytokines.[10–14] The first-line therapy is nonsteroidal anti-inflammatory drugs and corticosteroids, but antirheumatic drugs are used for chronic symptoms such as arthralgia.

Our patient's condition had been well controlled for 4 years with corticosteroids, which were tapered very slowly to avoid the reactivation of AOSD and hepatitis B virus. Recently, lamivudine therapy has been introduced as an optimal therapeutic choice for the prevention of immunosuppressive-induced HBV reactivation in HB carriers.[15] However, our patient refused this medication because she was concerned about possible pregnancy, and it is very likely that continued steroid therapy due to AOSD or underlying AOSD itself had paved the way for severe *Pseudomonas* infection.

It is likely that her course was part of an exacerbation of smoldering AOSD, considering the high levels of LDH, ferritin and sIL-2R, although the nature of skin eruptions was not typical and hemophagocytosis was absent at least in the bone marrow. The clinical significance of serum levels of IFN-γ, IL-6, and TNF-α is still unclear in AOSD due to limitations in the methods used for measurement and the lack of published information. Notably, her course was characterized by marked expansion of LGL. Several reports have demonstrated that the proliferation of LGL is caused by stimulation by bacteria.[16–21] However, in this case, *Pseudomonas* infection was only limited to a local area involving the bulbar conjunctiva, and the portal site appeared to be corneal infection with contaminated contact lenses. Moreover, it has been reported that expanded LGL observed during bacterial infection are mostly associated with NK phenotypes,[16–21] while in this case they were predominantly composed of CD8(+) T cells with a small fraction of CD56(+) cells. Our patient required a prompt initiation of treatment due to the risk of blindness by pressure secondary to severe orbital cellulitis, and hence, did not have time to undergo complete examinations including Ga scintigram, although general examination including chest X-ray and abdominal sonogram detected no other foci of suspected infection or lymph node swelling. Therefore, underlying malignancy is unlikely. The rare possibility of concomitant viral activation such as EB virus, HSV, CMV, and HHV-6 should still be investigated.

Taken together, we speculate that severe *Pseudomonas* infection induced exacerbation of AOSD, followed by hepatic dysfunction and DIC with underlying disturbance of immune function and stress response. As a consequence, marked expansion of LGL was induced. There have been no previous reports on LGL expansion in the course of AOSD.

References

1. Bowman SJ, Geddes GC, Corrigall V, Panayi GS, Lanchbury JS. Large granular lymphocyte expansions in Felty's syndrome have an unusual phenotype of activated CD45RA+ cells. Br J Rheumatol 1996;35:1252–5.
2. Ozaki M, Kawabe Y, Nakamura H, Migita K, Kawakami A, Tsukazaki K, et al. Elevated serum cytokine levels in a rheumatoid arthritis patient with large granular lymphocyte syndrome. Rheumatology 2001;40:592–3.
3. Loughran TP Jr, Starkebaum G. Large granular lymphocyte leukemia. Report of 38 cases and review of the literature. Medicine (Baltimore) 1987;66(5):397–405.
4. Shvidel L, Duksin C, Tzimanis A, Shtalrid M, Klepfish A, Sigler E, et al. Cytokine release by activated T-cells in large granular lymphocytic leukemia associated with autoimmune disorders. Hematol J 2002;3:32–7.
5. Yamaguchi M, Ohta A, Tsunematsu T, Kasukawa R, Mizushima Y, Kashiwagi H, et al. Preliminary criteria for classification of adult Still's disease. J Rheumatol 1992;19:424–30.
6. Bywaters EGL. Still's disease in the adult. Ann Rheum Dis 1971; 30:121–32.
7. Stephan JL, Zeller J, Hubert P, Herbelin C, Dayer JM, Prieur AM. Macrophage activation syndrome and rheumatic disease in childhood: a report of four new cases. Clin Exp Rheumatol 1993;11:451–6.

8. Arai Y, Handa T, Mitani K. Adult-onset Still disease presenting with disseminated intravascular coagulation (in Japanese). Jpn J Clin Hematol 2004;45:316–8.
9. Muta T, Yamano Y. Fulminant hemophagocytic syndrome with a high interferon γ level diagnosed as macrophage activation syndrome. Int J Hematol 2004;79:484–7.
10. Choi JH, Suh CH, Lee YM, Suh YJ, Lee SK, Kim SS, et al. Serum cytokine profiles in patients with adult onset Still's disease. J Rheumatol 2003;30:2422–7.
11. Madson KL, Moore TL, Lawrence JM 3rd, Osborn TG. Cytokine levels in serum and synovial fluid of patients with juvenile rheumatoid arthritis. J Rheumatol 1994;21:2359–63.
12. Lepore L, Pennesi M, Saletta S, Perticarari S, Presani G, Prodan M. Study of IL-2, IL-6, TNF alpha, IFN gamma and beta in the serum and synovial fluid of patients with juvenile chronic arthritis. Clin Exp Rheumatol 1994;12:561–5.
13. Mangge H, Kenzian H, Gallistl S, Neuwirth G, Liebmann P, Kaulfersch W, et al. Serum cytokines in juvenile rheumatoid arthritis. Correlation with conventional inflammation parameters and clinical subtypes. Arthritis Rheum 1995;38:211–20.
14. Rooney M, Varsani H, Martin K, Lombard PR, Dayer JM, Woo P. Tumour necrosis factor alpha and its soluble receptors in juvenile chronic arthritis. Rheumatology 2000;39:432–8.
15. Shibolet O, Iian Y, Gillis S, Hubert A, Shouval D, Safadi R. Lamivudine therapy for prevention of immunosuppressive-induced hepatitis B virus reactivation in hepatitis B surface antigen carriers. Blood 2002;100:391–6.
16. Michelini-Norris MB, Blanchard DK, Friedman H, Djeu JY. Involvement of HLA-DR+ large granular lymphocytes in the induction of tumor necrosis factor by *Mycobacterium avium*-intracellulare complex. J Leukoc Biol 1991;50:529–38.
17. Blanchard DK, Michelini-Norris MB, Pearson CA, Freitag CS, Djeu JY. *Mycobacterium avium*-intracellulare induces interleukin-6 from human monocytes and large granular lymphocytes. Blood 1991;77:2218–24.
18. Blanchard DK, McMillen S, Hoffman SL, Djeu JY. Mycobacterial induction of activated killer cells: possible role of tyrosine kinase activity in interleukin-2 receptor alpha expression. Infect Immun 1992;60:2843–9.
19. Tarkkanen J, Kosunen TU, Saksela E. Contact of lymphocytes with Helicobacter pylori augments natural killer cell activity and induces production of gamma interferon. Infect Immun 1993; 61:3012–6.
20. Pinola M, Saksela E. Adhesion molecules involved in the interaction of LGL/NK cells and human endothelial cells stimulated with Salmonella bacteria. Scand J Immunol 1992;36:671–9.
21. Tarkkanen J, Saksela E. Potentiation of human natural killer cell cytotoxicity by Salmonella bacteria is an interferon- and interleukin-2-independent process that utilizes CD2 and CD18 structures in the effector phase. Infect Immun 1991;59:2767–73.