Successful treatment of pure red cell aplasia of an 88-year-old case with cyclosporin A and erythropoietin after thymectomy

An 88-year-old Japanese female with pure red cell aplasia was treated safely and effectively by a combination of thymectomy, cyclosporin A, and erythropoietin. The thymoma was histologically classified as lymphocytic type or cortical type, which are uncommon in cases of a thymoma accompanied by pure red cell aplasia. Immunohistochemical analysis of the thymoma and bone marrow revealed a predominance of $CD8^+$ cells. Thymectomy alone was ineffective, but cyclosporin A treatment subsequent to thymectomy was safe and effective and resulted in the disappearance of a Vβ12 bearing T cell clone in the bone marrow. Additional treatment with erythropoietin enhanced the effects of cyclosporin A and recovered the patient's hemoglobin to normal levels. The beneficial effect of cyclosporin A may be attributed not to a broad immunomodulatory effect, but to a local effect on a limited T cell subset.

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Pure red cell aplasia (PRCA) is a rare hematological disorder characterized by severe anemia with reticulocytopenia and selective aplasia of the erythroid series in the bone marrow.[1-3] Acquired PRCA may occur secondarily to various underlying conditions such as carcinoma, thymoma, leukemia, autoimmune disease, viral infection, and drugs.[1-4] The pathogenesis of PRCA is not well understood, but an autoimmune mechanism is believed to be involved.[5] For this reason, thymectomy is routinely performed for thymoma–associated cases. Immunomodulatory agents, such as corticosteroids and immunosuppressants, have also been used to treat PRCA and are considered standard therapy for PRCA. We report here an 88-year-old case with PRCA accompanied by thymoma and discuss age-related problems with a combination of thymectomy and immunosuppressive treatment.

Case Report

On April 16, 2002, an 88-year-old female was admitted to the hospital with complaints of easy fatigability and exertional dyspnea. She had been previously medicated for hypertension before admission. Anemic palpebral conjunctiva and pretibial edema was noticed upon admission. Laboratory findings upon admission were the following: white blood cells, 4880/μL (neutrophils, 36%; eosinophils, 1%; basophils, 1%; monocytes, 4%; lymphocytes, 58%); red blood cells, 131×10^4/μL; hemoglobin (Hb), 4.4 g/dL; hematocrit, 12.9%; platelets, 25.7×10^4/μL; and reticulocytes, 0.1%. Other measurements were: serum creatinine, 0.9 mg/dL; fasting serum blood sugar, 111 mg/dL; hemoglobin A1c, 7.10% (normal range: 4.0-5.8%); serum iron, 171 microgram/dL. Parvovirus infection in this case was unlikely, since IgM specific for parvovirus B19 was undetectable and polymerase chain reaction (PCR) analysis for viral DNA was negative. Haptoglobin was within normal range, and the direct and indirect Coombs' test was negative. Levels of CD55 and CD59, markers for paroxysmal nocturnal hemoglobinuria (PNH), were within the normal limits. Erythropoietin (EPO) was 2860 mU/mL (normal range: 8-36 mU/mL). Examination of the patient's bone marrow showed severe hypoplasia specific for erythroid lines (erythroid, 0%) but leukemic blasts and no abnormality in other cell lineages, suggesting a diagnosis of pure red cell aplasia. Antinuclear antibody titer was ×640 (homogeneous and speckled pattern). The HLA-DRB1 alleles included 0101 and 0405. Chest roentgenogram revealed an enlarged heart with a cardiothoracic ratio of 54% and the presence of pleural effusion, suggesting impaired cardiac function by severe anemia. Computerized chest tomography revealed a relatively large thymic mass (3×3 cm) for her advanced age. Although all previous medication prior to the admission was withdrawn, anemia was not improved. In order to suppress the suspected autoimmune condition, a thymectomy was performed. The pathological diagnosis of the thymic mass was thymoma of lymphocytic type (the Lattes-Bernatz classification) or cortical type (the Muller-Hermelink classification), corresponding to type B of the WHO Classification.[1-6] We did not observe recovery of Hb levels after the thymectomy. We next administered cyclosporin A (CsA), which has been found to be the most effective agent in single treatments.[7] CsA was administered at 125 to 250 mg/day to keep serum CsA levels between 200 and 300 ng/mL, respectively. Oral hydration of 1.5-2.0 liters a day was supplied simultaneously. Reticulocyte numbers recovered within one week. We also observed a gradual rise in Hb levels that reached 8.5-9.5 g/dL. EPO reduced into a normal level (18.8IU/L). The CsA treatment did not result in any observable complication. The pretibial edema, enlarged heart, and pleural effusion gradually disappeared. However, the patient still complained of mild exertional dyspnea and dorsal edema. For this reason, we began EPO injections at 6000 IU/week. Approximately one month later, the patient's Hb levels rose to 14.0±0.5 g/dL, at which point she felt no exertional dyspnea and had no apparent edema. At this stage, erythroid cells in the bone marrow recovered to 15%. She has been followed as an outpatient for 11 months without relapse. The accompaniment of PRCA by a thymoma and increased antinuclear antibody (ANA) titer suggested that an autoimmune mechanism triggered the PRCA. The ANA titer, however, was not reduced after CsA treatment. Immunohistochemical staining of the thymoma tissue revealed expanded CD8 positive cells in the cortical zone and normal numbers of $CD4^-$, $CD20^-$, and CD56-positive cells.

Flow cytometric analysis of bone marrow cells showed that the CD4/CD8 ratio of lymphocytes was low, but within the normal range. After thymectomy, the CD4/CD8 ratio increased without remission of anemia. Unexpectedly, however, the ratio decreased with CsA administration even during the remission period. This effect of CsA would result from a localized influence rather than an inhibitory effect on the entire CD8 population in bone marrow.

To analyze further the alteration of T cell-mediated autoimmunity by CsA treatment, the T cell receptor (TCR) repertoires in the thymus and bone marrow were analyzed by reverse transcriptase polymerase chain reaction (RT-PCR) with TCR beta chain subfamily-specific primers and a subsequent single-strand conformation polymorphism (SSCP) analysis, according to methods previously described.[8] One of the SSCP bands for Vbeta12, which presumably corresponds to a T cell clone in the bone marrow, disappeared after the start of CsA administration (Figure 1).

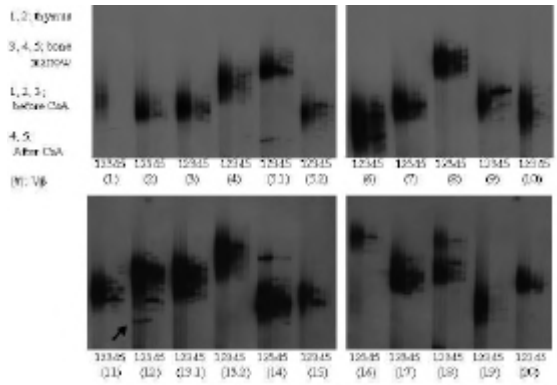

Figure 1. RT-SSCP analysis of T cell receptor Vbeta12 of thymoma and bone marrow of the patient. Two specimens were obtained separately from the patient's thymoma and subjected to RT-SSCP analysis. SSCP results of Vbeta1 to Vbeta20 are shown. Note that a band indicated by the arrow disappears after the commencement of CsA administration (see text and Figure. 1).

Discussion

With reduced reticulocyte levels and erythroid-specific hypoplasia accompanying high erythropoietin levels, PRCA was diagnosed under the medication-free condition of the patient. Immunosuppressive therapy is currently an accepted treatment for PRCA1. 10% to 15% of the PRCA patients are found to have thymoma, usually spindle cell (the Lattes-Bernatz classification) or medullary type (the Muller-Hermelink classification).[6] Thus far, it is uncertain if the accompaniment of a histopathologically unusual thymoma corresponds to a specific feature of this case, such as CD8 dominance, or bias of Vbeta12 clones.

Levels of circulating CD45RA$^+$ CD8$^+$ T cells were reported to be increased in thymoma patients and to decrease after thymectomy.[9] The observed cortical CD8(+) accumulation in this case may reflect activation of cytotoxic T cells against erythroid precursor cells.

Increase of the CD4/CD8 ratio by CsA therapy has been reported in autoimmunity-associated diseases, e.g., aplastic anemia with autoimmune thyroiditis, and pure red cell aplasia.[10-12] In the present case, CsA reduced the CD4/CD8 ratio in bone marrow. This discrepancy might reflect a heterogeneous pathogenesis of pure red cell aplasia. In this case, CsA did not ameliorate broad CD8 dominance.

A bias in T cell receptor Vbeta repertoires has also been reported in various autoimmune conditions such as rheumatoid arthritis, Sjogren's syndrome, myasthenia gravis, pure red cell aplasia, and multiple sclerosis.[12-18] T cell clonality, as evidenced by clonal Vbeta rearrangement, was reported in PRCA patients.[17] The disappearance of a Vbeta12 clone after CsA treatment supports the idea that immunosuppression of T cells resulted in a favorable clinical outcome. CsA likely suppresses a small population of CD8+ T cell in bone marrow.

Preferential Vbeta12 usage has been observed in Crohn's disease, rheumatoid arthritis, and multiple sclerosis.[19-22] To our knowledge, this is the first report of an altered Vbeta12 repertoire associated with a thymoma accompanied by PRCA. Analysis of the TCR repertoire over the course of treatment may be useful for evaluation of the therapeutic effect of immunosuppressants.

An aplastic anemia case was reported to bear a CD4$^+$ Vbeta21+ T cell clone capable of killing hematopoietic cells in an HLA-DRB*0405-restricted manner.[23] Because the present case also includes the DRB*0405 allele, HLA-DRB*0405 restriction may correlate with the concurrent autoimmune condition.

A recent report showing an associated expansion of CD3+ CD8+ granular lymphocytes expressing cytotoxicity against HLA-E+ cells in peripheral mononuclear cells of PRCA patients supports the idea that a subset of the CD8$^+$ T cell population was involved in the pathogenesis of PRCA24. Another PRCA patient was reported to bear CD3+, TCR-alpha+ beta+, TCR-Vbeta8+, CD8+, CD57+ large granular lymphocytosis that showed significant responsiveness to CsA therapy.[12] In the present case, CD8+ cells may be involved in erythropoietic impairment. Taken together, unknown CD8+ clones are likely involved in pathogenesis of PRCA in an HLA-DRB*0405 restricted manner.

Thymectomy, even in high-aged cases, is considered beneficial for either directly improving the disease or increasing its sensitivity to immunosuppressive treatment. A combination of thymectomy and CsA, however, was not enough to recover the patient's hematocrit to normal levels. This could partially have resulted from CsA itself, as one side effect of CsA is to suppress the level of endogenous erythropoietin;[25,26] this is the basis for providing additional EPO treatment. EPO therapy has been shown to be effective in treating PRCA accompanied by underlying autoimmunity such as systemic lupus erythematosus. The expectation is that the supplied EPO will compete with autoantibody by binding the erythropoietin receptor on immature erythroid cells27. In the present case, EPO could have provided a direct therapeutic effect in overcoming the speculative underlying autoimmunity. Further similar cases are necessary to confirm the effectiveness of the present combination therapy for the high-aged PRCA population.

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