Successful Treatment with Cyclosporine of Thymoma-related Aplastic Anemia

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Abstract. *Aplastic anemia is a rare immune-mediated complication of thymoma. Thymomas, especially of the cortical type, have the capacity to generate mature T-cells from their immature precursors. Furthermore, mature intratumorous T-cells have an increased autoantigen-specific potential. We present the case of a 75-year-old patient with an inoperable cortical thymoma who developed aplastic anemia 7 years after the initial diagnosis. The infiltration of the bone marrow by these cells was accompanied by the production of cytokines such as tumor necrosis factor alpha (TNF-α) in the microenvironment of bone marrow and in the serum sample. The patient was successfully treated with oral cyclosporine A for 14 months and when she died due to progression of the thymoma, 9 months after the discontinuation of cyclosporine, the aplastic anemia had not recurred.*

Thymoma, a relatively rare tumor, is the most usual diagnosis for a mass located in the anterior mediastinum. The mainstay of therapy for thymomas is complete surgical resection, which is an important predictor for long-term survival. The 5-year survival rates for stage III and IV are 69% and 50%, respectively (1). The patients of stage III and IV are treated with subtotal resection, radiation therapy and chemotherapy. Thymomas are often associated with autoimmune disorders including myasthenia gravis, polymyositis, hypogammaglobulinemia, agranulocytosis, pure red cell aplasia (PRCA) and aplastic anemia (AA) (1-7). Myasthenia gravis and PRCA are the most common disorders (40% and 5%, respectively) while hypogammaglobulemia and haemolytic anemia are less common (1, 6, 7). Aplastic anemia, a state of bone marrow (BM) failure, is characterized by hypocellular marrow and peripheral pancytopenia. Aplastic anemia (AA) is a rare complication and its incidence in thymomas is 0-1.4% (7, 8). Immunosuppressive therapy with antithymocyte globulin (ATG) or antilymphocyte globulin (ALG) with or without cyclosporine A (Cy-A) are the most efficient treatments in the majority of patients with idiopathic or thymoma-related aplastic anemia (TA-AA) (9-11). We present the case of AA which developed in a patient with an inoperable thymoma 7 years after the initial diagnosis who was successfully treated with Cy-A.

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Case Report

In September 2003, a 75-year-old woman with a history of an inoperable, stage IVa cortical thymoma, diagnosed in January 1996, presented with anemia and thrombocytopenia. At diagnosis of thymoma in 1996, the patient received 6 cycles of cisplatin, epirubicin and cyclophosphamide. Despite chemotherapy, the disease progressed and 6 additional cycles of ifosphamide, mitoxantrone and etoposide were administered, which resulted in a minor response. After the completion of chemotherapy the patient had regular follow-up every 3-4 months.

When she was admitted to the hospital in 2003, the thymoma remained stable. At her admission, a full blood count revealed normocytic anemia (hemoglobulin 8.8 g/dL) with reticulopenia (reticulocyte count $<5x10^9$/l), thrombocytopenia (platelets $13x10^3/mm^3$), neutropenia and lymphocytosis. The WBC was $9x10^3/mm^3$ with 29% neutrophils and 68% lymphocytes. Direct Coombs was ++ IgG-positive. The serum levels of haptoglobin, LDH, total and indirect bilirubin, iron, vitamin B12 and folate were normal. Protein electrophoresis showed no hypogammaglobulinemia. The serum erythropoietin levels were 98 mU/ml (normal values, 3.3-20 mU/ml). Other laboratory tests were within the normal limits. The radiograph and the

computed tomography (CT) of the chest revealed a large mass with calcifications in the upper anterior mediastinal which was extended throughout the pleura of the left hemithorax and diaphragm, and remained stable compared to that of 1999. The CT of the abdomen was normal. The bone marrow biopsy revealed granulocytic hypoplasia, red cell and megakaryocytic aplasia and infiltration of 60% the specimen by small mature lymphocytes, both in a diffuse pattern and in small nodules. Flow cytometry of the bone marrow specimen revealed a decreased CD4/CD8 ratio (0.2), due to an increase in CD8+ T lymphocytes. Eighty-three percent of the mature CD3+ T-lymphocytes of the bone marrow (CD3+ T-lymphocytes comprised 75% of the total T-lymphocyte population) were CD8+, while the remaining 17% were CD4+ T lymphocytes. Flow cytometry of the peripheral blood showed similar results. The CD4/CD8 ratio was 0.26 due to an increase in CD8+ cells (73% of total, 93% of the CD3+ T cells). Lymphocytosis of the peripheral blood was related to an increase in CD8+ T-lymphocytes. T-cell receptor gene rearrangement studies in blood samples excluded clonal abnormality.

At admission, TNF-α levels were measured by enzyme-linked immunosorbent assay (Rapid ELISA, Biosource International, CA, USA) in the supernatant of the blood marrow and in the serum. Serum samples from 8 healthy blood donors were tested for TNF-α. The detected TNF-α levels ranged between less than 5 and 66 pg/ml, with a mean level of 19.8 pg/ml. Two healthy bone marrow controls were used in order to assess the results of ELISA in the bone marrow supernatant. The level of TNF-α in the patient's serum sample was 280 pg/ml and in the bone marrow was 480 pg/ml. The respective numbers of the bone marrow supernatant in the controls were: control A: 90 pg/ml, control B: 110 pg/ml. Fifteen days after the patient's admission a second serum sample was collected and the level of TNF-α was similar to that of the first sample (260 pg/ml).

The patient was initially treated with several blood transfusions and subsequently with Cy-A at a daily dose of 200 mg. The patient weighed 50 kg. Until that time the blood analysis had been worsening (WBC: 2900/mm^3, neutrophils: 400/mm^3, lymphocytes 2500/mm^3, platelets: 4000/mm^3, Hb: 6 g/dL). One month later, the dose of Cy-A was reduced by half (100 mg) since the level of the drug in the serum was over the upper safety limit (400 ng/ml). Two months after the initiation of post-therapy, the hemoglobulin and the platelets number started to rise and by the end of the third month returned within the normal limits. The neutrophil count was elevated to over 1000/mm^3. The lymphocytosis and neutropenia remained for 1.5 years and then the differential of the WBC became normal. At that point, the CT and the continuously elevated tumour marker (Ca125) demonstrated worsening of the disease despite the improvement of the haematological picture of the patient. The patient died 24 months after the initiation of Cy-A due to the progression of thymoma in the mediastinum. The patient had stopped receiving Cy-A 9 months before she died during which the pancytopenia had not recurred.

Discussion

A clear correlation exists between the development of autoimmune phenomena and neoplasms of the cortical thymus. Thymoma-related aplastic anemia (TA-AA) is a rare immune-mediated complication. This association arises from the capacity of thymomas to generate mature CD4+ and CD8+ T-cells from immature precursors, producing autoreactive T-cell clones responsible for humoral and cytotoxic autoimmune diseases (12). Thymopoiesis and T-cell function correspond with immunologic alterations in the blood. The proportion of circulating CD8+ T-cells is significantly increased in patients with thymoma (12). In addition, in thymomas, especially the cortical type, even minimal thymopoiesis along with preferential generation of CD8+ T-cells is sufficient to skew the T-cell composition in the blood towards the CD8+ phenotype (12, 13). These T-cells are produced intratumorally and not by the residual thymus (12). Intratumoral T-cell maturation is considered abnormal since these cells often have increased autoantigen-specific potential (13). An excess of CD8+ T-cells both in the peripheral blood and bone marrow, as in our patient, represents the most consistent finding (2, 10, 12). Furthermore, most of the reported cytopenia cases related to thymoma (PRCA, thrombocytopenia, agranulocytosis, AA) have at least an inverted CD4/CD8 ratio towards the CD8+ phenotype (12, 14). In our patient the CD4/CD8 ratio, both in the bone marrow and in the blood, decreased. In most cases the absolute number of T-lymphocytes in the peripheral blood remains within the normal limits despite an increase in CD8+ T-cells. This phenomenon has been attributed to the peripheral homeostatic mechanism (15). In our patient, this mechanism was probably impaired since the absolute number of T-cells in the peripheral blood ranged between 7 and $9x10^9$/l.

Thymoma-related aplastic anemia, as well as most cases of idiopathic aplastic anemia, is attributed to a T-cell-mediated destruction of CD34+ hematopoietic stem and progenitor cells (16). Two mechanisms have been proposed to coexist in the pathogenesis of AA: the contact-dependent destruction of the bone marrow target cells and the production of cytotoxic cytokines (TNF-α and interferon-gamma, INF-γ) in the microenvironment of the bone marrow of patients with aplastic anemia (16-19). T-lymphocytes play a major role in destroying the stem cells in bone marrow by infiltrating the bone marrow and secreting excessive levels of the anti-hemopoietic

cytokines, IFN-γ and TNF-α (20). Hinterberger *et al.* (21, 22) reported that IFN-γ and TNF-α are overproduced as cytokines, potently inhibiting hematopoiesis in AA. Schultz and Sahidi (23) measured INF-γ and TNF-α in bone marrow plasma from patients with AA and found higher levels of these cytokines than in normal controls. In our patient, TA-AA was associated with the infiltration of the bone marrow by T- lymphocytes, mainly of the CD8+ phenotype, as well as with increased levels of TNF-α both in bone marrow supernatant and in blood serum. TNFα has been reported to inhibit multipotent colony formation of normal human hematopoietic stem cells and human leukaemia progenitor cells *in vitro* (24, 25). *In vitro*, TNF-α and INF-γ have been shown to act as potent suppressors of hematopoiesis by induction of apoptosis in early and late hematopoietic progenitors (19, 26). In AA patients, these cytokines have been shown to up-regulate the Fas antigen on CD34+cells (26). Hara T *et al.* (18) measured intracellular TNF-α in patients with aplastic anemia and in normal controls and found significantly increased TNF-α production in CD4 and CD8 bone marrow lymphocytes and CD8+ peripheral blood lymphocytes. Other studies have also shown increased TNF-α levels in the bone marrow supernatant and in the serum which decline after the administration of antithymocyte globulin and Cy-A (19, 23).

Most cases in the literature with TA-AA, TA- PRCA and idiopathic AA have been treated with Cy-A and anti-thymocyte globulin (ATG). A great proportion of patients with AA respond to this regimen, a fact that reinforces the hypothesis of immune-mediated stem cell damage. Cy-A has been reported to block cytotoxic lymphocytes while ATG causes profound lymphopenia (27). Our patient received only oral Cy-A at a dose of 2.5 mg/kg daily which resulted in long-term hematologic remission. It was encouraging that response to Cy-A was observed despite the bulk of the remaining thymoma.

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