

## SUCCESSFUL TREATMENT OF CHRONIC AUTOIMMUNE NEUTROPENIA WITH CYCLOSPORIN A

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### ABSTRACT

**Chronic primary autoimmune neutropenia (AIN) is a distinct clinical entity seen mostly in young children, characterized by persistent neutropenia with circulating anti-neutrophil antibodies and no associated disorders known to produce AIN. Herein we report a 22-year-old male who spontaneously developed severe chronic neutropenia with recurrent episodes of high fever and oral aphthous ulcers. Laboratory evaluations detected the presence of anti-granulocyte autoantibodies directed against the NA1 neutrophil-specific antigen. Clinical, laboratory and roentgenographic testing did not reveal any disorder known to be associated with AIN. The patient's severe neutropenia did not respond to therapy with prednisone alone, but resolved following treatment with prednisone and high-dose cyclosporin A.**

*Key words: chronic autoimmune neutropenia, cyclosporin A, prednisone*

Chronic neutropenia is an ill-defined clinical entity in which many different pathogenic mechanisms may play a role. In this setting the finding of autoreactive anti-neutrophil antibodies strongly suggests the presence of an autoimmune-mediated neutropenia.<sup>1</sup> This type of neutropenia can appear spontaneously,<sup>2,3</sup> be drug-induced or appear in the context of an autoimmune disease, an infectious disease, a lymphoproliferative disease<sup>1</sup> or following autologous or allogeneic bone marrow transplantation (BMT).<sup>4</sup> Herein we describe a patient who spontaneously developed severe autoimmune neutropenia that did not respond to high-dose steroid therapy and granulocyte colony-stimulating factor (G-CSF), but who achieved a long-term rise in neutrophil counts after treatment with cyclosporin A (CyA) was begun.

### Case report

A 22-year-old male with no significant personal or family medical history was first seen in our hospital in December, 1991 with fever, sore throat and aphthous oral ulcers. He had a six-month history of recurrent episodes of this type, which lasted two to three weeks and had been treated with oral antibiotics and antipyretics by his family doctor. Physical examination was unremarkable except for the presence of an axillary temperature of 38-40°C, well-tolerated, and 0.5 cm white aphthous ulcers on the oral mucosa.

The leukocyte (WBC) count was  $1.9 \times 10^9/L$  with 1% neutrophils [absolute neutrophil count (ANC)  $0.019 \times 10^9/L$ ], 2% monocytes and 98% lymphocytes. The hemoglobin level was 12.6 g/dL and platelet count  $189 \times 10^9/L$ .

Serologic studies for human immunodeficiency virus (HIV) and hepatitis B virus (HBV) were negative.

ciency virus types 1 and 2, cytomegalovirus, Epstein-Barr virus, HBsAb and Toxoplasma were negative. Rheumatoid factor (RF), antinuclear antibodies (ANA), circulating immune complexes, HLA B27 and a complete series of organ-specific autoantibodies were negative. Immunoglobulin and complement levels were within the normal range. A direct Coombs' test and Ham's and sucrose lysis tests were negative. Abdominal ultrasound and thoracic and abdominal CT revealed only the presence of moderate splenomegaly. A bone marrow aspirate yielded normocellular marrow with significantly reduced granulocyte precursors (16%), which showed a *maturational arrest* at the level of the myelocyte, relatively increased erythropoiesis (54%), 3% monocytes and 25% lymphocytes. Megakaryocytes were present in normal numbers. Bone marrow cultures were performed at diagnosis and eight months later, and both demonstrated normal growth of granulocyte-macrophage colony forming units (GM-CFU) that was not inhibited by the patient's acute-phase serum (fresh serum obtained during severe neutropenia), or by the addition of

acute-phase peripheral T lymphocytes to the culture medium. Bone marrow chromosome analysis showed a normal karyotype. Serial blood cultures and specimens from the aphthous ulcers were negative for bacteria, mycobacteria, viruses and parasites.

Treatment with cefixime, acyclovir and nistatin was begun; clinical response was good but severe neutropenia persisted ( $\text{ANC} < 0.2 \times 10^9/\text{L}$ ), and in January, 1992 (four weeks later) treatment with prednisone (PDN, 2 mg/kg daily) was started.

The patient's ANC did not rise above  $0.7 \times 10^9/\text{L}$ , and in February (six weeks of therapy with PDN) he began treatment with CyA (5 mg/kg daily in two divided doses). PDN was slowly tapered until its suspension in April. Three weeks later the ANC was  $1.4 \times 10^9/\text{L}$  (Figure 1). During the next 14 weeks the dose of CyA was gradually reduced to maintain a serum creatinine level  $< 140 \mu\text{mol}/\text{L}$ , and the ANC ranged from 0.9 to  $1.5 \times 10^9/\text{L}$ .

In July fever and aphthous ulcers reappeared; WBC were  $3.3 \times 10^9/\text{L}$  with an ANC of  $0.2 \times 10^9/\text{L}$ . The patient was then taking a daily

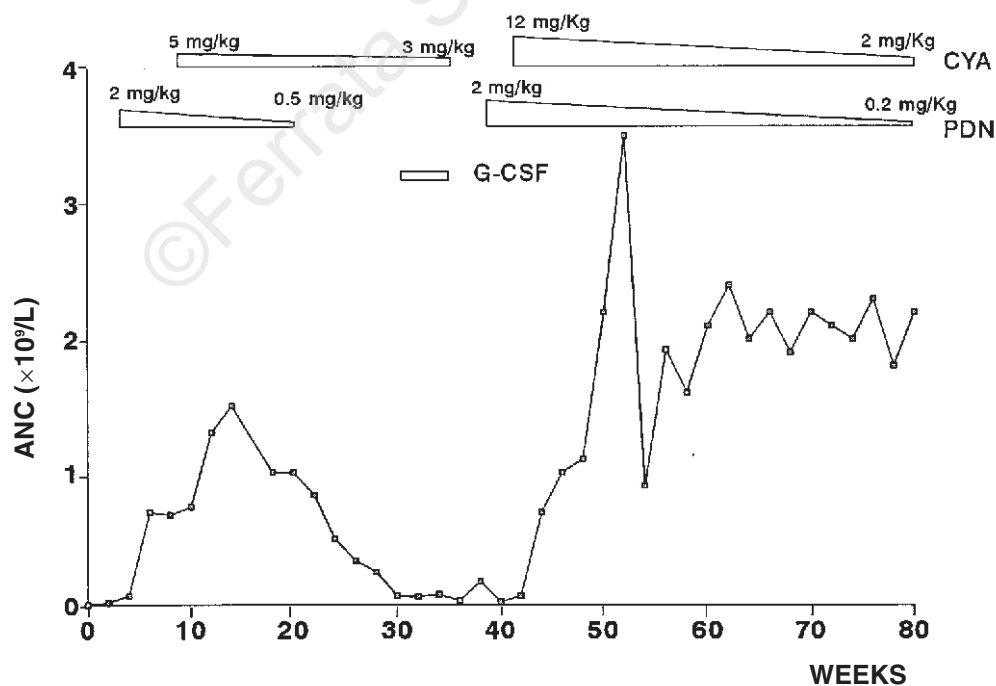


Figure 1. Changes in the patient's absolute neutrophil count (ANC) over time with respect to treatment received. CYA: cyclosporin A; PDN: prednisone; G-CSF: granulocyte colony-stimulating factor.

dose of 3.5 mg/kg of CyA. Therapy with G-CSF (5 µg/kg daily subcutaneously) was added and four weeks later the ANC was  $0.06 \times 10^9/L$ ; G-CSF was discontinued.

In August the patient was readmitted to the hospital for high fever (39-40°C) and aphthous oral ulcers. Upon admission CyA was discontinued. Serial blood cultures were negative and empirical therapy with ceftazidime and amikacin was followed by a disappearance of all symptoms in one week. All previous laboratory and roentgenographic studies were repeated with similar results. The patient continued to be severely neutropenic (ANC <  $0.2 \times 10^9/L$ ) for the following three weeks. PDN was then given at a dose of 2 mg/kg, but there was no response after four weeks of therapy.

In October, treatment with CyA was restarted at a higher dose than before (12 mg/kg daily in two divided doses). Ten days later the ANC rose to  $1.2 \times 10^9/L$ , and appropriate blood samples were obtained for anti-granulocyte antibody testing. An immunofluorescence test detected the presence of an IgG autoantibody in both the patient's serum (indirect test) and on the surface of his granulocytes (direct test). Using a panel of target granulocytes from normal donors typed for the neutrophil-specific antigens NA1, NA2 and NB1, these antibodies were shown to be specific for the NA1 antigen.

At the present time (follow-up of 11 months as of August, 1993) the patient is still being treated with CyA treatment and remains asymptomatic. CyA and PDN dosages were slowly tapered, and he currently receives 2 mg/Kg and 0.2 mg/kg daily, respectively. Bimonthly peripheral blood counts have shown that his ANC has generally been  $> 1.5 \times 10^9/L$ . On only one occasion has it fallen below  $1 \times 10^9/L$  (Figure 1), after the dose of CyA had been temporarily reduced faster than usual because of renal toxicity. Moderate impairment of renal function (serum creatinine 140-150 µmol/L), hypomagnesemia and moderate hypertension have been the only side effects observed.

### Discussion

Many chronic neutropenias are due to immune mechanisms, and patients are said to have primary autoimmune neutropenia (AIN) when this clinical entity appears spontaneously,

while secondary AIN are associated with other conditions.<sup>1-4</sup> Primary chronic AIN occurs mostly in young children but can be seen in any age group.<sup>1</sup> Our patient presented chronic neutropenia associated with recurrent episodes of high fever and aphthous ulcers. All laboratory and roentgenographic findings performed ruled out the presence of a disease that might be associated with secondary AIN.<sup>1-3</sup>

The *myeloid maturation* observed in the patient's bone marrow, the presence of IgG antibodies directed at the NA1 antigen, and the normal results of bone marrow cultures were all consistent with a diagnosis of primary AIN,<sup>1-3</sup> thus ruling out the existence of an autoimmune pure white cell aplasia or other abnormalities of early myeloid progenitors.<sup>5</sup> Splenomegaly has also been frequently reported in AIN,<sup>1,6</sup> as in other autoimmune cytopenias, presumably due to the destruction of opsonized neutrophils in the spleen.<sup>7</sup>

Treatment of patients with AIN usually involves management of infections and other supportive measures, since most have only moderate neutropenia (ANC 0.5- $1.5 \times 10^9/L$ ) and do not suffer life-threatening infections.<sup>1-3</sup> Our patient, however, presented severe chronic neutropenia requiring therapy directed at improving the granulocyte count. Several strategies for raising the ANC in these patients have been reported, including the use of splenectomy,<sup>1</sup> intravenous immunoglobulins,<sup>8</sup> cyclophosphamide<sup>9</sup> and corticosteroids,<sup>10</sup> the latter being the initial treatment of choice. Our patient showed no response to treatment with PDN alone on two separate occasions: after six and four weeks of therapy.

CyA is a potent immunosuppressive agent which has been shown to be effective in several autoimmune blood disorders,<sup>11</sup> including two cases of neutropenia in patients with large granular lymphocyte (LGL) lymphocytosis,<sup>12,13</sup> one patient with adult-onset cyclic neutropenia,<sup>14</sup> and two patients with Felty's syndrome.<sup>15</sup> G-CSF has also been effective in treating cyclic neutropenia,<sup>16</sup> but in our case it proved ineffective. In view of the refractoriness of our case to other immunosuppressive regimens, we introduced CyA initially at a dose of 5 mg/kg per day, with only a transient response, and finally at a dose of 12 mg/kg per day, which led to an 11-month rise in the ANC and no further febrile episodes.

To our knowledge, CyA has not been previously reported to be beneficial in the treatment of chronic primary AIN. Although our patient responded to a combination of PDN and CyA, we believe that the six and four weeks of ineffective therapy with PDN alone, followed by a rapid response to CyA on two separate occasions, strongly suggests that this latter agent was responsible for the sustained rise in the ANC.

In conclusion, we believe that CyA could be an effective alternative for the treatment of chronic AIN in those patients with severe neutropenia who suffer from repeated episodes of potentially life-threatening infections and do not respond to corticosteroids.

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