Partial Immunologic Reconstitution of a Patient with Acquired Agammaglobulinemia: A Transient Phenomenon Accompanying Therapeutic Plasmapheresis

By Barry Wenz and Arye Rubinstein

A patient with acquired agammaglobulinemia was treated with plasmapheresis. The rationale for this procedure was based on the presence of a cytotoxic autoantibody with specificity for helper (TH2) T lymphocytes. Plasmapheresis reduced the autoantibody concentration to undetectable levels, which resulted in an increase number of helper T cells. These T cells provided normal in vitro helper activity. Plasmapheresis did not correct a concomitant suppressor T-cell defect, and the clinical remission ended during the fifth month of exchange therapy.

ACQUIRED agammaglobulinemia (AAG) or common variable immunodeficiency disease (CVID) is a disorder characterized by a diminished quantity of circulating immunoglobulin, qualitatively abnormal B lymphocytes, and impaired cell-mediated immunity. Although individuals afflicted with AAG have a more favorable prognosis than those with other pathophysiologically related disorders, life-threatening infection and premature death are the ultimate sequela of the disease. The use of prophylactic antibiotics is of no avail. Temporizing measures, such as the administration of gammaglobulin, frequently improve the quality of life. Successful bone marrow transplantation offers the most definitive therapeutic results. During the past 7 yr, we had the opportunity to care for a patient with AAG. In addition to the customary immunologic deficiencies, the patient produced a cytotoxic antibody that reacted with autologous and histoidentical helper T lymphocytes. The presence of this potentially pathogenic immunoglobulin provided a rationale for the use of plasma exchange therapy.

PATIENT HISTORY AND IMMUNOLOGIC STATUS

D.L. was a 17-yr-old white male with confirmed AAG. Details of his medical history and clinical course have been reported previously.1 His early childhood was complicated by recurrent episodes of dacrocystitis, conjunctivitis, upper respiratory tract infections, and pneumonia. Pulmonary infections eventually progressed to bronchiectasis. Immunoglobulins were measured in early childhood and throughout adolescence. IgG levels averaged 34 mg/dl and never exceeded 70 mg/dl. Levels of IgM and IgA were consistently below detectable limits. He was anergic to Candida albicans, streptokinase-streptodornase, and dinitrochlorobenzene. His peripheral blood lymphocytes averaged 1200/cu mm, ranging from 800 to 2200/cu mm. T cells constituted 70% of the total lymphocyte count. His TH2 cell population (helper T cells) was 25%-30% of normal. Phytohemagglutinin, concanavalin-A, pokeweed mitogen, Candida albicans, staphylococcus protein A, and streptokinase-streptodornase failed to stimulate his lymphocytes in short-term culture. Coculture experiments using the patient's T lymphocytes proved that these cells suppressed the secretion of immunoglobulins by both autologous and normal donor B lymphocytes. His serum was shown to contain an IgG globulin that was cytotoxic to autologous and histoidentical helper T cells.1,3

The patient's clinical condition failed to improve with conventional therapy, including periodic administrations of gammaglobulin and fresh frozen plasma. Thymosin and transfer factor therapy were begun at age 13 with transient clinical improvement. Unfortunately, this favorable response lasted less than a year, terminating in repetitive episodes of severe pulmonary infection and suppurative otitis media.

The sustained reduction of TH2 lymphocytes and the presence of a circulating lymphocytotoxic immunoglobulin with autologous reactivity, provided rationale for the use of plasma exchange therapy. Plasmapheresis was performed every 2 wk for a total of 5 mo.

MATERIALS AND METHODS

Peripheral blood lymphocytes were quantitated by conventional differential counting and with a Technicon Hemalog D, which uses spectrophotometric principles.1 Immunoglobulins were detected by immunoelectrophoresis and quantitated by radial immunodiffusion. T and B lymphocytes were identified by sheep red blood cell (SRBC) rosette formation employing E and EAC cells. T cells were determined by rosetting with IgM-coated SRBC, as reported previously.1,4 Suspensions of T lymphocytes obtained by SRBC rosetting were incubated with horse anti-human thymocyte globulin, washed and reincubated with fluorescein-conjugated rabbit F(ab')2 antiserum to horse globulin, and analyzed with a fluorescence activated cell sorter (FACS II). The response of lymphocytes to mitogens was measured by the incorporation of tritiated thymidine into stimulated cells. Immunoglobulin secretion by B lymphocytes was measured by the double antibody precipitation technique of Litwin.1,4'9 Suppressor T cells were identified by the reverse plaque assay and by coculture experiments with homologous B-cell preparations in the presence of pokeweed mitogen. The lymphocytotoxic factor in the patient's serum was assessed by the Trypan blue dye exclusion technique of James et al.1,4 and the 51Cr-microcytotoxicity method of Brunner et al.7 Cell pellets containing either 106 peripheral blood lymphocytes or 104 T cells obtained by rosetting with sheep red blood cells were incubated for 1 hr at 37°C with 0.2-ml aliquots of the patient's serum or 0.2 ml of a purified IgG fraction of his serum obtained by ammonium sulfate precipitation followed by DE-52 ion exchange chromatography. The cells were washed free of the serum or the IgG-containing fraction and incubated with guinea pig red blood cells to measure the percentage of cells surviving in the presence of antibody.
pig complement for 18 hr at 37°C. Lymphocytotoxicity was considered significant at the 15% level. The effect of the patient's cytotoxic antibody on helper T-cell function was assessed in coculture experiments employing mixtures of B lymphocytes and T cells that had been irradiated to eliminate suppressor cell activity. Fluorescent-labeled anti-human IgG was used to enumerate the binding of the lymphocytotoxic autoantibody to T cells.

Plasmapheresis was performed on the Haemonetics Model 30 (discontinuous) blood processor. During each procedure, 650 ml of plasma, approximately 20 ml/kg, were removed and replaced with an equal amount of normal serum albumin (5%). Anticoagulant ACD formula B was used throughout the exchange procedures. Lymphocytapheresis was not performed during any of the procedures.

**RESULTS**

Following the first plasma exchange, a decrease in the activity of the cytotoxic autoantibody was observed. A decrease in antibody activity, as measured by indirect immunofluorescence, followed each subsequent procedure. Prior to plasmapheresis, the patient's serum reacted with 39% of histoidentical T cells. Reactivity was reduced to 16% following the second plasmapheresis and by the fifth procedure was no longer evident by fluorescence microscopy or FACS II analysis.

The fall in antibody concentration was accompanied by an increase in the patient's total lymphocyte count and an absolute increase in the T[μ] and TH[2] (helper) cell populations (Fig. 1). The absolute lymphocyte count rose from a nadir of 800–5000/µl. TH[2] cells increased from 20% to 42% of the total T-cell population. Prior to plasma exchange, T[μ] cells constituted 8%–16% of the lymphocyte population. Following the exchange procedures, this subset increased to 79%.

Prior to plasmapheresis, the patient's serum blocked the helper T-cell–B-cell interaction necessary for immunoglobulin secretion in vitro. This blocking activity was eliminated by the first plasma exchange. Both the serologic and the cytologic improvements persisted throughout the period of plasma exchange. However, 48 days after plasmapheresis therapy was discontinued, the TH[2] lymphocyte antibody was again detectable in the patient's serum, binding to 42% of the histoidentical helper T cells. This activity was not influenced by any subsequent therapy.

Clinical improvement was evident during the first 4 mo of plasmapheresis therapy, but terminated in the fifth month with recurrent bouts of pneumonia and suppurative otitis media. A thymectomy was performed. The postoperative course was complicated by osteomyelitis of the sternum. The infection did not clear with intensive specific antimicrobial therapy. A bone marrow transplant was performed using a histoidentical sibling as the donor, but the patient died of sepsis shortly afterwards.

**DISCUSSION**

Acquired agammaglobulinemia is a heterologous group of disorders characterized by altered humoral and cellular immunity. The pathophysiology of the disease is unknown and in many instances is unrelated to either an inherent stem cell or B-lymphocyte defect. Patients with normal numbers of T and B cells fail to develop "normal B cell function." In previous reports this defect has been associated with aberrant suppressor T-cell activity and with a functionally abnormal helper T-cell subset.11 Our patient had both a markedly reduced helper (TH[2]) T-cell population and an increased number of activated suppressor T cells.1
The patient's B cells secreted gammaglobulin when cocultured with histoidentical T cells obtained from a sibling. Similarly, the addition of autologous helper T cells to a culture of the patient's B cells initiated the secretion of gammaglobulin. These in vitro observations suggested that the patient's helper T-cell defect was a quantitative and not a qualitative problem, and that this problem, at least in part, was attributable to the circulating anti-TH_{2} lymphocytotoxic autoantibody. Pathophysiologically, the patient's disease could be considered an autoimmune disorder. It was therefore of great interest to ascertain what effect the elimination of the autoantibody would exert on the helper T-cell population. Extrapolating from in vitro data, restoration of a phenotypic balance between the T-cell subsets could alleviate the agammaglobulinemia.

Plasma exchange has been used in the supportive therapy of a variety of autoimmune disorders, including Goodpasture's syndrome, myasthenia gravis, idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia, and "acquired" hemophilia. In most instances a direct relationship has been noted between the level of circulating autoantibody and the clinical response. Although plasmapheresis is neither specific nor definitive, it provides sufficient time to initiate a more conventional form of therapy.

REFERENCES

Partial immunologic reconstitution of a patient with acquired agammaglobulinemia: a transient phenomenon accompanying therapeutic plasmapheresis

B Wenz and A Rubinstein