Transmission of Toxoplasmosis by Leukocyte Transfusion

By Stuart E. Siegel, Milford N. Lunde, Albert H. Gelderman, Roger H. Halterman, James A. Brown, Arthur S. Levine and Robert G. Graw, Jr.

Four patients with acute leukemia developed toxoplasmosis following leukocyte transfusions from donors with chronic myelogenous leukemia. Serologic data, obtained from the donors retrospectively, revealed elevated antitoxoplasma antibody titers, suggesting that the transfused leukocytes were the source of the organism in the recipients.

STUDIES IN RABBITS HAVE SHOWN that the protozoan *Toxoplasma gondii* is able to parasitize peripheral blood leukocytes.[1] In humans, parasitemia caused by this organism has been demonstrated in an asymptomatic blood donor,[2] and in a woman who delivered a congenitally infected infant.[3] Despite these observations, no cases of acute toxoplasmosis acquired via transfusion of blood products have been reported previously. We have recently observed four patients with acute leukemia who developed toxoplasmosis following transfusion of leukocytes (WBC) from donors with chronic myelogenous leukemia (CML). These transfusions had been given in an attempt to treat granulocytopenia complicated by bacterial infection.[4] Serologic data, obtained retrospectively, suggest that the transfused leukocytes were the source of the parasite in the recipients.

Materials and Methods

Peripheral blood leukocytes were collected from the CML donors using either a conventional single-unit leukapheresis technique[5] or the NCI-IBM continuous-flow blood cell separator.[6] An average of 5×10^{10} leukocytes were given with each transfusion. The leukocytes were rapidly transfused intravenously through a platelet recipient set (Fenwal HB-182).

Sera were obtained at first admission from all patients admitted to the Leukemia Service. Serial samples were drawn as clinically indicated or obtained from the Leukemia

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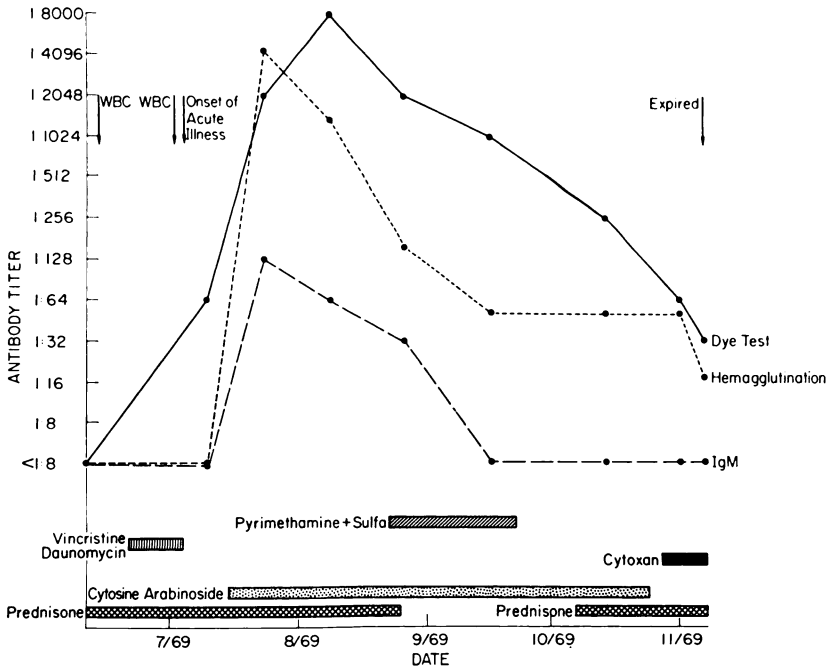

Fig. 1.—Recipient No. 1. Clinical course and antitoxoplasma antibody titers.

Service serum bank. Antitoxoplasma Sabin-Feldman dye test (DT) and hemagglutination (HA) antibody titers were determined by methods previously described.[7,8] The indirect fluorescent antitoxoplasma IgM antibody test was performed as described by Remington et al.[9] Titers of less than 1:8 were considered negative. Isolation of toxoplasma by mouse inoculation was accomplished by the technique of Jacobs et al.[10]

Case Reports

Recipient No. 1 was a 5-year-old girl with acute lymphocytic leukemia (ALL) diagnosed in June 1967. She received various schedules of combination chemotherapy and had achieved two remissions prior to relapse in June 1969. At that time, she received five courses of daunomycin in combination with vincristine and prednisone, and developed severe bone marrow hypoplasia accompanied by an infraorbital cellulitis. Chemotherapy was withheld and she was given, in addition to antibiotics, three leukocyte transfusions from CML Donor No. 1. Four weeks after the initial transfusion she developed fever, rash and pneumonia, followed rapidly by bilateral pleural effusion, hepatitis, grand mal seizures and congestive heart failure. Repeated bacterial, fungal and viral cultures were sterile, and her pneumonia and fever did not respond to broad-spectrum antibiotic therapy. Antitoxoplasma HA, Sabin-Feldman DT, and indirect fluorescent IgM antibody titers, which had been negative, rose to 1:4374, 1:8000, and 1:128 respectively (Fig. 1).

She recovered from this acute episode with supportive therapy over a 4-week period. A bone marrow remission was subsequently induced with cytosine arabinoside therapy. Because of persistent hepatomegaly, abnormal liver function tests, low-grade fever, and elevated antitoxoplasma titers, toxoplasmosis was suspected and she was treated with a 4-week course of pyrimethamine and sulfadiazine. Fever and hepatomegaly resolved and liver function tests approached normal values within two weeks of the start of this therapy. Simultaneously, the antitoxoplasma HA titer fell to 1:54 and the IgM titer to less than 1:8.

Three months later, the patient's leukemia relapsed. She developed congestive heart

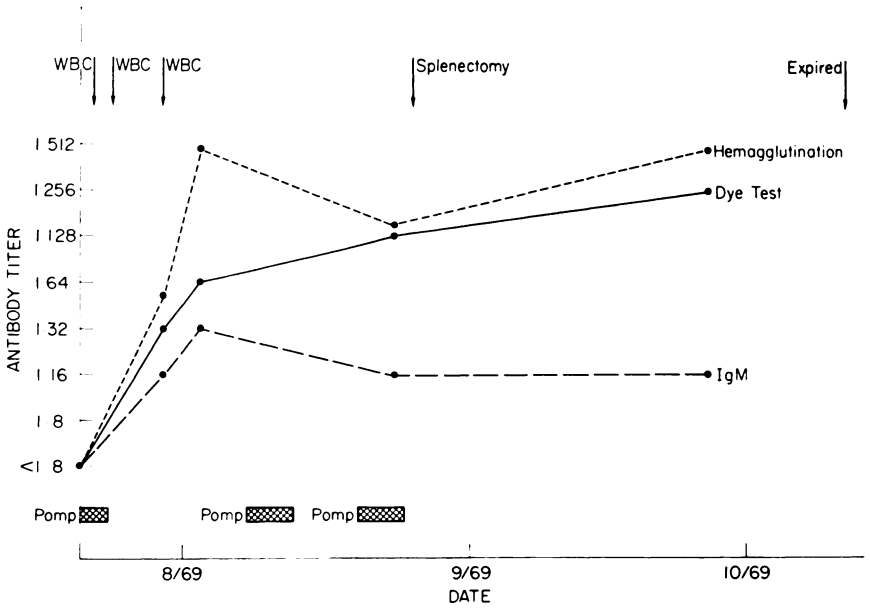

Fig. 2.—Recipient No. 2. Clinical course and antitoxoplasma antibody titers.

failure, progressive liver failure and grand mal seizures, expiring on November 7, 1969. During her final illness, the antitoxoplasma HA titer remained 1:54 and the IgM titer did not rise.

At postmortem examination, toxoplasma cysts and trophozoites were seen in histologic sections of the patient's heart, pancreas, brain, bone marrow, lymph nodes, and peripheral nerve ganglia. Intracellular organisms were also seen in leukemic infiltrates of these tissues. Cell suspensions of tissues obtained at autopsy were inoculated intraperitoneally into 20–25-Gm. NIH mice. Toxoplasma were subsequently isolated from the peritoneal fluid of mice inoculated with brain, liver, lung and spleen.

Recipient No. 2 was an 11-year-old girl with ALL diagnosed in July 1969. Although she obtained a remission with combination chemotherapy (POMP: prednisone, vincristine, methotrexate, 6-mercaptopurine),[11] granulocytopenia persisted and *Escherichia coli* septicemia developed. Antibiotic therapy was instituted and she received three leukocyte transfusions from CML Donor No. 1 over a 1-week period. Blood cultures subsequently became sterile. Four weeks following the initial leukocyte transfusion, her fever recurred, accompanied by massive splenomegaly and diffuse abdominal pain. A sulfur colloid scan of the spleen suggested a space-occupying lesion. Laparotomy was performed on August 25, 1969, and a large splenic abscess was found from which *E. coli* was cultured. A single toxoplasma cyst was seen in histologic sections of the spleen. Toxoplasma cysts were seen in the brains of mice inoculated with splenic tissue. Postoperatively, the patient developed Gram-negative septicemia and expired on October 12, 1969.

Postmortem histologic sections revealed toxoplasma cysts in the lung and heart. Antitoxoplasma titers, which had been negative prior to the leukocyte transfusion, rose, the HA titer reaching 1:486, the DT titer 1:64, and the IgM titer 1:32 three weeks following the initial transfusion (Fig. 2). Terminally, her titers remained elevated.

Donor No. 1 was a 49-year-old woman with CML diagnosed in May 1969. She had no history suggestive of toxoplasmosis; however, sera obtained 2 months prior to her first leukocyte donation and examined retrospectively, revealed an HA titer of 1:4374, DT titer of 1:2048, and negative IgM titer. When it became apparent that two recipients of her leukocytes had developed toxoplasmosis, sera obtained at the time of the donations

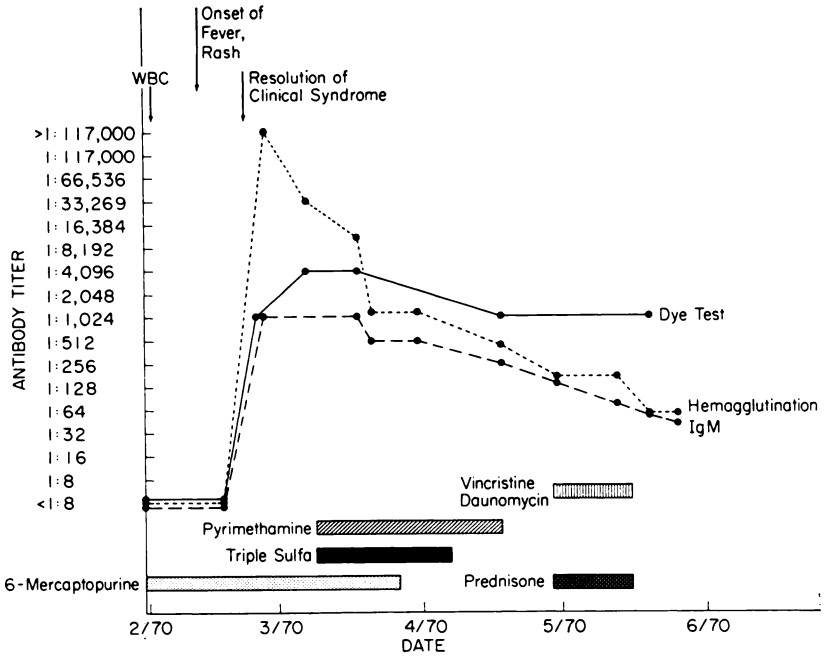

Fig. 3.—Recipient No. 3. Clinical course and antitoxoplasma antibody titers.

were examined. The HA titer was 1:4374, the DT titer 1:4096, and the IgM titer 1:16. Attempts to isolate the organism from the buffy coat of the donor have been unsuccessful.

Recipient No. 3 was a 6-year-old girl with ALL diagnosed in August 1968. She achieved initial remission with POMP therapy and continued on POMP maintenance therapy until relapse in January 1970. Following an unsuccessful attempt to induce bone marrow remission, she developed marked granulocytopenia accompanied by multiple areas of *Staphylococcus aureus* cellulitis. In addition to antibiotics, she received a single leukocyte transfusion from CML Donor No. 2. Her fever and cellulitis resolved and her peripheral granulocyte count returned to normal. Three weeks following the leukocyte transfusion, she developed fever, rash, and abnormal liver function tests. Antitoxoplasma titers had been negative immediately prior to the leukocyte transfusion. At the time of the acute illness, the antitoxoplasma HA titer rose to 1:117,000 or greater, the DT titer to 1:1024, and the IgM titer to 1:1024 (Fig. 3). A percutaneous liver biopsy was performed and toxoplasma were isolated from the peritoneal exudate of mice inoculated with cell suspensions of the biopsy specimen. The patient was treated subsequently for six weeks with triple sulfa and pyrimethamine therapy. Following completion of therapy, the antitoxoplasma HA titer had fallen to 1:486, and the IgM titer to 1:256.

Recipient No. 4 was a 48-year-old man with acute myelogenous leukemia diagnosed in February 1969. He obtained an initial remission with cytosine arabinoside and was maintained on this drug until relapse in December 1969. Reinduction was attempted with various schedules of combination chemotherapy. Severe pancytopenia resulted, accompanied by *Klebsiella* hidradenitis and septicemia. He was given antibiotics and three leukocyte transfusions from CML Donor No. 2. Subsequently, he became afebrile and the hidradenitis cleared. Three weeks following the first leukocyte transfusion, he developed fever, rash, congestive heart failure, and encephalopathy. Antitoxoplasma antibody titers, which had been negative prior to this illness, rose. The HA titer reached 1:162, and the DT titer 1:64 (Fig. 4). The IgM titer remained negative. Toxoplasma were isolated from the peritoneal exudate of mice inoculated with heparinized peripheral blood from the patient. Sulfadiazine

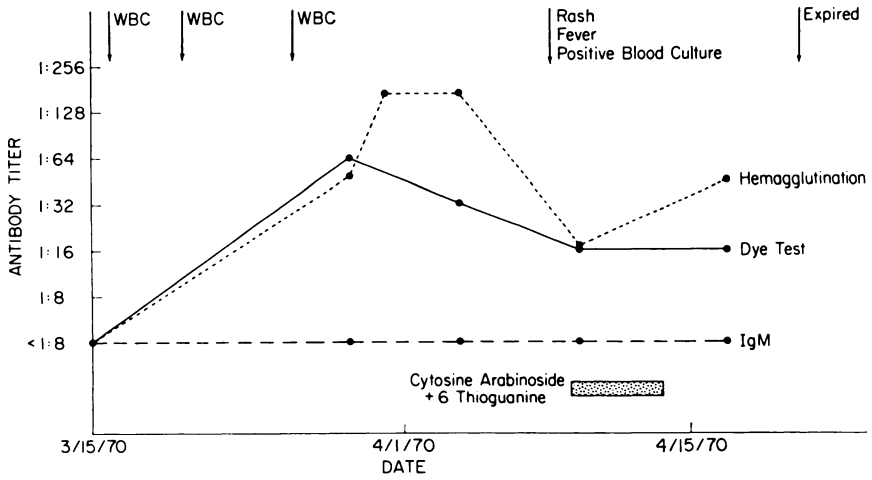

Fig. 4.—Recipient No. 4. Clinical course and antitoxoplasma antibody titers.

and pyrimethamine therapy was instituted, but the patient developed progressive renal and hepatic failure, expiring on April 21, 1970.

Toxoplasma were isolated from the peritoneal exudate of mice inoculated with postmortem specimens of brain and heart.

Donor No. 2 was a 23-year-old man with CML diagnosed in October 1966. No previous history suggestive of toxoplasmosis was elicited. He was treated with myeleran and P-32 and at the time of the donations he was receiving myeleran 8 mg/day. His antitoxoplasma HA titer, which had been 1:1450 2 months prior to the leukocyte donations, rose to 1:117,000 at the time of the donations. The IgM titer remained negative until 2 weeks following the donations when a titer of 1:8 was obtained. Attempts to culture the organism from the donor's peripheral blood leukocytes have been, so far, unsuccessful.

Two additional patients with acute leukemia received one or more leukocyte transfusions from Donor No. 1; however, both died within 10 days of the transfusions. No toxoplasma were seen in postmortem histologic sections of tissues from these patients. No tissues were obtained for toxoplasma isolation.

Discussion

The possibility of transmission of toxoplasmosis via blood transfusions was suggested by Kimball et al.,[12] but these authors were unable to show an increased incidence of elevated dye test titers in multiply transfused patients with thalassemia major as compared with a group of normal untransfused controls of the same age. However, since toxoplasma organisms retain their viability after suspension in citrated blood and storage at 5°C for up to 50 days,[13] and since organisms have been recovered from the buffy coat of patients with toxoplasmosis,[3] it appears likely that toxoplasmosis could be acquired in this fashion, particularly if large concentrations of leukocytes are given from donors who, themselves, are likely to be infected with toxoplasma.

The significance of the various antitoxoplasma antibody titers in relation to possible parasitemia of blood donors has not been evaluated; nor has the maximum duration of parasitemia in asymptomatic donors been determined. However, Miller et al.[3] have isolated toxoplasma from the blood of a woman who 14 months earlier had delivered a congenitally infected infant. Since anti-

bodies detected by the dye test and hemagglutination test may remain elevated for years after an active infection,[14] an isolated positive titer in either of these tests is not proof of active disease or parasitemia. The indirect fluorescent antibody test for toxoplasma specific IgM antibody is a more useful means of differentiating between active and inactive infection; but even with this test, elevated levels may persist for months after active infection.

Evaluation of titers in patients receiving blood transfusions is also complex, in view of passive transfer of antibody.[15] Indeed, Feldman estimates that 20–50 per cent of adults have detectable HA or DT antibody.[16] HA and DT titers acquired in this manner are, however, usually low and indirect fluorescent IgM antibody titers are negative.

In the four cases presented, serologic evidence suggests the transmission of toxoplasmosis via the transfusion of leukocytes. The recipients had no demonstrable antibodies to the organisms prior to their leukocyte transfusions. Each of the recipients developed rising antibody titers after receiving the leukocyte transfusions and toxoplasma were isolated from their tissues. The donors had, at the time of the transfusions, antitoxoplasma DT and HA titers greater than 1:4000.

These results also suggest that the risk of toxoplasma transmission may be increased if parasitized leukocytes are transfused in a high concentration. Furthermore, patients given large amounts of leukocyte-rich blood products are often receiving cancer chemotherapy or immunosuppressive agents and are especially susceptible to disseminated toxoplasmosis. We would, therefore, propose that the use of blood donors with high antitoxoplasma DT and HA titers and/or positive IgM titers at any dilution be discouraged, especially if the host defenses of the recipient are compromised.

REFERENCES

1. Huldt, G.: Experimental toxoplasmosis. Effect of corticosteroids on rabbits with varying degrees of immunity. Acta Path. Microbiol. Scand. 68:604, 1966.

2. Neto, A. V., Cotrim, J. X., Laus, W. C., and DeO. Gomes, M. C.: Note on the finding of toxoplasma gondii in blood intended for transfusion. Rev. Inst. Med. Trop. S. Paulo 5:68, 1963.

3. Miller, M. J., Aronson, W. J., and Remington, J. S.: Late parasitemia in asymptomatic acquired toxoplasmosis. Ann. Int. Med. 71:139, 1969.

4. Graw, R. G., Jr., Henderson, E. S., and Perry, S.: Leukocyte procurement and transfusion into leukopenic patients. Proc. Internat. Symp. of the Centre National de la Recherche Scientifique on White Cell Transfusions, 1969, Paris, France (in press).

5. Morse, E. E., Carbone, P. P., Freireich, E. J, Bronson, W., and Kliman, A.: The transfusion of leukocytes from donors with chronic myelocytic leukemia. Tranfusion 6:175, 1966.

6. Buckner, D., Graw, R. J., Jr., Eisel, R. J., Henderson, E. S., and Perry, S.: Leukapheresis by continuous flow centrifugation (CFC) in patients with chronic myelocytic leukemia (CML). Blood 33: 353, 1969.

7. Sabin, A. B., and Feldman, H. A.: Dyes and microchemical indicators of a new immunity phonomenon affecting a protozoan parasite (toxoplasma). Science 108: 660, 1948.

8. Jacobs, L., and Lunde, M. N.: Hemagglutination test for toxoplasmosis. J. Parasit. 43:308, 1957.

9. Remington, J. S., Miller, M. J., and Brownlee, I.: IgM antibodies in acute toxoplasmosis. II. Prevelance and significance in acquired cases. J. Lab. Clin. Med. 71: 855, 1968.

10. Jacobs, L., Remington, J. S., and Melton, M. L.: A survey of meat samples from swine, cattle, and sheep for the presence of encysted toxoplasma. J. Parasit. 46: 23, 1960.

11. Henderson, E. S.: Treatment of acute leukemia. Sem. Hema. 6:271, 1969.

12. Kimball, A. C., Kean, B. H., and Kellner, A.: The risk of transmitting toxoplasmosis by blood transfusion. Transfustion 5:447, 1965.

13. Talice, R. V., Gurri, J., Royol, J., and Perez-Moreira, L.: Investigaciones sobre la *Toxoplasma gondii* en sangue destinado a transfusao. Fac. Med. Montevideo 42:143, 1957.

14. Sabin, A. B., Eichenwald, H., Feldman, H., and Jacobs, L.: Present status of clinical manifestations of toxoplasmosis in man. JAMA 150:1063, 1952.

15. Vogel, C. L., and Lunde, M. N.: Toxoplasma serology in patients with malignant diseases of the reticuloendothelial system. Cancer 23:614, 1969.

16. Feldman, H. A.: Toxoplasmosis. New Eng. J. Med. 279:1431, 1968.

blood 1971 37: 388-394

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