Survival of *Borrelia burgdorferi* in blood products

S. J. Badon, R. D. Fister, and R. G. Cable

The incidence of Lyme disease is rapidly increasing in the United States. To assess the potential of transmission of the disease through blood transfusion, we studied the survival of *Borrelia burgdorferi* in blood products under blood bank storage conditions. Two units of whole blood, separated into red cells (RBCs), fresh-frozen plasma (FFP), and platelet concentrates (PCs), were inoculated with *B. burgdorferi* (strain B31) in concentrations of approximately 3000 organisms per mL of RBCs and FFP and 200 organisms per mL of PCs. Products were then stored under blood banking conditions and sampled at several storage times. The viability of the spirochete in blood components was determined by darkfield microscopic examination of cultures in modified Kelly's medium. The organism was shown to survive in RBCs (4° C) and FFP (below −18° C) for 45 days and in PCs (20–24° C) for 6 days. The results of this study do not exclude the possibility of transmission of Lyme disease through blood transfusion. **TRANSFUSION** 1989;29:581–583.

In the United States, Lyme disease, caused by the spirochete *Borrelia burgdorferi,* is transmitted by the *Ixodes dammini* tick in the Northeast (Connecticut, Delaware, Maryland, Massachusetts, New Jersey, New York, Pennsylvania, and Rhode Island) and the Midwest (Minnesota and Wisconsin) and by the *Ixodes pacificus* tick in the West (California, Nevada, Orgeon, and Utah).[1,2] In certain towns in Connecticut, the incidence has increased 163 percent since 1977, and there is evidence that the disease is spreading inland.[3]

Available data suggest that there is a spirochetemic phase. The organism has been cultured from blood and serum,[4–6] and it has been identified in the myocardium,[7] eye,[8] skin,[4,6,9,10] synovium and synovial fluid,[10–12] and cerebrospinal fluid (CSF).[4,6,10] Transplacental infection of the fetus has also been demonstrated.[13]

This spirochetemia may be of significance in blood transfusion, although no cases of transfusion-transmitted Lyme disease have been reported to date. To determine whether the spirochete can survive in blood products, we inoculated red cells (RBCs), fresh-frozen plasma (FFP), and platelet concentrates (PCs), stored them under appropriate blood banking conditions, and cultured them.

Materials and Methods

B. burgdorferi (strain B31) was grown in 500-mL bottles containing modified Kelly's medium.[4] Spirochete densities of 10^{10} organisms per mL were reached after 7 days of incubation. The organisms were then washed three times in 50 mL of phosphate-buffered saline (PBS) at pH 7.2, pelleted by centrifugation at 10,000 × *g* for 20 minutes at 4° C (Sorvall RC5 centrifuge, DuPont, Wilmington, DE), washed three times in PBS at pH 7.4, and resuspended in PBS to a final concentration of 10^6 organisms per mL.

From the American Red Cross Blood Services, Connecticut Region, and the Department of Clinical Microbiology, University of Connecticut Health Center, Farmington, Connecticut.

Received for publication January 4, 1989; revision received April 20, 1989, and accepted April 20, 1989.

Whole blood from two donors was collected into triple blood bags (Fenwal Laboratories, Deerfield, IL) in CPDA-1. Donor history cards were reviewed and found to be negative for evidence of tick bite or history of Lyme disease. The units of whole blood were processed using a light spin (2800 rpm for 3 min at 20–24° C in a Sorvall RC3C centrifuge) into RBCs and platelet-rich plasma (PRP). We added 100 mL of ADSOL (Fenwal Laboratories, Deerfield, IL) to the RBCs. PRP was processed using a hard spin (3700 rpm for 6 min at 20–24° C) into PCs and FFP. *B. burgdorferi* (strain B31) was injected aseptically into each blood product so that the final concentration was approximately 3000 organisms per mL of RBCs and plasma and 200 organisms per mL of platelets. The plasma from each donor was then aliquoted into two additional transfer bags (Fenwal Laboratories) using a sterile connecting device (SCD 312, Haemonetics, Braintree, MA), so that there were three bags containing equal amounts of plasma. At the same time, a plasma sample from each donor was tested by an enzyme-linked immunosorbent assay (ELISA) for the presence of antibodies to the spirochete.

The ELISA method for antibody detection was similar to that of Magnarelli et al.[14] Donor sera, diluted 1:160 to 1:5120 along with positive and negative controls, were added to microtiter plates (Nunc-Immuno Plate, Marsh Biochemical Products, Rochester, NY) containing antigen-coated and nonspecific control wells. After an incubation period of 1 hour at 37° C, the plates were washed five times with a solution of PBS with 0.05 percent Tween. After the final wash, a peroxidase-conjugated anti-human goat antisera for either IgM or IgG (Sigma, St. Louis, MO) was added to each plate and allowed to incubate for 1 hour at 37° C and washed five times with PBS with 0.05 percent Tween; then a substrate chromagen, made from equal volumes of 2,2-azino-di(3-ethyl-benzthiazoline sulfonate) and hydrogen peroxide (Kirkegaard and Perry Laboratories, Gaithersburg, MD), was added to each well. The plates were read at 414 nm on a spectrophotometer (Titertek Multiskan Plus, Flow Laboratories, McLean, VA).

A serum dilution was considered positive if the net absorbance of the antigen minus the nonspecific wells was 3 standard deviations(SD) or more above the mean absorbance of the negative control wells.

The RBCs were stored at 4° C, the FFP at below −18° C, and the PCs at 20 to 24° C with continuous agitation on a platelet agitator (Helmer Labs, St. Paul, MN). RBCs and FFP were cultured 2, 7, and 45 days after injection. For each plasma sampling, one of the three aliquots from each donor was thawed and discarded after sampling. Platelets were cultured 2 and 6

days after inoculation. At each sampling period, 2 mL of each blood product was withdrawn aseptically, injected into 3 mL of modified Kelly's medium, and incubated at 30° C for 14 days. At the end of each incubation period, 0.1 mL of the culture suspension was examined under a microscope (Kramer Scientific Corporation, Yonkers, NY) with a darkfield condenser (Kramer Scientific Corporation, Yonkers, NY) for the presence of organisms and motility.

Results

The ELISA was negative for the presence of antibodies to *B. burgdorferi* in both donors. Numerous viable, motile spirochetes were seen under a darkfield microscope from cultures taken from RBCs and FFP at 2, 7, and 45 days and from cultures taken from PCs at the end of 2 and 6 days. Careful attention was paid to distinguish brownian movement from the full-length movements of live organisms.

Discussion

The results of these experiments demonstrate that *B. burgdorferi* is able to survive at 20 to 24° C in PCs, at 4° C in RBCs, and at below − 18° C in FFP. The implication of this finding is that, if blood is drawn from an individual who is spirochetemic at the time of donation, the organism could be transmitted by stored blood products. The relatively large bacterial inoculum selected in this study was designed to overcome bactericidal properties that white cells or plasma proteins may have against the spirochete. Two recent reports[15,16] have reported that the organism was capable of survival in RBCs (in test tubes) and whole blood (in flasks) at 7 and 4° C, respectively, when inoculated at final concentrations above 10^2 organisms per mL. However, at final concentrations of 10^2 organisms per mL, the organism was largely inhibited.[15]

A reading of the available literature infers that the magnitude and duration of spirochetemia in persons infected with Lyme disease are limited. Benach et al.[5] were able to culture the organism from the blood of only 2 of 36 persons with Lyme disease. One patient was culture positive 4 days after a tick bite and the second 7 days after the appearance of erythema chronicum migrans, the typical skin lesion found in 83 percent of cases of Lyme disease.[3] Both patients were in the initial acute symptomatic phase. Steere et al.[4,6] described 4 patients of 65 with Lyme disease from whom the organism could be cultured from spun plasma pellets but not concurrently from whole blood or plasma. The patients with positive blood cultures were symptomatic at the time a specimen was taken for culture, whereas those patients from whom the organism was recovered from the CSF and skin lesion had negative blood cultures.

An important factor involved in the limitation of spirochetemia may be the development of antibodies. To exclude this variable, we tested the two donors by ELISA and found them negative. IgM antibody reaches its highest point 3 to 6 weeks after the onset of erythema chronicum migrans, whereas IgG antibodies are highest when arthritis is present, months later.[4,17] In treated patients without complications, the IgM and IgG antibodies can drop to undetectable levels, but in patients with complications, both antibodies can persist for years.[4] It is likely that these antibodies would be inhibitory to the organisms.

Thus, the danger of blood-borne transmission appears to be greatest during the first week after the onset of symptoms. Symptomatic donors should be eliminated during the routine health history interview. There may, however, be patients who do not have erythema chronicum migrans, fever, or malaise. If they became donors they could possibly transmit the spirochete with their donation, if the organism can survive blood bank storage. Our study has confirmed the survival of *B. burgdorferi* under these conditions and hence the possibility of transmitting Lyme disease.

To date, there have been no cases of Lyme disease attributed to blood transfusion. However, the clinical manifestations of the disease are protean and, except for erythema chronicum migrans (which may go undetected by donors, especially if the tick bite is in an inconspicuous place), are not diagnostic. Posttransfusion cases may not have this diagnostic rash, in which case, they may go undetected.

With the information that is currently available, it would not seem appropriate to eliminate donors on geographic grounds. Diagnostic tests for the antibody to *B. burgdorferi* are least sensitive in the early stages of the disease,[17-21] yet that is the time when the donor is presumably more likely to be spirochetemic. Hence, antibody testing may do nothing to eliminate the donors who would be most likely to pass on the spirochete with their blood donation.

On the basis of this study, the possibility of transmission of Lyme disease through blood transfusions has not been excluded. Additional study of the nature and degree of spirochetemia following tick bite and of the survival of low inoculations of *B. burgdorferi* in blood products is warranted.

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Stanley J. Badon, MD, Assistant Medical Director, American Red Cross, Connecticut Region; and Instructor, Department of Laboratories, University of Connecticut, 263 Farmington Avenue, Farmington, CT 06032. [Reprint requests]

Richard D. Fister, PhD, Fellow, Department of Clinical Microbiology, University of Connecticut.

Ritchard G. Cable, MD, Medical Director, American Red Cross, Connecticut Region, and Associate Professor, Department of Laboratories, University of Connecticut.

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