Survival of *Borrelia burgdorferi* in human blood stored under blood banking conditions

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Hematogenous dissemination of organisms occurs in many spirochetal diseases, including Lyme disease and syphilis. Although syphilis has been transmitted by transfusion, in the vast majority of cases, only fresh blood products were involved, in part because *Treponema pallidum* survives poorly when refrigerated in citrated blood. Because of the rising incidence of Lyme disease in certain areas, whether its causative agent, *Borrelia burgdorferi*, could survive under blood banking conditions was studied. Dilutions of stock cultures of two strains of *B. burgdorferi* were inoculated into samples of citrated red cells (RBCs). Viable spirochetes were recovered from RBCs inoculated with 10^6 organisms per mL, after refrigeration for as long as 6 weeks. It is concluded that *B. burgdorferi* may survive storage under blood banking conditions and that transfusion-related Lyme disease is theoretically possible. **TRANSFUSION** 1990;30:298-301.

BORRELIA BURGDORFERI infection, or Lyme disease, is endemic in much of the United States,[1] as well as in Canada,[2] Europe,[3] Asia,[4] and Australia.[5] The disease has certain similarities to syphilis,[6,7] including a period of hematogenous dissemination.

Syphilis has been transmitted via contaminated blood transfusions,[8-16] which has prompted routine serologic screening by blood banks. However, experiments have demonstrated that *Treponema pallidum* does not survive more than a few days under present blood banking conditions.[12,16-21]

Transfusion-associated Lyme disease has not been reported, although another spirochete, *B. recurrentis*, has been reported to cause transfusion-associated relapsing fever.[22,23] The study described herein was designed to evaluate whether *B. burgdorferi* can survive under standard conditions of blood storage used in the United States.

Materials and Methods

We drew blood from three healthy human volunteers who had no history of Lyme disease and who had not taken antibiotics for the 30 days before phlebotomy. Lyme serologies, done by the fluorescent immunoassay (FIAX) method,[24] were negative for all subjects. One person was group A, Rh positive, and two persons were group O, Rh positive on standard blood typing technique.

Using aseptic technique, we drew blood into a sterile syringe containing a known amount of the anticoagulant/preservative CPDA-1, in concordance with the standard conditions used by blood collection agencies in the United States.[25] We centrifuged the mixture for 15 minutes at 2200 rpm and removed the plasma with a sterile pipette, leaving red cell (RBC) concentrates.

We used aliquots from two stock cultures of *B. burgdorferi* to inoculate RBC samples. Strain 297 (provided by Dr. Russell Johnson) was originally cultured from the cerebrospinal fluid of a patient with Lyme disease in Connecticut,[6] and strain CB had been recovered from the blood of a patient with Lyme disease in Westchester County, New York.[26] Both isolates had been maintained (4 years for 297, 1 month for CB) in serial culture using modified Barbour-Stoenner-Kelly (BSK II) medium.[27]

Dilutions of stock cultures were added to 2-mL samples of RBCs cultured in plastic snap-cap tubes (leaving an air space of 2-5% by volume) to make final concentrations of 10^1, 10^2, 10^4, and 10^6 organisms per mL. Organism concentration was determined by the enumeration method of Fieldsteel et al.[28] Each strain was used to inoculate a sample of RBCs at each of the four dilutions.

Controls consisting of aliquots from each stock *B. burgdorferi* culture were added to 2-mL samples of BSK II media to make final concentrations of organisms identical to those in the RBCs. We then refrigerated controls and RBCs at 4°C. Subcultures were performed as follows: we removed 0.1-mL aliquots from RBC and control cultures at intervals of 6 and 12 hours; 1, 2, 3, 4, and 5 days; and 1, 2, and 6 weeks. We added these aliquots to 1 mL of Bsk II media, and incubated them at 33°C for 6 weeks. At the end of this time, we visually inspected the subcultures, first for characteristic color changes in media and then for spirochetes, under a fluorescent microscope after staining with acridine orange.[29] All subcultures were then subcultured again by identical methodology to ensure the viability of organisms, as judged by their ability to replicate. After 6 weeks of incubation following the second subculture, we again performed visual inspection under fluorescent microscopy. The presence, at this time, of multiple spirochetes per field was considered indicative of organism viability.

Statistical analysis was performed using the Fisher's exact test and chi-square methods.

Results

Viable *B. burgdorferi* spirochetes from RBCs given large inocula of organisms were recovered at each interval for up to

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Table 1. Borrelia burgdorferi *growth* in red cells*

	Incubation interval									
	Hours		Days					Weeks		
Inoculum†	6	12	1	2	3	4	5	1	2	6
10^6	+	+	+	+	+	+	+	+	+	+
10^4	+	+	+	−	+	+	+	+	+	−
10^2	−	−	−	−	−	+	−	−	−	−
10^1	−	−	−	−	−	−	−	−	−	−

* + Indicates growth of either strain in any donor's red cells; − indicates no growth.
† Organisms per mL.

Table 2. Borrelia burgdorferi *growth* in controls (BSK II media)*

	Incubation interval									
	Hours		Days					Weeks		
Inoculum†	6	12	1	2	3	4	5	1	2	6
10^6	+	+	+	+	+	+	+	+	+	−
10^4	+	+	+	+	+	+	+	+	−	−
10^2	+	+	+	+	+	+	−	+	−	−
10^1	−	−	−	+	−	−	+	+	−	−

* + Indicates growth of either strain; − indicates no growth.
† Organisms per mL.

6 weeks (Table 1) and from controls at each interval except at 6 weeks (Table 2). Every case of spirochete growth in subculture was preceded by characteristic media color changes (i.e., from light purple to straw yellow). In several cases in which media color change was noted without spirochete growth, we observed contamination by other bacteria.

Spirochete recovery from both RBCs and controls decreased as an inverse function of inoculum size (Tables 1-3). Samples containing inocula of 10^6 organisms per mL yielded organisms from 16 of 20 control and 48 of 59 RBC subcultures. (One RBC subculture was lost in a laboratory accident.) However, when smaller inocula were used, recovery of the organisms was significantly lower in RBCs than in controls in BSK II media (Table 3). Only 3 of 20 control and 0 of 59 RBC subcultures yielded viable organisms when a concentration of 10^1 organisms per mL was used as an inoculum. It is of interest that subcultures from controls were not uniformly positive, even with high inocula.

We observed no significant differences in the viability of the two strains of *B. burgdorferi*. Both strains were able to grow up to 6 weeks in control media and in RBCs.

Table 3. Borrelia burgdorferi *growth in controls (BSK II) and in human red cells*

	Number positive/total samples (%)		
Inoculum*	Controls	Human red cells	p value
10^6	16/20 (80)	48/59 (81)	0.5†
10^4	14/20 (70)	14/59 (24)	<0.01‡
10^2	11/20 (55)	1/59 (2)	<0.01†
10^1	3/20 (15)	0/59 (0)	<0.02†

* Organisms per mL.
† Fisher's exact test.
‡ Chi square.

Discussion

Lyme disease has frequently been compared to syphilis because of its spirochetal etiology and because of the clinical similarities of the two infections. Syphilis has been well documented to be transmitted by the transfusion of fresh blood products, although there have been only rare reports of *T. pallidum* infection transmitted by transfusion of stored blood.[16,21] Storing citrated blood for 48 to 72 hours,[12,17,18] 120 hours,[19,20] or 168 hours[21] at 2 to 6°C has been shown virtually to eliminate *T. pallidum* viability.

In contrast, our study has demonstrated that *B. burgdorferi* can persist in human RBCs under blood banking conditions (at least when large inocula of organisms are used). We isolated viable spirochetes from specimens of RBCs stored for up to 6 weeks at 4°C. Both strains of *B. burgdorferi* tested showed ability to survive under these conditions.

In the present study, the initial inoculum greatly influenced the recovery of viable organisms from both RBCs and BSK II (control) media. Although *B. burgdorferi* grew readily from stored RBCs and from BSK II media at inocula of 10^6 organisms per mL (a much higher concentration than one might expect in the blood of a patient with Lyme disease), there was progressively less recovery from both sources when smaller inocula were used, suggesting either an inherent insensitivity of the culture methods or diminution of survival under these test conditions. At inocula of 10^1, 10^2, and 10^4 organisms per

mL, recovery of the organisms was significantly lower from RBCs than from controls (Table 3). (No organisms were recovered from RBCs inoculated with 10^1 organisms per mL). This might be attributed, in part, to the bactericidal properties of stored blood.[30,31] Sampling error (i.e., the failure to gather small numbers of organisms while pipetting during subculture, as a result of uneven organism distribution in media) may also have accounted for the poor recovery of spirochetes at low inocula from both RBC and control cultures.

In a preliminary report from France,[32] one strain of *B. burgdorferi* survived in human whole blood for up to 60 days with inocula of 10^6 organisms per mL. However, the effect of varying inocula of organisms was not reported.

The contrasting capability of *B. burgdorferi* and *T. pallidum* to survive in RBCs under blood banking conditions is consistent with the much greater ability of *B. burgdorferi* to survive and multiply under in vitro conditions, generally. *T. pallidum* still has not been successfully maintained for prolonged periods in vitro, and organism reproduction in vitro has been even more difficult to demonstrate.[28,33]

Other bacteria, particularly certain gram-negative rods,[30,31,34] have been found to survive under blood banking conditions. Resistance to the bactericidal properties of stored blood,[30,31] the use of citrate in the anticoagulant as a carbon source,[30,34] and an ability to grow in cold temperatures[30,31,34] were considered to be important factors in the survival of these organisms. What specific role these play in the survival of *B. burgdorferi* in stored RBCs remains to be defined.

The ability of *B. burgdorferi* to survive in stored blood raises the possibility of transmission of Lyme disease by blood transfusion. Whether transfusion-related Lyme disease will occur clinically is also dependent on the frequency and quantity of spirochetemia with *B. burgdorferi*.

It is not clear whether certain stages of Lyme disease are particularly likely to be associated with spirochetemia (and thus with transfusion-induced illness). Whereas syphilis has been transmitted via transfusion from donors with incubating illness[11,12,15,16], with early disease,[9,12,13,15,16] and with asymptomatic latent illness,[8,14] transmission has not been found to occur in late illness.[15,35,36] This is probably a result both of donor screening on clinical and serologic grounds and of decreased organism concentration in blood in late disease.[8,37] As *B. burgdorferi* spirochetemia has thus far been noted only in patients with relatively recent onset of illness,[6,26,38,39] there is reason to believe that the risk of transfusion-related Lyme disease would be highest early in the course of infection.

A mean spirochetal blood concentration of 1.5×10^4 organisms per mL was demonstrated by Stanek[40] in white mice infected with *B. burgdorferi*. However, we are unaware of quantitative data on the level of spirochetemia in humans with Lyme disease, as organisms have only rarely been isolated on culture from these patients. *B. burgdorferi* has not been visualized on peripheral blood smears from patients with Lyme disease, although the reverse is true in cases of relapsing fever. This would imply a lower organism concentration for *B. burgdorferi* than for *B. recurrentis* in the blood of infected patients. A low organism burden would probably affect the occurrence of transfusion-associated Lyme disease.

Since the minimum infectious dose of *B. burgdorferi* in humans is unknown, it is possible that even small numbers of organisms, undetectable by current culture methods, might result in disease. Syphilis has been transmitted to human volunteers by as few as 10 organisms injected subdermally, with a 50 percent infectious dose ID_{50} of 57 organisms.[41]

Lyme disease is endemic in many parts of the world. In view of the findings presented, transfusion-associated Lyme disease must be considered theoretically possible. Further laboratory and epidemiologic studies are warranted to determine whether such transmission actually occurs in a clinical setting.

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