Interaction of *Borrelia burgdorferi Sensu Lato* with Epstein-Barr Virus in Lymphoblastoid Cells

(lymphoblast / viruses / borrelia / interaction)

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Abstract. Since the possibility of interruption of latent EBV infection has been suggested by the induction of the lytic virus cycle with chemical substances, other viruses, and by immunosuppression, we hypothesized that the same effect might happen in *B. burgdorferi sensu lato* infection as happens in Lyme disease patients with positive serology for both agents. We have observed EBV replication in lymphoblastoid cells after superinfection with *B. garinii* and *B. afzelii* strains after 1 and 4 h of their interaction. We found that viral and borrelial antigens persisted in the lymphoblasts for 3 and 4 days. Morphological and functional transformation of both agents facilitate their transfer to daughter cells. Association with lymphoblasts and internalization of *B. garinii* by tube phagocytosis increased replication of viruses more successfully than *B. afzelii* and chemical inductors. Demonstration of such findings must be interpreted cautiously, but may prove a mixed borrelial and viral cause of severe neurological disease.

The Epstein-Barr virus (EBV) as a human lymphotropic herpesvirus may participate in evoking severe neurologic symptoms (Strauss, 1993) in immunodeficient patients. *Borrelia garinii* (*B. garinii*), serotype 4, was also considered to be a neurotropic agent (Marconi et al., 1999).

Unlike viruses, borreliae as a helical form are present in human cerebrospinal fluid (Busch et al., 1996), blood and tissue (Moter et al., 1994) only in low amounts, but under suitable conditions they are able to grow, colonize cell surfaces and form immunocomplexes (Coyle et al., 1995). This allows them to live as intracellular parasites and thus persist in the host tissue (Wang et al., 2000). The association of Borrelia with lymphoblastoid cells and possible interaction with EBV that are in latent phase in these cells was not studied. Serological cross-reaction between anti-viral and anti-borrelial IgM antibodies has been mentioned by Harris (1998). Adherence of spirochetes *Borrelia burgdorferi (B. burgdorferi) sensu lato* to monocytes (Wang et al., 2000), neutrophilic leucocytes (Hulínská et al., 1995), macrophages (Linder et al., 2001) and human B lymphocytes (Dorward et al., 1997) could explain this hypothesis of cross-reactivity in cases of superinfection or co-infection with different bacteria and viruses in the host. Knutson and Sugden (1989) described immortalization of B lymphocytes by the EBV. Borrelial adherence, internalization by fibroblasts (Georgilis et al., 1992) and reactivity with decorin (Guo et al., 1995) allow them to become hidden against antibiotics and the defence immunological reactivity of the host. The uptake of *B. burgdorferi sensu lato* occurs predominantly by the coiling process, which can influence the course of the infection (Linder et al., 2001). The lymphoblastoid P3HR-1 line derived from Burkitt lymphoma (Klein, 1989) was used as a model for the study of the interaction of EBV and spirochetes *B. burgdorferi sensu lato*. EBV pathogenic effects are connected with its capacity of inducing latent infection in B lymphocytes, when the lytic function of the virus is suppressed and several non-structural virus proteins with the regulatory function are formed. This ensures long-term persistence of viral DNA in the infected cells (Thorley-Lawson, 1988). Long-term persistence of borrelial antigen or nucleic acids was observed in patients with Lyme arthritis (Hulínská et al., 1999) and in the central nervous system (Luft et al., 1992).

The state of latent EBV infection can be interrupted by factors that induce a lytic virus cycle, for instance a-butyrate (Klein, 1989), phorbol-ester (TPA), antibody against IgG, hydrocortisone. The interruption of EBV latency can also occur upon superinfection of the cell with other viruses. Latent borrelial infection has also been demonstrated (Karch et al., 1994).

Although serological tests using ELISA and IFA are considered to be sensitive both for EBV and *B. burgdorferi sensu lato*, they are not highly specific for both agents. Possible serological cross-reactions

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Abbreviations: EBV – Epstein-Barr virus, Mab(s) – monoclonal antibodies.

between Borrelia and EBV have been presumed. The possible involvement of *B. burgdorferi sensu lato* infection by activation of EBV in Lyme disease patients can be revealed by experimental study of the conditions of their interaction.

Material and Methods

Spirochetes *B. garinii*, strains 192M and 61E, *Borrelia afzelii* (*B. afzelii*), strains 97 M and Kc90 were isolated from cerebrospinal fluids (CSF) and plasma of patients with clinically and serologically estimated Lyme borreliosis in BSK-H medium (Sigma-Aldrich GmbH, Steinheim, Germany) and kept at –80˚C in aliquots. Borrelial cells were cultured from aliquots in volumes of 10 to 250 ml until they reached the log phase, approximately 3–5 days after inoculation, making up a 1 : 100 dilution. Culture density, approximately 10^6/ml bacteria, was determined by dark-field microscopy by counting in a Petroff-Hausser chamber. Viability of the spirochetes was assessed by their typical corkscrew motility.

Lymphoblastoid P3HR-1 cells were passaged twice weekly in RPMI 1640 medium (SeVac, Prague, Czech Republic) supplemented with antibiotics (100 i.u./ml penicillin, 100 µg/ml streptomycin, and 25 µg/ml gentamycin), sodium bicarbonate, and 10% foetal calf serum. Before infecting, the lytic virus cycle was induced in a part of the culture: the third day after passaging, the cells were spun down at 1500 *g* for 10 min and seeded into the induction medium containing 1 mM sodium a-butyrate (Merck, BRAND GmbH, Wertheim, Germany) and 20 ng/ml 12-o-tetradecanoyl-phorbol acetate (TPA, Sigma), to obtain a density of 3 x 10^5 cells/ml.

The other part of the culture was seeded similarly into the culture medium containing no inductors. After 24 h the cells were spun down, washed in the culture medium containing no antibiotics, seeded into a new medium in a density 3 x 10^5 cells/ml and infected with borreliae.

Association and internalization of *B. garinii* and *B. afzelii* with P3HR-1 cells was investigated by light and fluorescent microscopy. A freshly prepared bacterial culture in 0.1 ml of BSK-H medium (about 2 x 10^5 cells) was mixed with 0.4 ml of P3HR1 cells (about 3 x 10^5 cells) and added into 4 ml of complete RPMI medium and then incubated for 1 h and 4 h at 36˚C in a CO_2 incubator with regular rocking. Instead of the microbial suspension, 0.1 ml of filtered (Microcon YM-10, YM50, Millipore Carrigtwahille, Co., Cork, Ireland) borrelia-spent medium BSK-H was added to control mock-infected cultures. To remove non-attached bacteria from P3HR-1 cells the cultures were spun down at 3000 *g* for 2 min. Supernatants containing free borreliae were spun out into other tubes and the numbers of borreliae were counted. Sedimented cells were washed in sterile PBS and seeded into antibiotics-free RPMI medium at a density of 3 x 10^5 cells/ml and incubated at 36˚C in a CO_2 incubator; on days 3, 4, and 6 the cells were subjected to study. Cells after incubation were briefly centrifuged (at 2300 *g*) and washed with PBS. The supernatant with non-attached bacteria was serially diluted (10-fold) with BSK-H medium and the number and viability of bacteria were detected by dark-field microscopy. The sedimented cells with bacteria were then fixed on a glass slide for immunofluorescent examination. Slides with cells were fixed for 5 min by ice-cold methanol. Another part of the samples were taken for electron microscopy.

Virus antigens were detected in cells by indirect immunofluorescence using a) human immune serum containing antibody against early and capsid EBV antigen and b) monoclonal antibody against early EBV antigen, which was non-reactive to Borrelia (anti-ZEBRA, DAKO, GmbH, Copenhagen, Denmark). Porcine immunoglobulin against human and murine IgG tagged with fluorescein-isothiocyanate (SwAHu-IgG-FITC, SwAM-FITC, SeVac, Prague, Czech Republic) served as the second antibody. The number of cells containing virus antigens was determined in *B. burgdorferi sensu lato*-invaded cells by viewing at least 600 cells on each slide. The same calculation was made in mock-infected and in chemically induced control cell cultures.

Assessment of association and internalization of Borrelia was expressed as the ratio of cell-associated bacteria (C) to extracellular supernatant bacteria (S), i.e. the number of free bacteria present in the supernatant after 1 h, 4 h and 3 days of incubation with P3HR-1 cells to the number of cell-associated bacteria. All experiments were performed with multiple observations and were reported as the means ± S.D. Results from experiments were analysed by Student's *t*-test with $P \leq 0.05$ considered to be statistically significant.

For the slide immunofluorescence assay, spirochetes were separated from supernatant by centrifugation at 12 000 *g* for 10 min and washed in PBS supplemented with 5 mM $MgCl_2$. Borreliae attached or inside cells (as mentioned above) and borreliae from supernatant were applied to slides and fixed by drying overnight at 4˚C and then stored at –80˚C. Monoclonal antibodies (Mab) against BmpA (H1141), OspA (336,184) and flagellin (H9724) from CDCP were used. Slides were incubated with Mabs for 1 h at 37˚C in moist chambers, washed in two changes of PBS + $MgCl_2$. Incubation with secondary antibodies, SwAM IgG-FITS or SwAM IgG-gold (Jenssen, Redding, CA) was for 1 h at 37˚C with two washes in PBS following incubation. Slides were mounted with Slow Fade (Molecular Probes Inc., Eugene, OR).

For electron-microscopic examination, the following material was used: a) P3HR-1 culture cell suspension, b) P3HR-1, mock-infected, c) induced P3HR-1 infected

with *B. garinii*, or other strains for 1 h and for 4 h, sampled on days 3 and 4. One part of each material was processed for the negative staining method (IEM) and one part for preparation of ultrathin sections as published previously (Hulínská et al., 1995, 1999). Drops of the sediment were stained on grids with 1% phosphotungstic acid (PTA) or the material was fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer at 4°C, postfixed in 1% OsO_4, dehydrated and embedded in Lowicryl K4M for immunocytochemistry as was described elsewhere (Hulínská et al., 1995). Examination was made under a Jeol 200CX electron microscope (Tokyo, Japan).

Results

Borrelia strains used for experiments were: *B. garinii* strain 192M, low-passaged isolate from CSF, OspA serotype 5 and strain 61E, high-passaged isolate from erythema migrans, OspA serotype 4, which have a sequence similarity with strains SL14 and Pbi; strain Kc90, high-passaged strain which was isolated from the blood, and strain 97M, low-passaged strain isolated from CSF, both with sequence similarity with *B. afzelii* strain BVI. All strains were in the log phase in BSK-H medium.

Visualization of *B. garinii* and *B. afzelii* interaction with P3HR-1 lymphoblastoid cells investigated by light and fluorescence microscopy showed significant differences in the behaviour of both strains. Lymphoblastoid cells incubated with selected pairs of Borrelia strains for 1 and 4 h at 36°C contained a number of associated bacteria. *B. garinii* displayed much higher association with P3HR-1 cells than *B. afzelii* strains. Quantitation of Borrelia association and internalization by lymphoblastoid P3HR-1 cells was expressed as the average C/S ratio. At 4 h this was also higher for the low-passaged *B. garinii* 192M strain (0.448 ± 0.048) as compared with the low-passaged *B. afzelii* strain 97M (0.117 ± 0.041) (shown in Table 1).

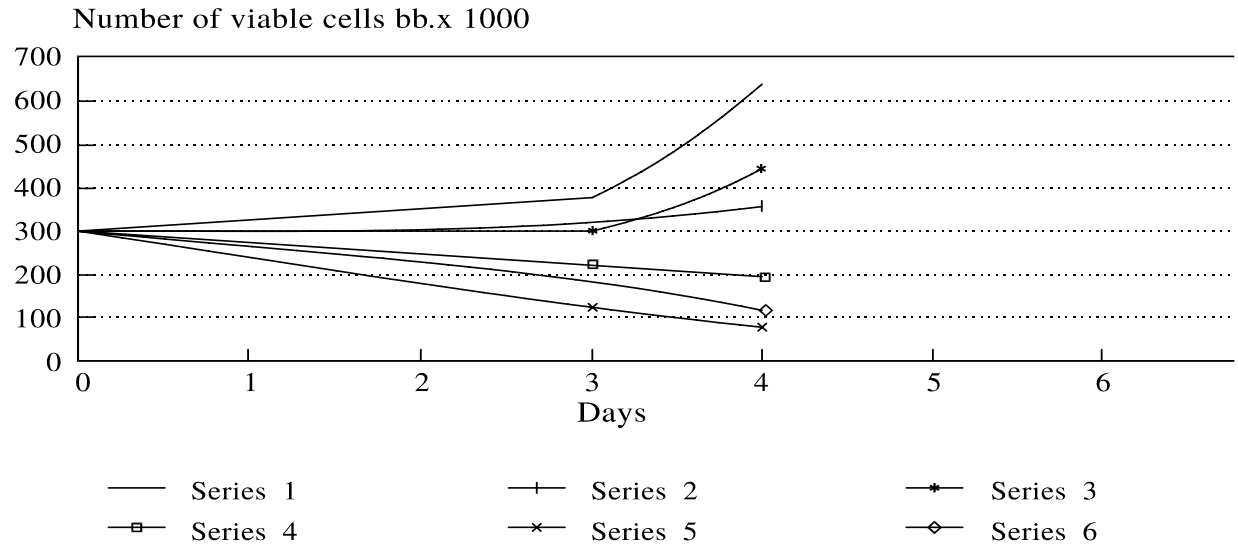
Light and fluorescence microscopic examination showed that both *B. garinii* and *B. afzelii* strains influenced the induction of the lytic cycle of EBV in lymphoblastoid P3HR-1 cells. The approximate numbers of viable P3HR-1 cells in different experiments either after chemically induced replication of EBV or after infection or induction of the viral lytic cycle with *B. garinii* were different as illustrated in Fig. 1. In all experiments we started with 3 x 10^5 cells infected 1 and 4 h and sampled on days 3 and 4. The number of viable P3HR-1 cells infected 1 or 4 h with borreliae diminished and after 3- and 4-day incubation they reached only 2 x or 1 x 10^5 cells. On the other hand, the number of viable mock-infected or chemically induced cells sampled on days 3 and 4 increased (Fig. 1).

The percentage of cells containing EBV antigens was higher in *B. garinii*-infected than in *B. afzelii*-infected or in the control mock-infected cultures. However, it has come to light that just the BSK-H medium alone in which *B. garinii* had been cultured for four hours also had an inducing effect on EBV. Growing Borrelia released microvesicles with outer surface proteins and decreased pH of the medium. In each preparation we inspected about 500–600 cells and about one hundred high-power fields. Viral antigens were seen in 14.0% induced P3HR-1 cells infected for 1 h with *B. garinii*, sampled on 3rd day, and in 4.8% cells infected with *B. afzelii* low-passaged strains in IFA observations with polyclonal and monoclonal antibodies (Table 2). Observations of EBV antigens in P3HR-1 cells expressed as the approximate ratio of the number of cells containing the EBV antigen and the number of cells not containing the virus antigen was higher for *B. garinii* (0.275 ± 0.070) than for *B. afzelii* (0.103 ± 0.041) on day 3 of incubation. Depending on the duration of infection with both viral and borrelial antigens on day 4, cell viability decreased and 50–60% of cells were lysed. A longer 6-day period of incubation with Borrelia caused lysis of P3HR-1 cells approximately in 70%. Growing Borreliae decreased the pH of the medium from pH 7.2 to pH 6.9.

Table 1. Time course of Borrelia association in induced lymphoblastoid P3HR-1 cells

			Average ratio of cell-associated bacteria (C) to bacteria in supernatant (S)		
Strain	**Origin**	**Phenotype**	**1 h**	**4 h**	**3 days**
Kc90	blood	*B. afzelii*, type-2 high-passaged	0.030 ± 0.002	0.040 ± 0.018	0.076 ± 0.010
97M	CSF	*B. afzelii* low-passaged	0.161 ± 0.017	0.117 ± 0.041	0.268 ± 0.072
192M	CSF	*B. garinii*, type-5 low-passaged	0.310 ± 0.070	0.448 ± 0.048	0.376 ± 0.134
61E	skin	*B. garinii*, type-4 high-passaged	0.300 ± 0.040	0.348 ± 0.072	0.301 ± 0.172

Association of *B. afzelii* and *B. garinii* strains was expressed as the ratio ± (S.D.) of cell-associated bacteria (C) to bacteria in the supernatant (S) after 1 and 4 h incubation with H3PR-1 cells and sampled on 3rd day of interaction. CSF, cerebrospinal fluid.



1: P3HR-1, 2: P3HR-1-MOI, 3: Induc. P3-MOI, 4: P3+B.g., 5: Induc. P3+B.g.1h, 6: Induc. P3+B.g. 4h

Fig. 1. B. garinii infection of P3HR-1 cells

Electron microscopic examination of the P3HR-1 cells incubated for 1 h at 36˚C with *B. garinii* and sampled on day 3 showed viral particles in the nucleus (Fig. 2). Borreliae and EBV were also visible in the cytoplasm (Fig. 3). The cell surface formed numerous projections. Borreliae were associated with cells individually by one apical end, but small clusters of bacteria were also found. Most P3HR-1 cells induced 4 h with *B. garinii* low-passaged strains, sampled on day 3, contained viruses on the plasma membrane and in the phagocytic vesicles. Negative staining and immunocytochemical reaction with anti-viral monoclonal antibody and protein A-gold conjugate showed numerous viral particles in the cytoplasm and in the nucleus of P3HR-1 cells infected with *B. garinii* (Fig. 4). Observation of the same preparation by dark-field microscopy suggested that a number of cells were clumped into syncytium-like formations. Viruses and borreliae were tightly closed between cells. Growing borreliae formed clusters inside and outside the syncytium and caused disintegration of cells on day 4 (Fig. 5).

Table 2. Effect of B. garinii and B. afzelii, low-passaged strains, on the production of EBV antigens in P3HR-1 cells detected with human serum

Culture	**Sampled on 3rd day % of EBV-positive cells**	**Sampled on 4th day % of EBV- positive cells**
P3HR-1 cells	2.50 (0.80)	0.54
P3HR-1, mock-infected	5.90 (2.00)	6.80
Induced P3HR-1 cells mock-infected	8.80 (1.60)	7.60
Induced P3HR-1 cells infected with *B. garinii*, 1 h	14.00 (2.80)	13.30
Induced P3HR-1 cells infected with *B. garinii*, 4 h	9.00 (2.10)	11.30
Induced P3HR-1 cells infected with *B. afzelii*, 1 h	4.80 (2.75)	4.07 (2.35)
Induced P3HR-1 cells infected with *B. afzelii*, 4 h	2.90 (1.75)	2.28 (1.56)
Control P3HR-1 cells infected with *B. afzelii*	1.14	2.04 (1.05)
Control P3HR-1 cells infected with *B. garinii*	2.00 (2.35)	2.39 (1.56)

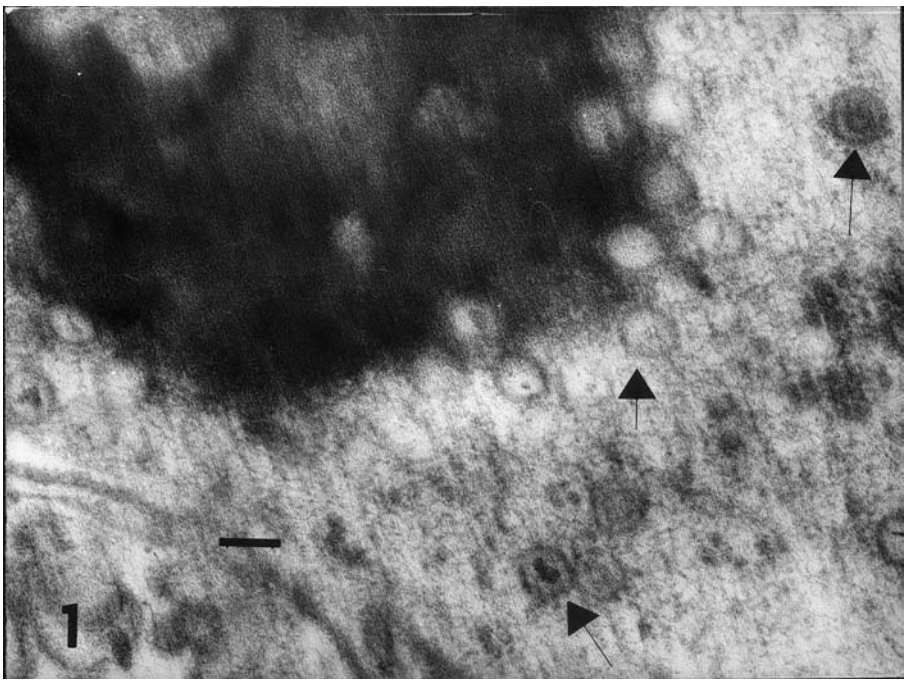


Fig. 2. Formation of EBV particles within the cell nucleus. Some capsids are in the process of assembling, some in the cytoplasm are complete (arrow). Cores of varying density are within the capsids (original magnification 78 000x, Uranyl acetate – Lead citrate / Ua-Lc/). Bar = 110 nm.

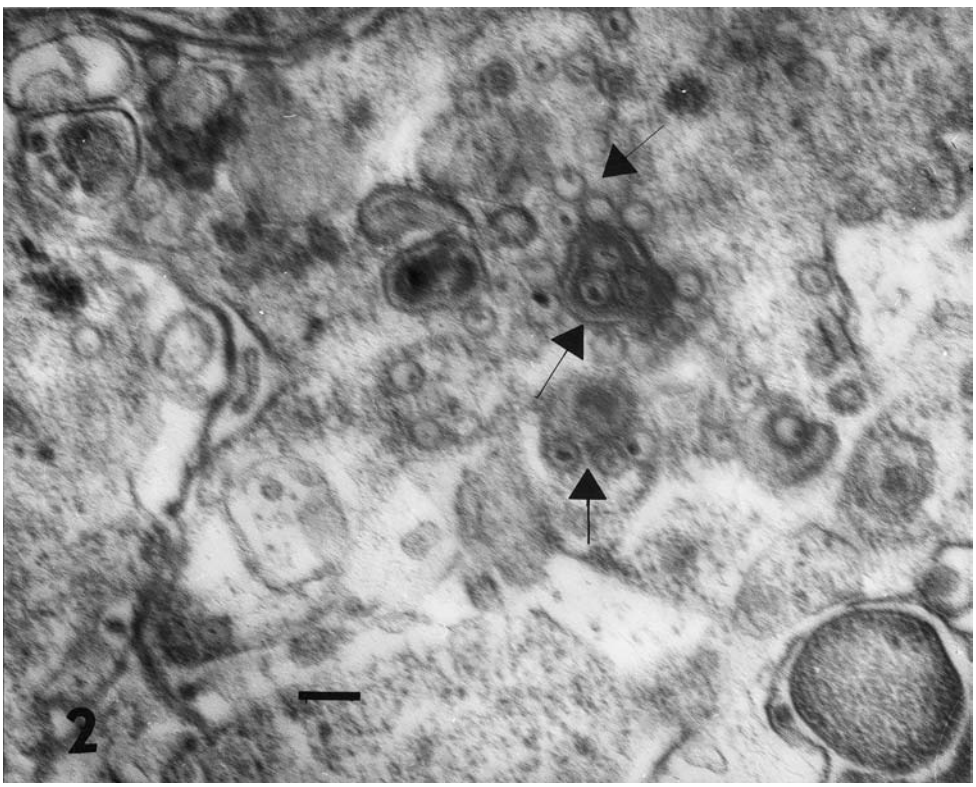


Fig. 3. Complete virus particles are free or in contact with the plasma membrane of a P3HR-1 cell and some are enclosed in a ribosome-rich structure (arrow). Distinct cores probably represent viruses cross-sectioned at one margin, some with loss of density of the overlapping structure (original magnification 68 000x, Ua-Lc). Bar = 180 nm.

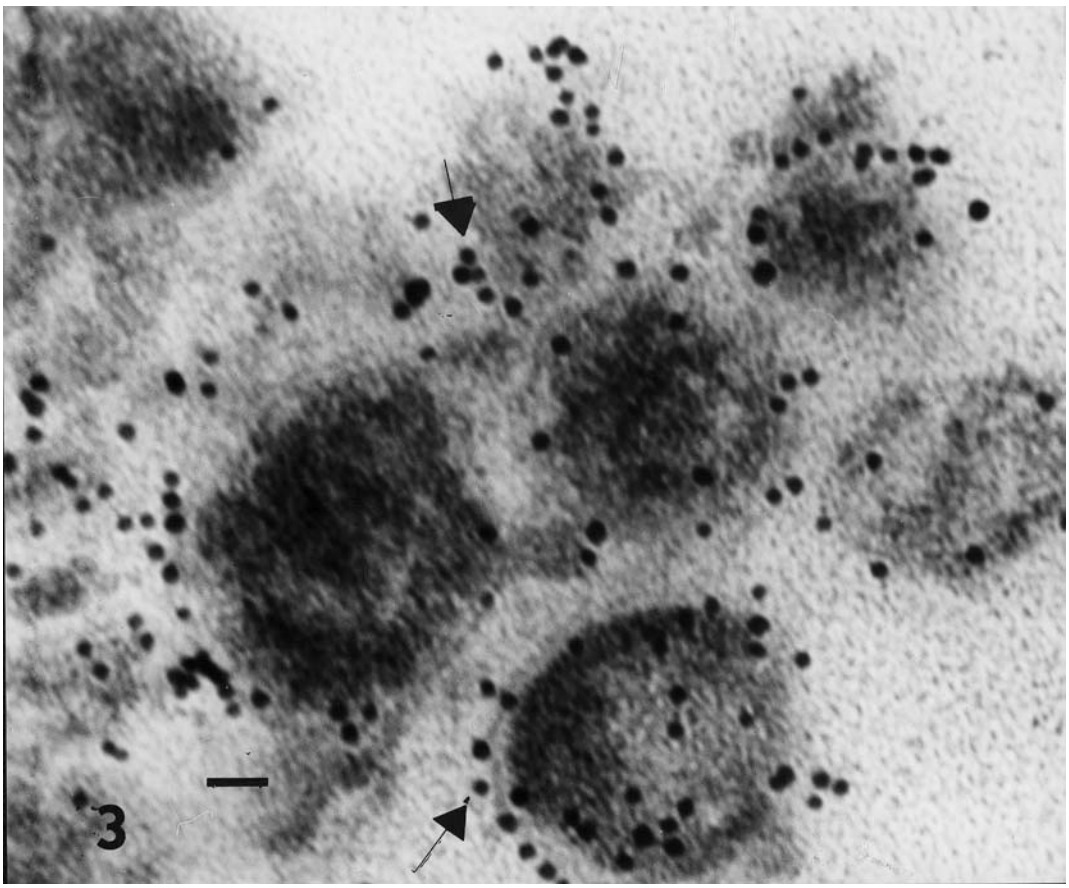


Fig. 4. Immunocytochemistry of EBV particles in the cytoplasm and nucleus of native P3HR-1 cells induced 4 h with *B. garinii* and sampled on day 4. Gold particles (10 nm) labelled the capsid and envelope surrounding the anti-ZEBRA EBV inner core (magnification 60 000x, 1% PTA, SwAM IgG-Au). Bar = 150 nm.

Examination of serial ultrathin sections revealed that borreliae persisted 3 and 4 days inside tube-like structures which were formed by invagination of the cytoplasmic membrane (Fig. 5). Borreliae caused the surface adhesion site to get thinner and pushed it inside the cell. This part of the plasma membrane surrounded the tube-like channels engulfing borreliae as well as viruses (Fig. 6). Viral capsids in the cell nucleus attained their envelope at the nuclear membrane, and migrated through reduplication of the nuclear membrane into cytoplasmic vesicles. Following attachment the viral envelope appeared to fuse with the cell plasma membrane, which invaginated by the action of borreliae. Immunocytochemical reaction with Mab against nonstructural EBV revealed the presence of viral antigens around and on the helical borreliae inside the invagination resembling tube-like structures (Fig. 6). Later, on day 4, mature viral particles with envelope, capsid and nucleoid together with convoluted spirochetes were found in the rounded bottom of these tubes in both experiments with *B. garinii* and *B. afzelii*. These membrane-bound vesicles in the cytoplasm of cells resembled „cellules“ or „niches“ which surround intracellular agents.

Viruses were liberated from the membrane into the borrelia-containing „cellules“ in the lytic phase on day 6, when membrane „ cellules“ containing viruses and borreliae (Fig. 7) were released from disintegrated cells. On day 6 free borreliae in the medium developed cysts and „gemme“, which could be retransformed in the BSK-H medium, where they immediately grew as small, thin 5.5 μm or longer spirochetes. Viruses from the lytic phase of infection freed into the medium replicated again in a new cell culture without inductors.

Discussion

The aim of our present work was to characterize the properties of *B. garinii* and *B. afzelii* strains originating from patients in association with lymphoblastoid P3HR-1 cells and their possible effect on replication of EBV in 1 and 4 h induced cells after 3 and 4 days of common incubation. Related bacterial strains did not share the same genotype and phenotype characteristics and they also differed in their interaction with cultures. Both light, fluorescence and electron microscopy indicated that *B. garinii* were associated and internalized more extensively in the P3HR-1 cells than *B. afzelii* strains. Our quantitative assessment of the interaction

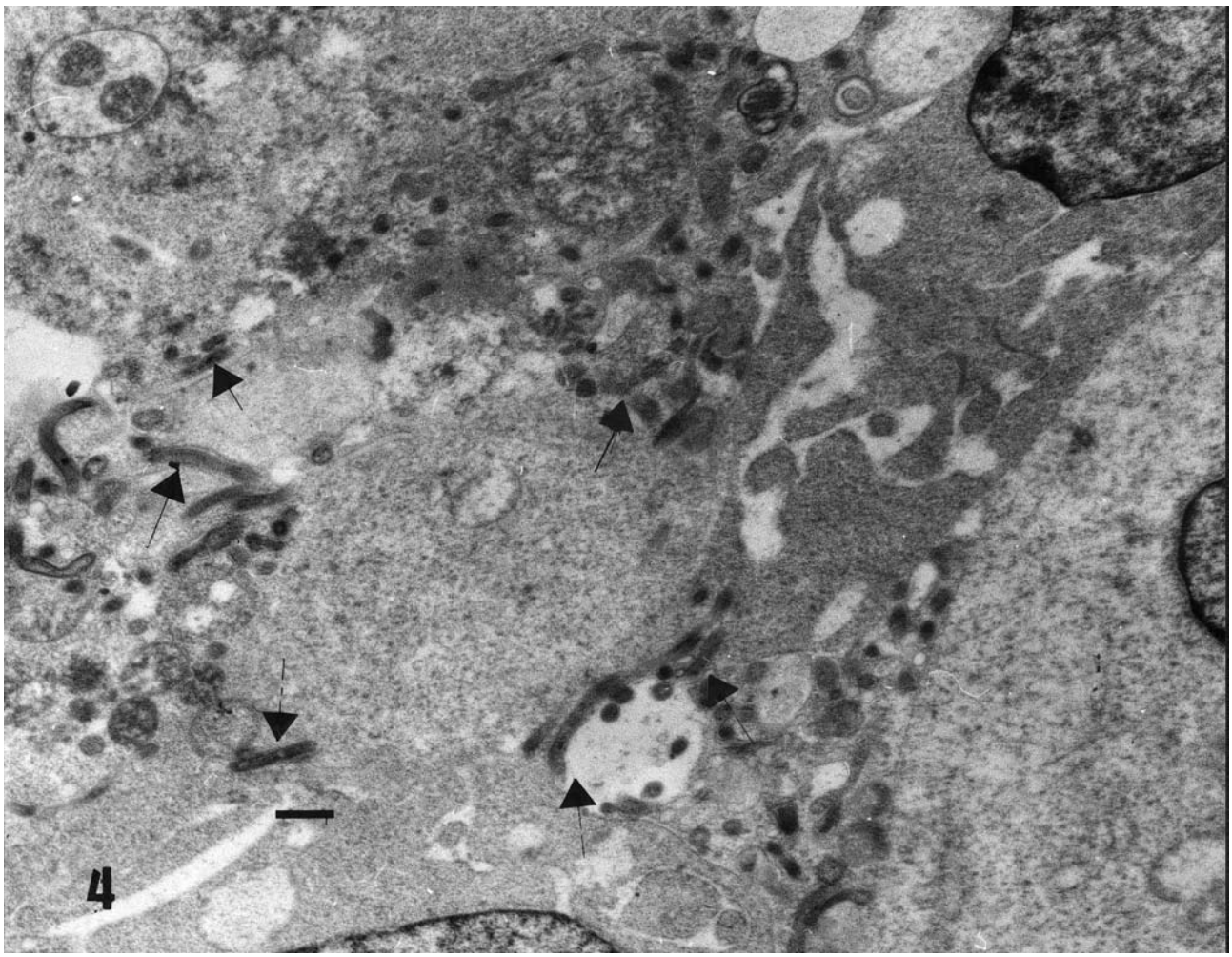


Fig. 5. Micrograph shows a transverse section of P3HR-1 cells clumped in syncytium-like formation and invagination or extension of borreliae into the cytoplasm. Some borreliae were inside (arrow) the host vacuoles (original magnification 14 500x, Ua-Lc). Bar = 600 μm.

of borrelial strains with P3HR-1 cells confirmed these observations. It has been found that *B. burgdorferi sensu stricto* cause lysis of B lymphocytes (Dorward et al., 1997). Epstein-Barr virus is considered to be a human B-lymphotropic virus (Strauss, 1993). Our present findings of the different behaviour and effect of different strains of *B. burgdorferi sensu lato* (Busch et al., 1996; Vasiliu et al., 1998) on replication of EBV in lymphoblastoid cells conform to some earlier studies. Norris et al. (1995) reported differences in virulence between low- and high-passaged borrelial strains. Distinct interactions have been described for various strains of Borrelia with cultured phagocytes, dependent on the presence of the outer-membrane proteins (Carrol and Gherardini, 1996). Different genospecies are associated with distinct clinical symptoms (Van Dam et al., 1993).

Neuroborreliosis as found by Marconi et al. (1999) should be associated with *B. garinii*-type 4. Primary infection by EBV occurs in early chilhood, is mostly asymptomatic (Roubalová et al., 1997) and serves for the development of specific antibodies, whereas primary infection with tick-borne Borrelia is mostly symptomatic but very often without development of specific antibodies, namely in the course of antibiotic treatment. After primary infection EBV is latently present in the organism life-long. Latent Lyme neuroborreliosis (Pfister et al., 1989) was also proved by detection of Borrelia in healthy skin and in the cerebrospinal fluids. Tai et al. (1994) demonstrated that Borreliae have the capacity to attach to different cells and that carbohydrate receptors are involved in their adhesion to eukaryotic cells. Coiling phagocytosis of Borreliae as a basic mechanism of borrelial internalization is controlled by actin polymerization (Linder et al., 2001).

It appears that the clinical outcome of borreliosis depends on a lot of factors also including the presence of viruses in the host, which can complicate the course of infection when they are activated. We have demonstrated that association and internalization of Borrelia by lymphoblastoid cells can cause induction of the lytic virus cyclus. *B. garinii* had a stronger inducing effect on EBV than *B. afzelii.*

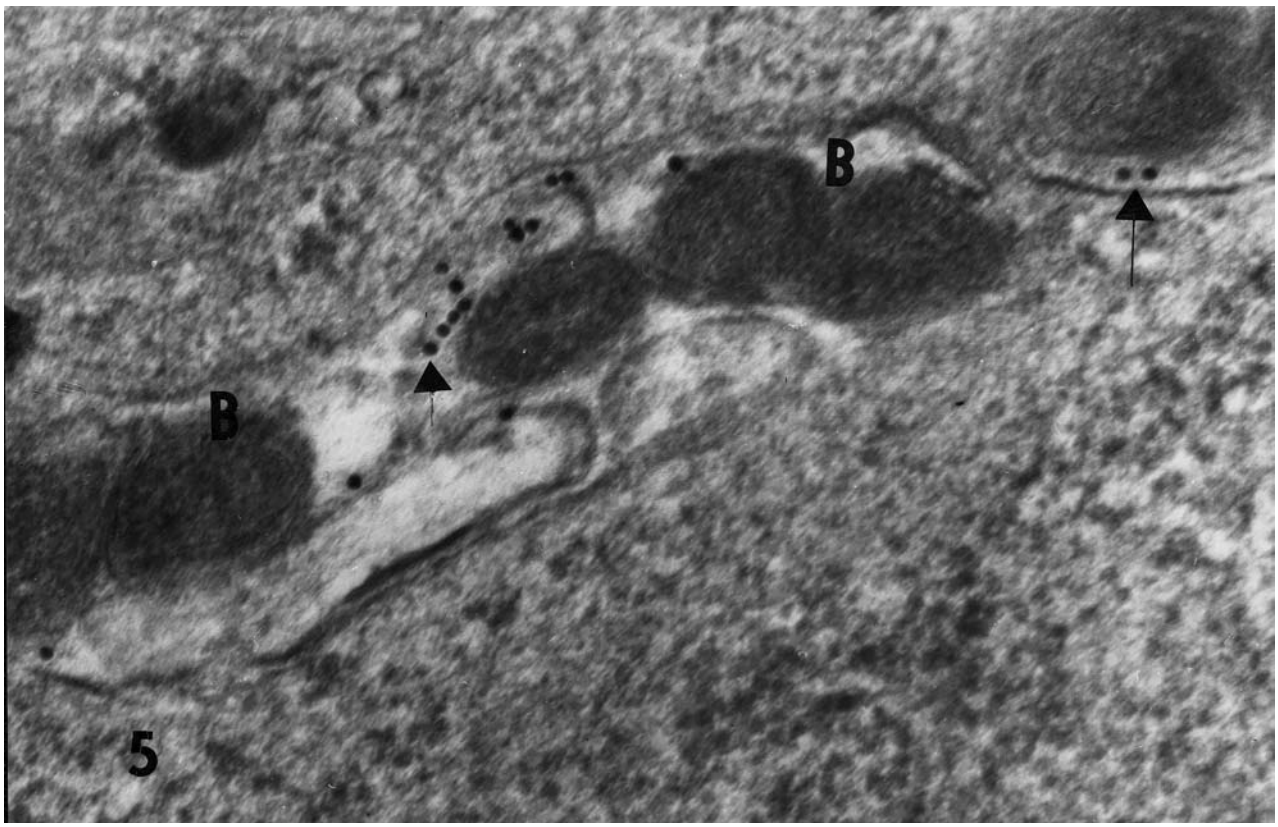


Fig. 6. Thin section micrograph of cross-sectioned *B. garinii* inside the invagination of the cytoplasmic membrane, which forms a tube-like structure protruding nearly to the nucleus of a P3HR-1 cell. EBV particles are marked by gold particles (5 nm) in the reaction with monoclonal anti-ZEBRA antibody (magnification 45 000x, Ua-Lc). Bar = 300 nm.

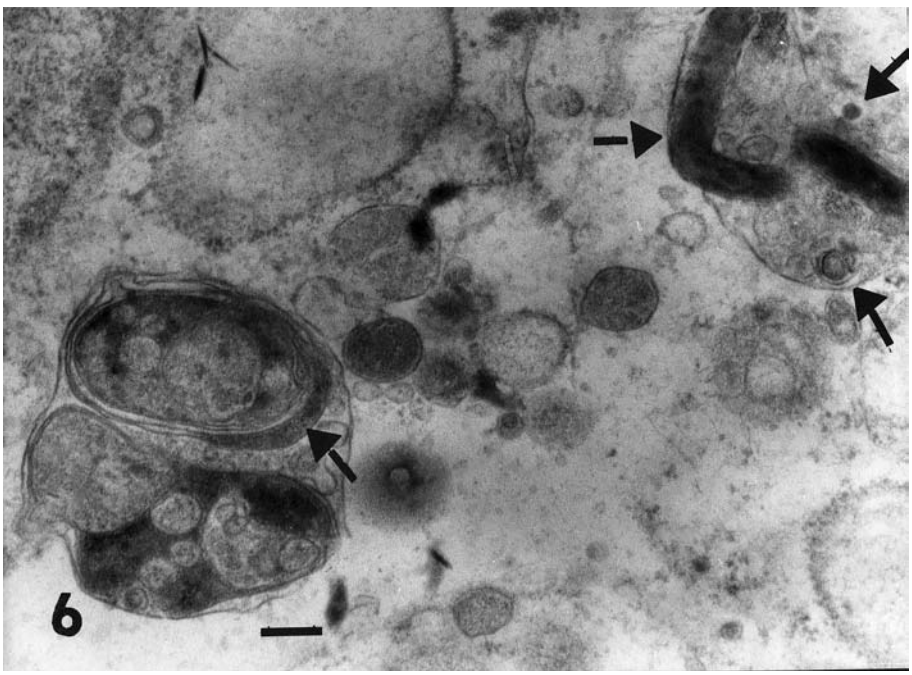


Fig. 7. Convoluted borreliae (arrow) and viruses (arrow) are visible inside the „cellule“ on day 4 of incubation (magnification 34 000x, Ua-Lc). Bar = 280 nm.

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References

Busch, U., Teufel, C. H., Boehmer, R., Fingerle, W., Nitschko, H., Wilske, B., Preak-Mursic, V. (1996) Three species of Borrelia burgdorferi sensu lato (B. burgdorferi sensu stricto, B. afzelii and B. garinii) identified from cerebrospinal fluid isolates by pulse-field gel electrophoresis and PCR. *J. Clin. Microbiol.* **34,** 1072-1078.

Carcia-Monco, J. C., Villar, B. F., Szcepanski, A., Benach, J. L. (1991) Cytotoxicity of Borrelia burgdorferi for cultured rat glial cells. *J. Infect. Dis.* **163,** 1362-1366.

Carrol, J. A., Gherardini, F. C. (1996) Membrane proteins variations associated with in vitro passage of Borrelia burgdorferi. *Infect. Immun.* **64,** 392-398.

Coyle, P. K., Schutzer, S. E., Deng, Z., Krupp, L. B., Benach, J. L., Luft, B. J. (1995) Detection of Borrelia burgdorferi-specific antigen in antibody-negative cerebrospinal fluid in neurologic Lyme disease. *Neurology* **45,** 2010-2015.

Dorward, D. W., Fischer, E. R., Brooks, D. M. (1997) Invasion and cytopathic killing of human lymphocytes by spirochetes causing lyme disease. *Clin. Infect. Dis.* **25,** 52-58.

Georgilis, K., Peacocke, M., Klempner, M. S. (1992) Fibroblasts protect the lyme disease spirochete, Borrelia burgdorferi, from ceftriaxome in vitro. *J. Infect. Dis.* **166,** 440-445.

Guo, B. P., Norris, S. J., Rosenberg, L. C., Hook, M. (1995) Adherence of Borrelia burgdorferi to the proteoglycan decorin. *Infect. Immun.* **63,** 3467-3472.

Halperin, J. J., Little, B.W., Coyle, P. K., Dattwyler, R. J. (1987) Lyme disease: cause of a treatable peripheral neuropathy. *Neurology* **37,** 1700-1706.

Harris, N. S. (1998) An understanding of laboratory testing for Lyme disease. *J. Spiro. Tick. Diseases (JSTD)* **5,** 16-24.

Hulínská, D., Basta, J., Murgia, B., Cinco, M. (1995) Intracellular morphological events observed by electron microscopy on neutrophil phagocytosis of Borrelia garinii. *J. Spiro. Tick. Diseases (JSTD)* **2,** 1-6.

Hulínská, D., Votypka, J., Valešová, M. (1999) Persistence of Borrelia garinii and Borrelia afzelii in patients with Lyme arthritis. *Zbl. Bakt.* **289,** 301-318.

Karch, H., Huppertz, H. I., Bohme, M., Schmidt, H., Wiebecke, D., Schwarzkopf, A. (1994) Demonstration of Borrelia burgdorferi DNA in urine samples from healthy humans whose sera contain B. burgdorferi-specific antibodies. *J. Clin. Microbiol.* **32,** 2312-2313.

Klein, G.(1989) Viral latency and transformation strategy of Epstein-Barr virus. *Cell* **58,** 5-8.

Knutson, J. C., Sugden, B. (1989) Immortalization of B-lymphocytes by Epstein-Barr virus. *Adv. Viral Oncol.* **8,** 151-156.

Linder, S., Heimerl, Ch., Fingerle, V., Aepfelbacher, M., Wilske, B. (2001) Coilling phagocytosis of Borrelia burgdorferi by primary human macrophages is controlled by CDC 42 Hs and Rac 1 and involves recruitment of Wiskott-Aldrich syndrome protein and Arp 2/3 complex. *Infect. Immun.* **69,** 1739-1746.

Luft, B. J., Steinman, C. R., Neimark, H. C., Muralidhar, B., Rush, T., Finkel, M. F., Kunkel, M., Dattwyler, R. J. (1992) Invasion of the central nervous system by Borrelia burgdorferi in acute disseminated infection. *J. Am. Med. Assoc. (JAMA)* **267,** 1364-1367.

Marconi, R. T., Hohenberger, S., Jouris-Heipke, S., Schulte-Spechtel, Lavore, C. P., Röbler, D., Wilske, B. (1999) Genetic analysis of Borrelia garinii OspA serotype 4 strains associated with neuroborreliosis; evidence of extensive genetic homogeneity. *J. Clin. Microbiol.* **37,** 3965-3968.

Montgomery, R. R., Nathanson, M. H., Malawista, S. E. (1994) Fc and non-Fc-mediated phagocytosis of Borrelia burgdorferi by macrophages. *J. Infect. Dis.* **170,** 890-893.

Moter, S. E., Hofmann, H., Wallich, R., Simon, M. M., Kramer, M. D. (1994) Detection of Borrelia burgdorferi sensu lato in lesional skin of patients with erythema migrans and acrodermatitis chronica atrophicans by OspA-specific PCR. *J. Clin. Microbiol.* **32,** 2980-2988.

Norris, R. J., Howell, J. K., Garza, S. A., Fedows, M. S., Barbour, A. G. (1995) High- and low-infectivity phenotypes of clonal populations of in vitro cultured Borrelia burgdorferi. *Infect. Immun.* **63,** 2206-2212.

Pfister, H. W., Preac-Mursic, V., Wilske, B., Einhaupl, K. M., Weinberger, K. (1989) Latent Lyme neuroborreliosis: presence of Borrelia burgdorferi in the cerebrospinal fluid without concurrent inflammatory signs. *Neurology* **39,** 1118-1120.

Roubalová, K., Schramlová, J., Piťha, J., Bojar, M., Effler, J. (1997) Laboratory diagnosis of Herpetic infection in patient with impairment of CNS. *Klin. Biochem. Metab.* **5,** 19-23.

Strauss, S. E. (1993) Epstein-Barr virus infections: pathogenesis and management. *Ann. Internal. Med.* **118,** 45-58.

Tai, K. F., Ma, Y., Weis, J. (1994) Normal B lymphocytes and mononuclear cells respond to the mitogenic and cytokine-stimulatory activities of Borrelia burgdorferi and its lipoprotein OspA. *Infect Immun.* **62,** 520-528.

Thorley-Lawson, D. A. (1988) Immunological response to Epstein-Barr virus infection and pathogenesis of EBV–induced disease. *Biochem. Biophys. Acta* **948,** 263-286.

Van Dam, A., Kuiper, H., Vos, K., Widjojokusumo, A., de Jongh, B. M., Spanjaard, L., Kramer, M. D., Dankert, J.(1993) Different genospecies of Borrelia burgdorferi are associated with distinct clinical manifestations of Lyme Borreliosis. *Clin. Infect. Dis.* **17,** 708-717.

Vasiliu, V., Herzer, P., Rössler, D., Lehnert, G., Wilske, B. (1998) Heterogeneity of Borrelia burgdorferi sensu lato demonstrated by an OspA-type specific PCR in synovial fluid from patients with Lyme arthritis. *Med. Microbiol. Immunol.* **187,** 97-102.

Wang, P., Gartenhaus, R., Sood, S. K., DeVoti, J., Singer, C., Dorante, G., Hilton, E. (2000) Detection of Borrelia DNA in circulating monocytes as evidence of persistent Lyme disease. *J. Spiro. Tick. Diseases (JSTD)* **7,** 16-19.