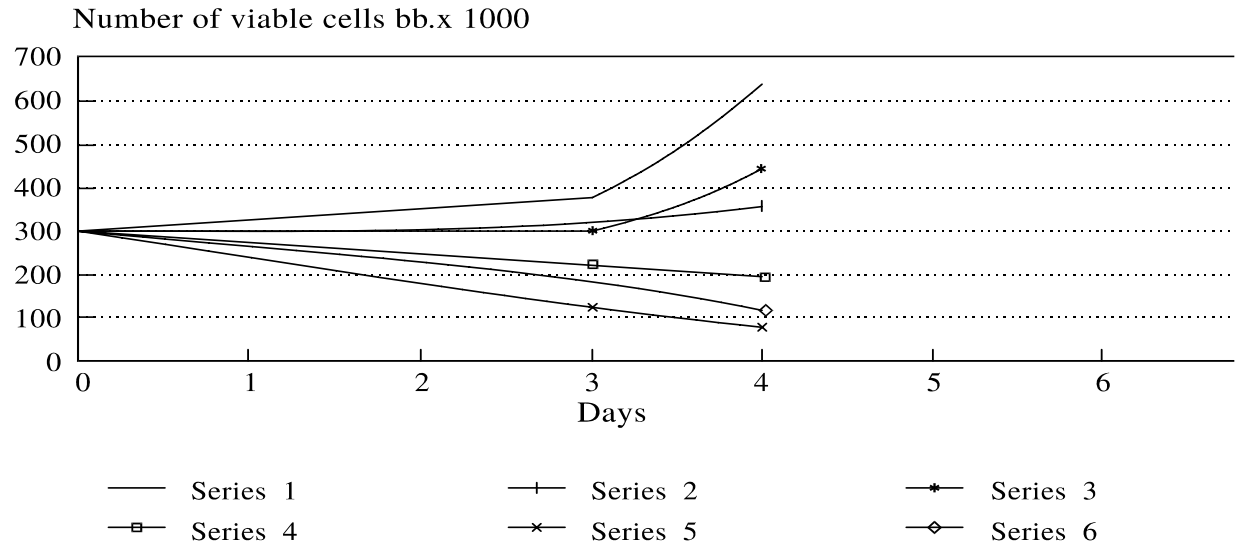

1: P3HR-1, 2: P3HR-1-MOI, 3: Induc. P3-MOI, 4: P3+B.g., 5: Induc. P3+B.g.1h, 6: Induc. P3+B.g. 4h

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)

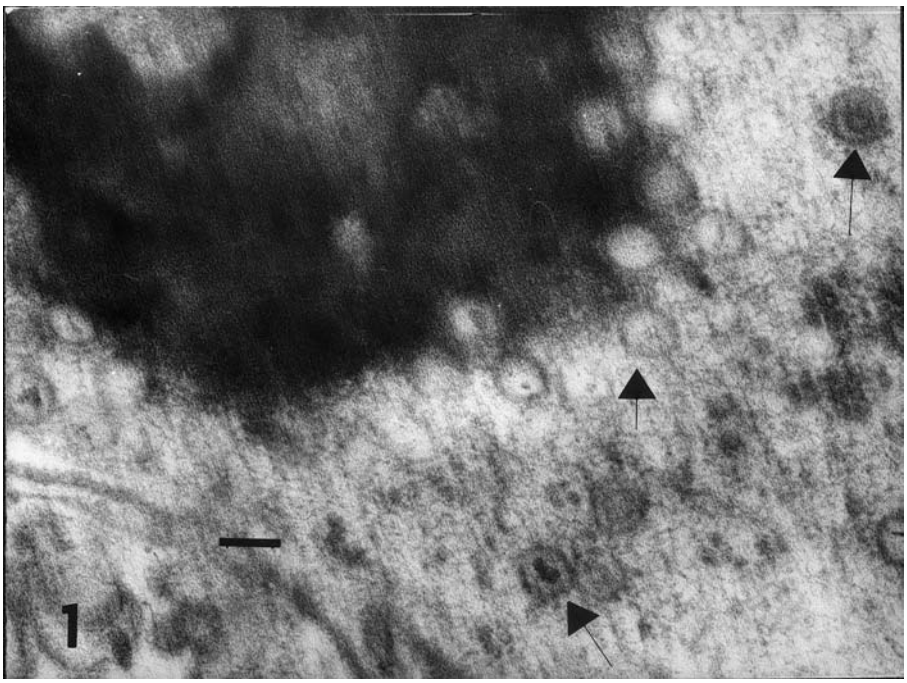

Fig. 2. Formation of EBV particles within the cell nucleus. Some capsids are in the process of assembling, some in the cytoplasm are complete (arrow). Cores of varying density are within the capsids (original magnification 78 000x, Uranyl acetate – Lead citrate / Ua-Lc/). Bar = 110 nm.

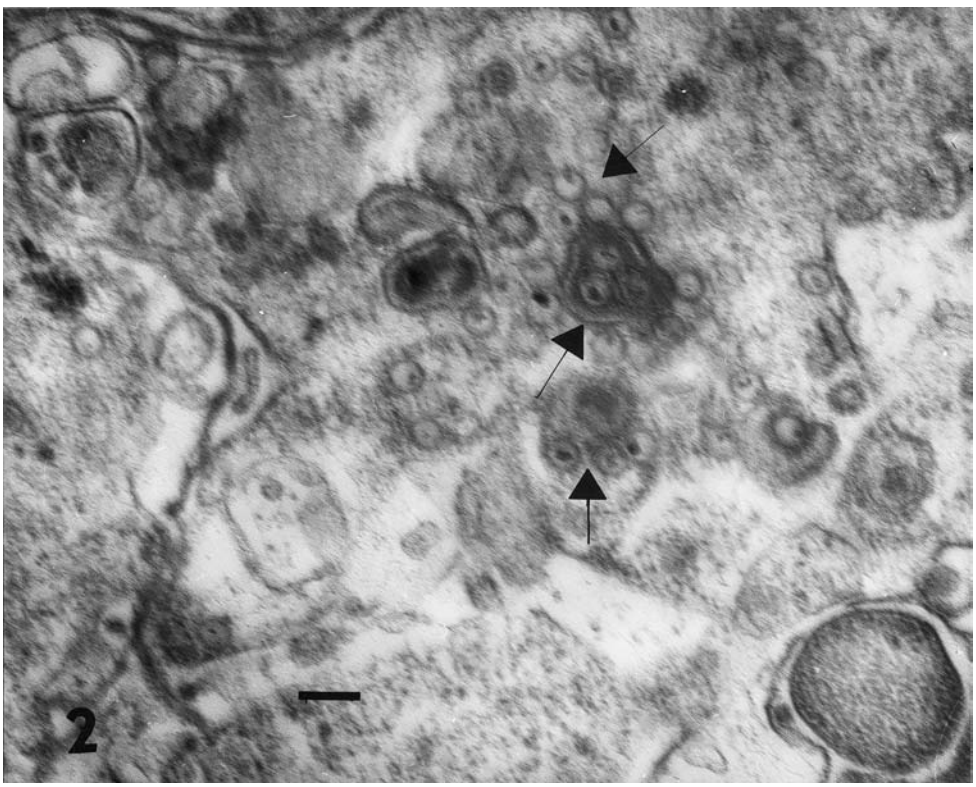

Fig. 3. Complete virus particles are free or in contact with the plasma membrane of a P3HR-1 cell and some are enclosed in a ribosome-rich structure (arrow). Distinct cores probably represent viruses cross-sectioned at one margin, some with loss of density of the overlapping structure (original magnification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

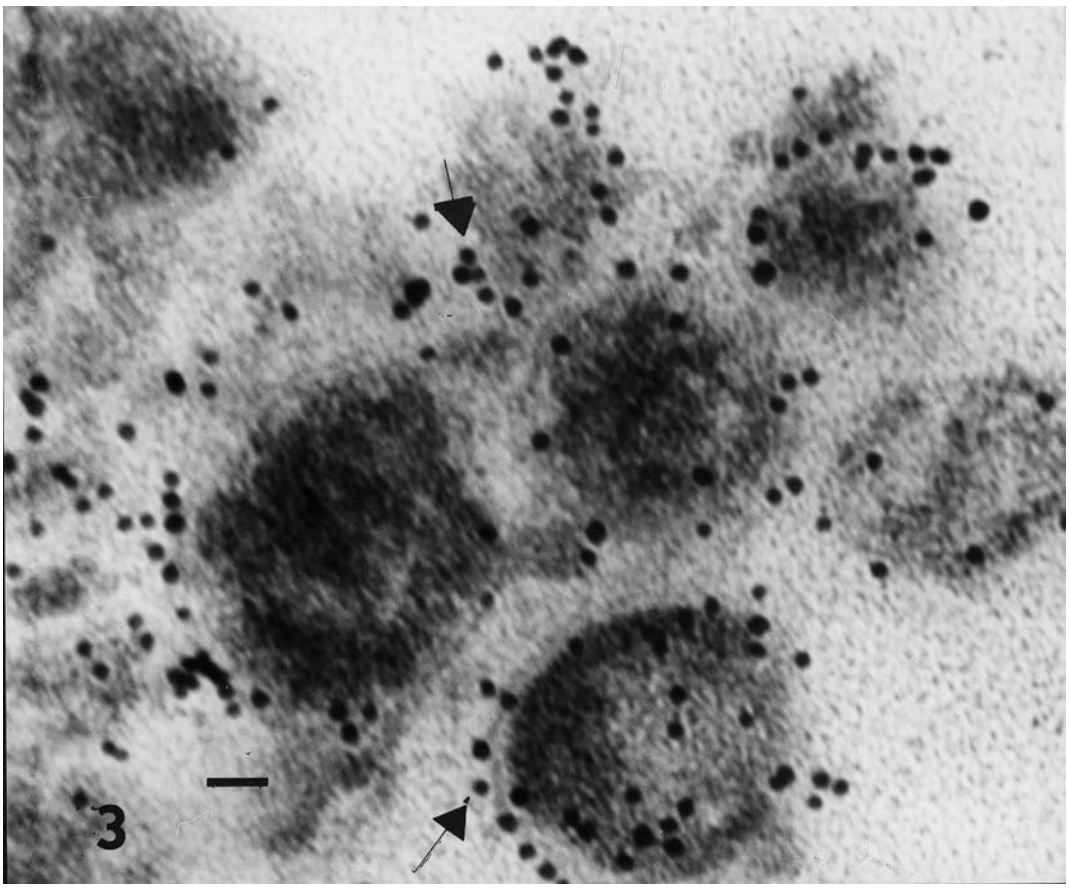

Fig. 4. Immunocytochemistry of EBV particles in the cytoplasm and nucleus of native P3HR-1 cells induced 4 h with *B. garinii* and sampled on day 4. Gold particles (10 nm) labelled the capsid and envelope surrounding the anti-ZEBRA EBV inner core (magnification 60 000x, 1% PTA, SwAM IgG-Au). Bar = 150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