

Effects of lactoferrin on viral replication in B cell lines corresponding to various stages of B cell differentiation

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Key words: lactoferrin, A-20, WEHI-231, 7TD1, EMCV, HSV-1, VSV.

Abstract

Introduction: Lactoferrin inhibits viral replication in many cell types.

Aim of the research: The aim of this study was to evaluate the protective effects of lactoferrin (LF) on susceptibility of B cell lines, representing different stages of differentiation, to viral infection.

Material and methods: Native bovine LF (bLF) and recombinant mouse LF (rmLF) were applied. Mouse cell lines: WEHI-231 (immature B cells), A-20 (mature B cells), and 7TD1 (plasma cells) were used for infection with encephalomyocarditis virus (EMCV), herpes virus type 1 (HSV-1), and vesicular stomatitis virus (VSV).

Results: A-20 cells were most sensitive to infection, WEHI-231 cells were less sensitive, while 7TD1 cells were most resistant to the infection. Both lactoferrins showed significant protective effects in WEHI-231 cells against EMCV, and in the case of A-20 cell line this effect was strongest. A potent antiviral effect of the lactoferrins was found in A-20 cells infected with HSV-1.

Conclusions: The susceptibility of B-cell lines to viral infection, at various stage of cell differentiation, was differential. The protective effects of rmLF were higher in comparison to bLF.

Introduction

B cells are a major target of viral infections, leading to the development of neoplastic and autoimmune diseases [1, 2]. Viruses access cell targets via a variety of receptors including heparin sulphate [3], DC-SIGN molecule [4], CD150 [5], CD21 [6], co-receptors [7], and other, nonspecific routes [8].

The susceptibility of B cells to viruses, at defined stages of development, may vary and lead to various consequences. Transformation of immature B lymphocytes with Abelson murine leukaemia virus, expressing the v-abl oncogene, induced pre-B cell lymphomas and kappa light rearrangement [9], but the target cells belonged to different developmental stages. On the other hand, the ABL-MYC virus, expressing both v-abl and c-myc, produced only plasmacytomas [10]. In another murine model, pim 1 and myc proto-oncogenes transformed precursor but not mature B lymphocytes [11]. Epstein-Barr virus infection of immature human B cells resulted in a switch of complement receptors [12]. Although gamma herpes viruses are detected in bone marrow pro-pre B cells and immature B cells during early and long-term latency, their possible access to the mature B cell compartment is also suggested [13]. Depletion of bone marrow during influenza infection in mice, associated probably with apoptosis, uses also indirect

mechanisms such as involvement of $\text{TNF-}\alpha$ and lymphotoxin α [14].

Lactoferrin (LF) is one of the proteins involved in iron metabolism and is contained in excretory fluids of mammals and secondary granules of neutrophils [15]. The protein is known for its protective properties against all classes of pathogens, including viruses [16]. The antiviral actions of LF include neutralisation of virus [17], competition with cell receptors for viruses [18], and interference with viral replication by various mechanisms [19]. Recent studies showed that iron-saturated LF was more efficient in inhibition of virus replication than iron-depleted LF [20]. In addition, LF encapsulated in liposomes exerted a stronger antiviral action than free LF [21].

The present investigations were performed on 3 B-cell lines differing in their differentiation stage. WEHI-231 is a mouse B-cell lymphoma with an immature cell phenotype, highly susceptible to apoptosis upon crosslinking of IgM surface receptors and by viruses that can overcome a survival signal by CD40 receptor ligation on these cells [22]. A-20 mature B-cell line is highly susceptible to viral infection, so this feature is of value in exploring oncolytic activities of viruses in *in vivo* models [23]. In addition to WEHI-231 and A-20 B cell lines, 7TD1, IL-6-dependent plasmacytoma cells [24] were included in the study because data on susceptibility of plasmacytoma cells

to viruses are lacking. Encephalomyocarditis virus (EMCV), vesicular stomatitis virus (VSV), and human herpes virus type 1 (HSV-1) were applied for cell infection. Bovine (bLF) and recombinant mouse (rmLF) lactoferrins were used to evaluate antiviral protection measured by a cytopathic effect.

Aim of the research

The aim of this investigation was to evaluate the susceptibility to viral infection of B cell lines, representing different stages of B cell differentiation, and to determine the protective effects of mouse and bovine lactoferrins on viral replication in these cells.

Material and methods

Cell lines

All cell lines and viruses were derived from the cell bank of the Institute of Immunology and Experimental Therapy, Wrocław, Poland. WEHI-231 cells – a mouse B cell lymphoma (ATCC CRL 1702), A-20 cells – a murine B cell lymphoma (ATCC TIB-208), 7TD1 cells – murine plasmacytoma (ATCC CRL-1851), L929 cells – a murine fibroblast-like cell line (ATCC CCL 1), and A549 cells – human adenocarcinoma lung cells (ATCC CCL185) were used and were maintained according to the manufacturer's instructions. L929 cells were applied as a reference line for titrated EMCV and VSV viruses, and the viral cytopathic effect (CPE) is shown in Figures 1 A–E. The CPE of the HSV-1 in the reference A549 cells is shown in Figures 2 A–C.

The viruses

EMCV – encephalomyocarditis virus (ATCC VR-129BTM); *Picornaviridae*; non-enveloped RNA virus was propagated in L929 cells.

VSV – vesicular stomatitis virus (ATCC VR-1238); Indiana strain, *Rhabdoviridae*, enveloped RNA virus was propagated in L929 cells.

HSV-1 – human herpes virus type 1 (ATCC VR-539TM); McIntyre strain, *Herpesviridae*, enveloped DNA virus was propagated in A549 cells.

The titres of the viruses were expressed in reference to the value of TCID₅₀ (tissue culture infectious dose), based on the cytopathic effect caused by this virus in about 50% of infected cells. EMCV and VSV viruses were used at the dose of 10¹ TCID₅₀/ml, and HSV-1 at the dose of 10⁴ TCID₅₀/ml.

Infection of cell lines and treatment with lactoferrins

WEHI-231, A-20, and 7TD1 (1 × 10⁵ cells/ml) were infected with a dose of 10¹ TCID₅₀/ml of EMCV or VSV and 10⁴ TCID₅₀/ml of HSV-1. Following a 45-min incubation at 37°C, the medium was removed and the LFs were added (50, 25, 12, or 6 µg/ml). Non-treated

and cells treated with viruses served as controls. Following 24 h, 48 h, and 96 h incubation, the supernatants were collected and stored at –80°C until virus titration using a standard TCID₅₀ method with a two-fold serial dilution. Briefly, the supernatants were diluted and adsorbed to L929 or A549 cells at a density of 1 × 10⁵ cells/ml in 96-well culture plates. Following 24 h, 48 h, and 96 h of incubation at 37°C the viral cytopathic effect (CPE) was analysed under a microscope (Figures 1 A–E and 2 A–C). The TCID₅₀ was calculated by determining the end-point dilution of the virus, where 50% of the infected cells displayed CPE. The antiviral activity was determined by comparing the logarithmic reduction factor (log 10) of the viral titre with the control. The results obtained from 3 independent experiments are presented as log TCID₅₀ mean values ± standard deviation (SD). When considering significance of the antiviral activity of LF, we refer to a commonly accepted definition that ≥ 4 Log 10 reduction of virus titre is typical for the action of chemical disinfectants, resulting in 99.99% elimination of the virus.

The photographs show some typical morphological changes in cells caused by the virus, such as loss of monolayer integrity, lysis, granulation, and vacuolisation in the cytoplasm. These pathological changes were absent upon addition of bLF at a concentration of 50 µg/ml (Figures 1 A–E and 2 A–C).

Results

A-20 cells were most prone to viral infection. The titre of EMCV in these cells after 24 h, 48 h, and 96 h was 8-log, of HSV-1: 7; 7.8 and 7.7-log, respectively, and that of VSV: 7.5; 7.7 and 7.7-log in the respective time points (Figure 3 A).

The titres of EMCV in WEHI-231 were 6.2; 7.5 and 8-log at respective time intervals. The titres of HSV-1, in turn, attained 3.7-log after 24 h and 7.5-log after 48 h and 96 h. VSV virus, at these time intervals reached titres of 5.2; 5.2 and 5.7-log (Figure 3 B).

The levels of viral replication in 7TD1 cells were much lower for EMCV after 24 h and 48 h equal to 5.6-log and 6.3-log after 96 h (Figure 3 C). The HSV-1 titres at respective time points attained 2.7-log (24 h, 48 h) and 3.0-log at 96 h. VSV titre was 2.6; 4.3 and 5.3-log at measured incubation times.

Both LFs lowered the viral titres, with the highest inhibitory effect in A-20 cells. In these cells bLF (50 µg/ml) reduced the HSV-1 level from 7.8-log to 2.5-log, at 25 µg/ml from 7.8-log to 3.0-log, and at 12.5 µg/ml to 4.5-log after 48-h incubation. bLF significantly decreased HSV-1 in a dose-dependent manner. Also, a significant reduction of HSV-1 titre by rmLF occurred after 24 h, from 7.0-log to 2.3-log at 50 µg/ml (Figure 4 A).

The inhibitory effects of LFs on virus replication in WEHI-231 and 7TD1 cells was also registered, albeit at

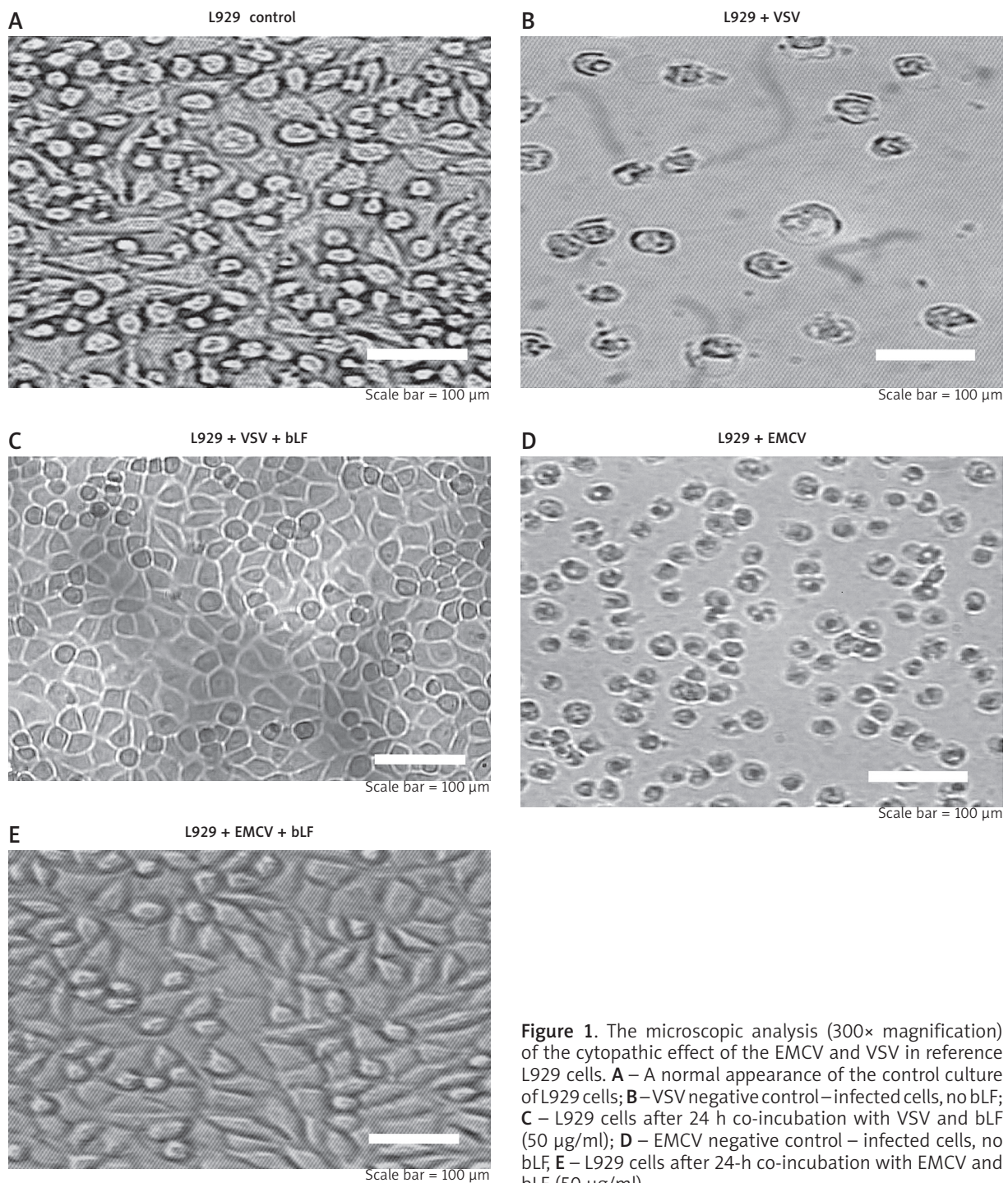


Figure 1. The microscopic analysis (300× magnification) of the cytopathic effect of the EMCV and VSV in reference L929 cells. **A** – A normal appearance of the control culture of L929 cells; **B** – VSV negative control – infected cells, no bLF; **C** – L929 cells after 24 h co-incubation with VSV and bLF (50 µg/ml); **D** – EMCV negative control – infected cells, no bLF; **E** – L929 cells after 24-h co-incubation with EMCV and bLF (50 µg/ml)

a lower efficacy, where bLF exhibited a better potency than rmLF (Figures 4 B, C).

Discussion

In this investigation the lactoferrins differentially inhibited multiplication of EMCV when applied after viral infection. Hence, these actions consisted of in-

terference of LF with the viral multiplication and were not dependent on interaction of LF with surface cell receptors for viruses. Recently, we demonstrated significant effects of both types of LF on inhibition of EMCV replication in B-cell-enriched splenocyte populations in young versus old mice [25]. Such splenocytes may be considered as an enriched population of mature B cells, so these results with resident cells

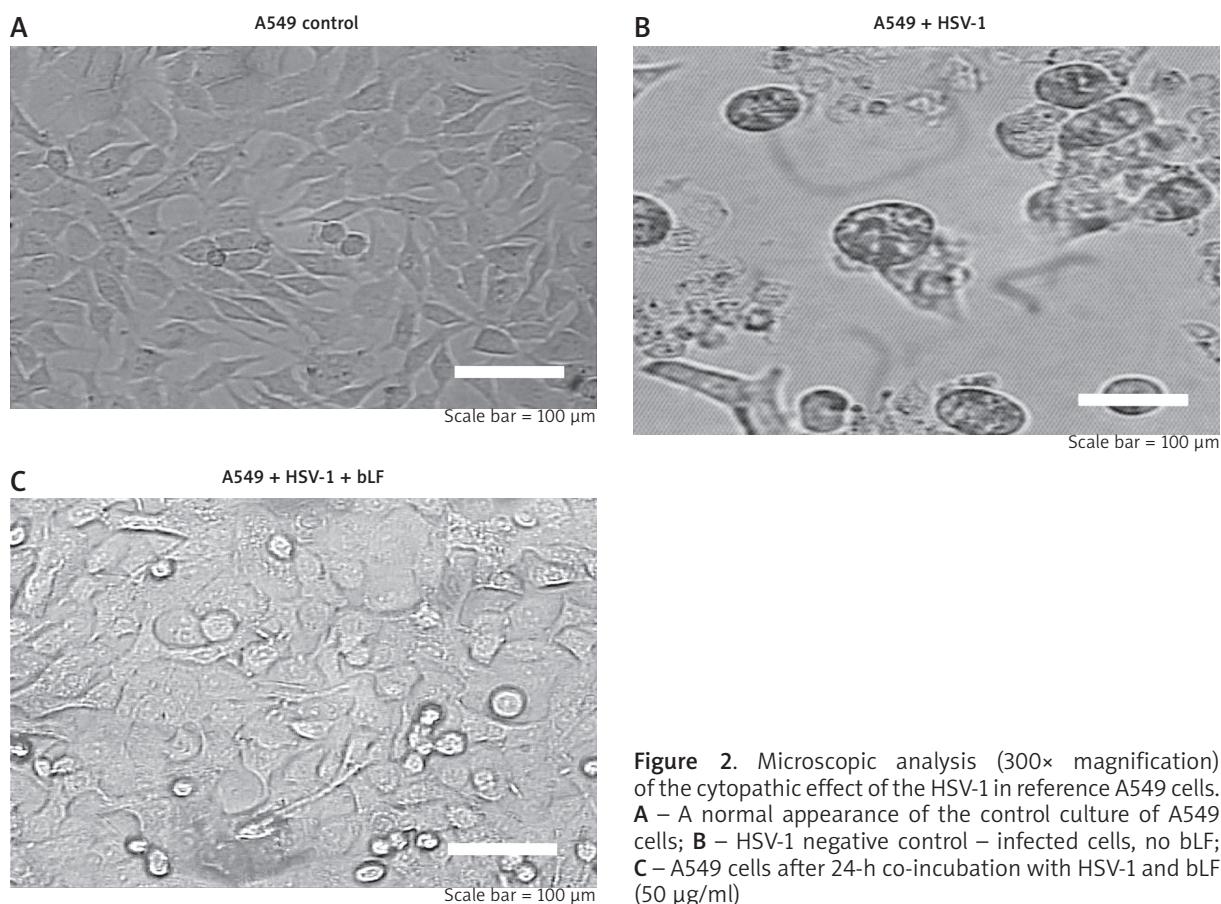


Figure 2. Microscopic analysis (300× magnification) of the cytopathic effect of the HSV-1 in reference A549 cells. **A** – A normal appearance of the control culture of A549 cells; **B** – HSV-1 negative control – infected cells, no bLF; **C** – A549 cells after 24-h co-incubation with HSV-1 and bLF (50 µg/ml)

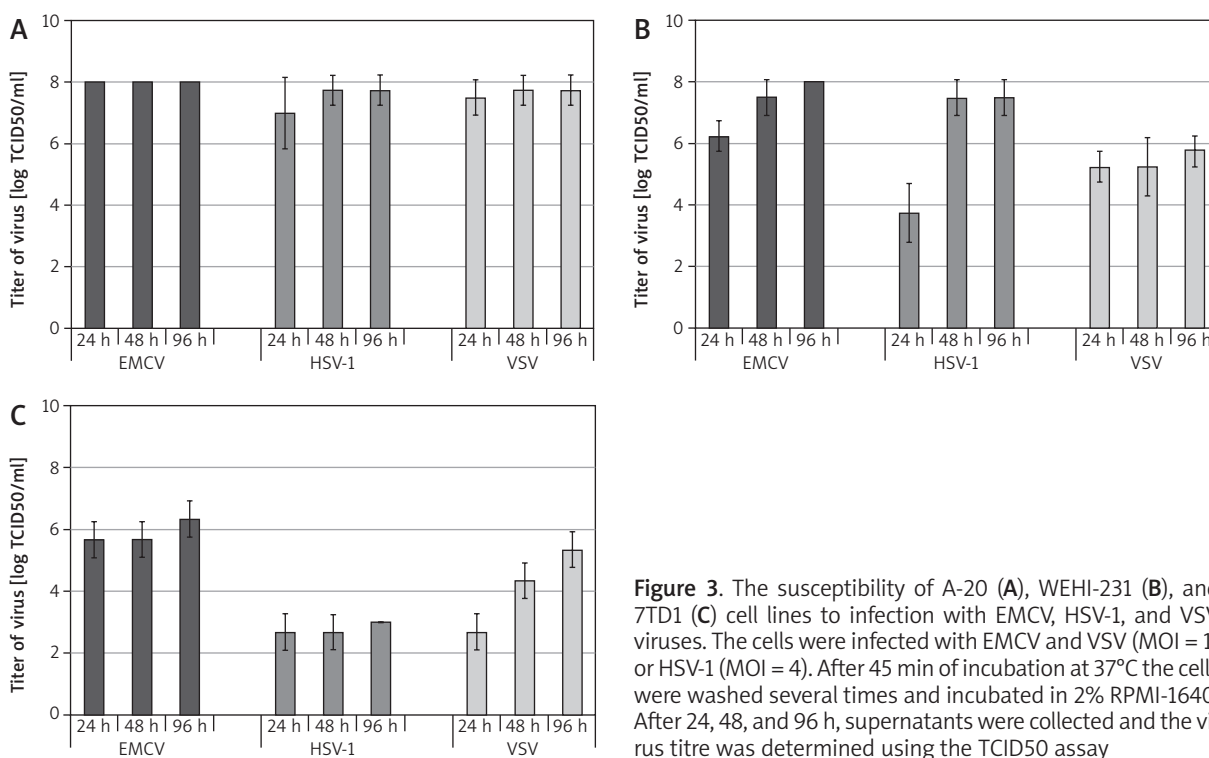


Figure 3. The susceptibility of A-20 (**A**), WEHI-231 (**B**), and 7TD1 (**C**) cell lines to infection with EMCV, HSV-1, and VSV viruses. The cells were infected with EMCV and VSV (MOI = 1) or HSV-1 (MOI = 4). After 45 min of incubation at 37°C the cells were washed several times and incubated in 2% RPMI-1640. After 24, 48, and 96 h, supernatants were collected and the virus titre was determined using the TCID50 assay

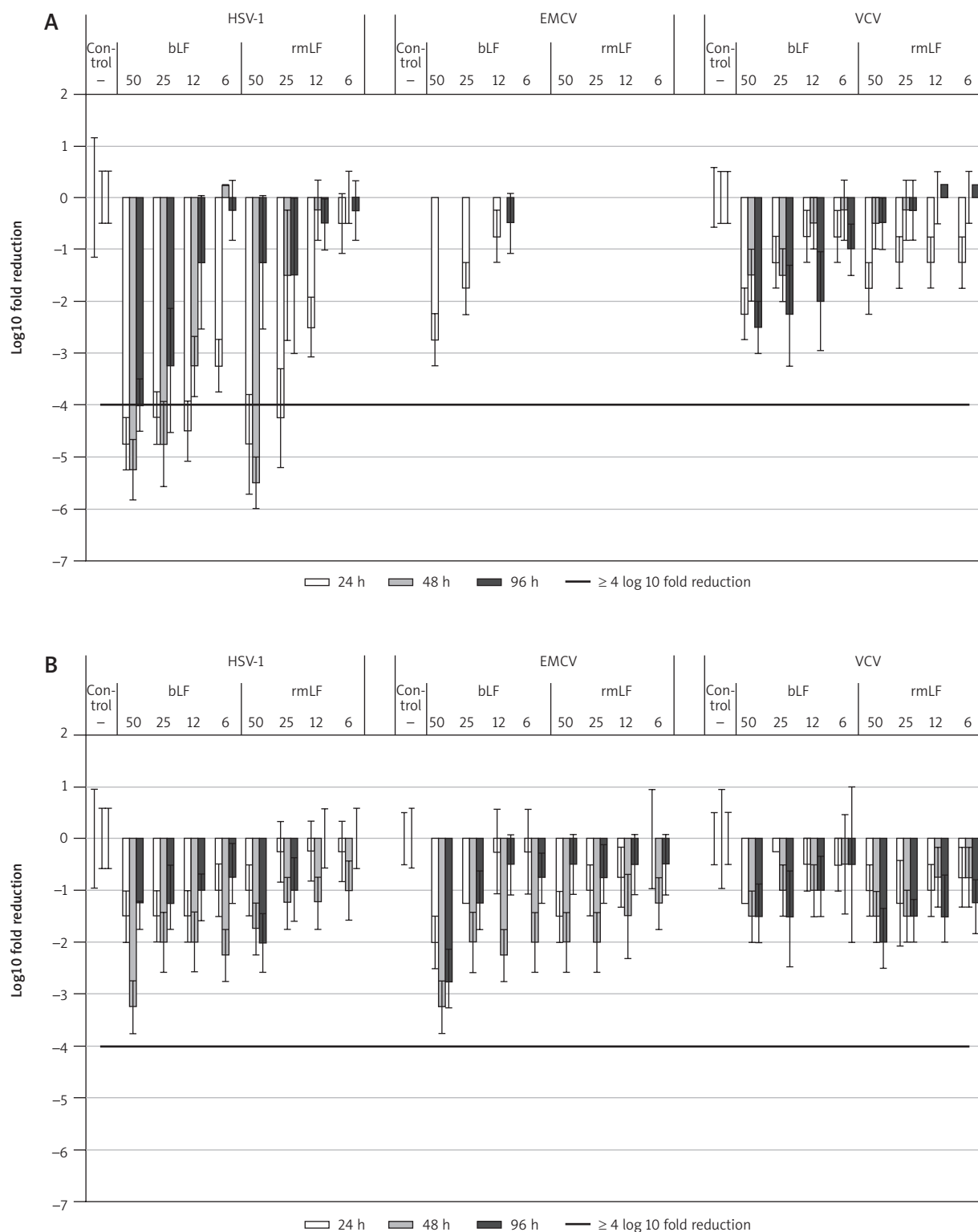


Figure 4. A-20 (A), WEHI-231 (B), and 7TD1; (C) cells were infected with EMCV and VSV (MOI = 1) or HSV-1 (MOI = 4). After 45 min at 37°C the virus inoculums were removed and the cells were incubated with bLF or rmLF. The cells treated only with the virus served as controls. Following incubation the viral cytopathic effect (CPE) was analysed. The corresponding negative controls (infected cells no LF-s) are marked as 0. Bold line: virucidal activity, ≥ 4 Log₁₀-fold reduction. Bars represent mean $\pm$ SD of 3 experiments

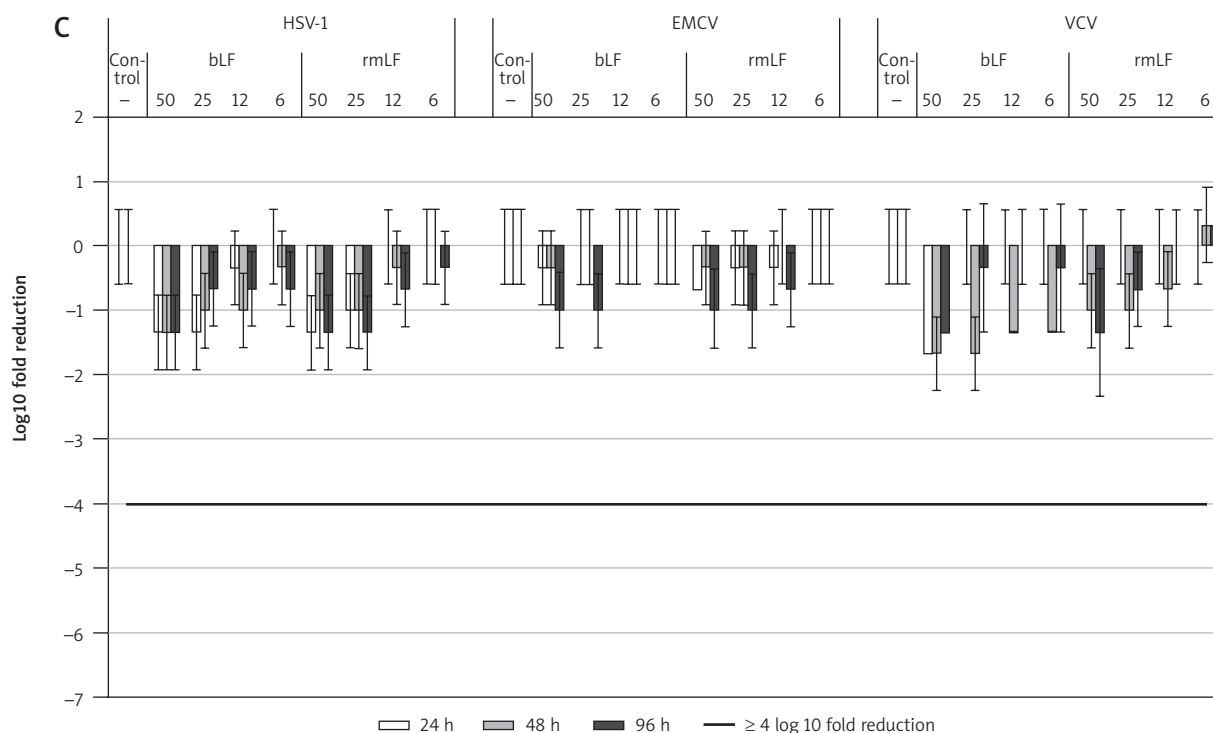


Figure 4. Cont.

correlate with the present study on A-20 cell line. Our results also confirmed other findings that A-20 cells are highly susceptible to viral infection [23]. In the present work bLF was much more effective in inhibiting viral replication when applied in culture after the infection, as compared to a pretreatment of the cells with LF. Similar results were obtained with resident splenic cells [25]. Because the main common cell receptor for LF and viruses is heparin sulphate [3, 17], other routes of viral entry, not interfered by LF, may account for this difference in LF antiviral actions.

The inhibitory effects of LF on virus replication and the susceptibility of A-20 cells to infection varied, depending on the type of virus. In the evaluation of the antiviral activity of LF we must consider a commonly accepted measure that ≥ 4 Log₁₀ reduction of virus titre is virucidal for chemical disinfectants because it leads to 99.99% reduction of viral titre [26]. A much deeper inhibition of viral replication was registered with HSV-1 than with VSV virus. This discrepancy could be due to different cell receptors used by these viruses, where VSV uses angiotensin-converting enzyme 2 (ACE2) [27] and HSV-1 – heparan sulphate [28] efficiently blocked by LF, as shown by others [17]. Although the target cells were washed after preincubation with LF, such a procedure cannot reverse firm attachment of LFs with cellular heparin sulphate receptors and subsequent block of virus entry.

In turn, the low susceptibility of 7TD1 cell to the virus probably results from a lack of most relevant receptors on these cells in the terminal stage of B-cell

differentiation. Thus, in theory, antibody-producing plasma cells may be less susceptible to viral infection. On the other hand, at this stage of investigation, it is hard to explain why homologous rmLF was effective in WEHI-231 but not in A-20 cell line.

Considering the ability of lactoferrin to enter cells, the protein may affect viral RNA. In fact, bLF significantly decreased the content of intracellular viral RNA in bovine kidney MDBK cells infected with enterovirus E [28, 29]. However, the effective concentration of LFs, leading to reduction of the cytopathic effect in our model (50 µg/ml), was many times lower than reported by these authors (1 mg/ml). This difference could be due to another experimental model used in their study.

Conclusions

The degree of infection was dependent on a stage of B-cell maturation. Mature B cells (A-20) were most susceptible to viral infection. Lactoferrins suppressed multiplication of 3 types of viruses to various degrees, and bLF was more effective than rmLF. We postulate that lactoferrin has a potential therapeutic utility in viral infections of B cells, applied together with classical antiviral drugs.

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Ethical approval

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

References

- Rowe M, Fitzsimmons L, Bell AI. Epstein-Barr virus and Burkitt lymphoma. *Chin J Cancer*. 2014; 33: 609-619.
- Márquez AC, Horwitz MS. The role of latently infected B cells in CNS autoimmunity. *Front Immunol*. 2015; 6: 544.
- Akula SM, Wang FZ, Vieira J, Chandran B. Human herpesvirus 8 interaction with target cells involves heparan sulfate. *Virology*. 2001; 282: 245-255.
- Marzi A, Möller P, Hanna SL, Harrer T, Eisemann J, Steinkasserer A, Becker S, Baribaud F, Pöhlmann S. Analysis of the interaction of Ebola virus glycoprotein with DC-SIGN (dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin) and its homologue DC-SIGNR. *J Infect Dis*. 2007; 196: 237-246.
- Erlenhoefer C, Wurzer WJ, Löffler S, Schneider-Schaulies S, ter Meulen V, Schneider-Schaulies J. CD150 (SLAM) is a receptor for measles virus but is not involved in viral contact-mediated proliferation inhibition. *J Virol*. 2001; 75: 4499-44505.
- Yang L, Maruo S, Takada K. CD21-mediated entry and stable infection by Epstein-Barr virus in canine and rat cells. *J Virol*. 2000; 74: 10745-10751.
- Haan KM, Kwok WW, Longnecker R, Speck P. Epstein-Barr virus entry utilizing HLA-DP or HLA-DQ as a coreceptor. *J Virol*. 2000; 74: 2451-2454.
- Colin M, Mailly L, Rogée S, D'Halluin JC. Efficient species C HAdV infectivity in plasmocytic cell lines using a clathrin-independent lipid raft/caveola endocytic route. *Mol Ther*. 2005; 11: 224-236.
- Whitlock CA, Ziegler SF, Treiman LJ, Stafford JI, Witte ON. Differentiation of cloned populations of immature B cells after transformation with Abelson murine leukemia virus. *Cell*. 1983; 32: 903-911.
- Weissinger EM, Mischak H, Goodnight J, Davidson WE, Mushinski JF. Addition of constitutive c-myc expression to Abelson murine leukemia virus changes the phenotype of the cells transformed by the virus from pre-B-cell lymphomas to plasmacytomas. *Mol Cell Biol*. 1993; 13: 2578-2585.
- Bouquet C, Melchers F. Pim1 and Myc reversibly transform murine precursor B lymphocytes but not mature B lymphocytes. *Eur J Immunol*. 2012; 42: 522-532.
- Kudo T, Tachibana T. Phenotype characteristics of human B cells studied by Epstein-Barr virus infection. II. C3 receptor switching during human B cell differentiation. *Immunol Lett*. 1984; 8: 343-348.
- Coleman CB, Nealy MS, Tibbetts SA. Immature and transitional B cells are latency reservoirs for a gammaherpesvirus. *J Virol*. 2010; 84: 13045-13052.
- Sedger LM, Hou S, Osvath SR, Glaccum MB, Peschon JJ, van Rooijen N, Hyland L. Bone marrow B cell apoptosis during in vivo influenza virus infection requires TNF-alpha and lymphotoxin-alpha. *J Immunol*. 2002; 169: 6193-6201.
- Légrand D. Overview of lactoferrin as a natural immune modulator. *J Pediatr*. 2016; 173 Suppl: S10-S15.
- Redwan EM, Uversky VN, El-Fakharany EM, Al-Mehdar H. Potential lactoferrin activity against pathogenic viruses. *C R Biologies*. 2014; 337: 581-595.
- Välimaa H, Tenonuo J, Waris M, Hukkanen V. Human lactoferrin but not lysozyme neutralizes HSV-1 and inhibits HSV-1 replication and cell-to-cell spread. *Virol J*. 2009; 6: 53.
- Di Biase AM, Pietrantonio A, Tinari A, Siciliano R, Valenti P, Antonini G, Seganti L, Superti F. Heparin-interacting sites of bovine lactoferrin are involved in anti-adenovirus activity. *J Med Virol*. 2003; 69: 495-502.
- Zheng Y, Qin Z, Ye Q, Chen P, Wang Z, Yan Q, Luo Z, Liu X, Zhou Y, Xiong W, Ma J, Li G. Lactoferrin suppresses the Epstein-Barr virus-induced inflammatory response by interfering with pattern recognition of TLR2 and TLR9. *Lab Invest*. 2014; 94: 1188-1199.
- Zhou H, Zhu Y, Liu N, Zhang W, Han J. Effect of iron saturation of bovine lactoferrin on the inhibition of hepatitis B virus in vitro. *Peer J*. 2024; 12: e17302.
- Andreu S, Ripa I, Bello-Morales R, López-Guerrero JA. Liposomal lactoferrin exerts antiviral activity against HCoV-229E and SARS-CoV-2 pseudoviruses in vitro. *Viruses*. 2023; 15: 972.
- Hirai H, Adachi T, Tsubata T. Involvement of cell cycle progression in survival signaling through CD40 in the B-lymphocyte line WEHI-231. *Cell Death Differ*. 2004; 11: 261-269.
- Weaver EA, Chen CY, May SM, Barry ME, Barry MA. Comparison of adenoviruses as oncolytics and cancer vaccines in an immunocompetent B cell lymphoma model. *Hum Gene Ther*. 2011; 22: 1095-1100.
- Van Snick J, Cayphas S, Vink A, Uytendhove C, Coulie PG, Rubira MR, Simpson RJ. Purification and NH₂-terminal amino acid sequence of a T-cell-derived lymphokine with growth factor activity for B-cell hybridomas. *PNAS*. 1986; 83: 9679-9683.
- Zaczyńska E, Artym J, Kocięba M, Burster T, Kruzel M, Paprocka M, Zimecki M. Antiviral resistance of splenocytes in aged mice. *Pol J Microbiol*. 2017; 66: 131-134.
- Jonsdottir HR, Zysset D, Lenz N, Siegrist D, Ruedin Y, Ryter S, Züst R, Geissmann Y, Ackermann-Gäumann R, Engler OB, Weber B. Virucidal activity of three standard chemical disinfectants against Ebola virus suspended in tripartite soil and whole blood. *Sci Rep*. 2023; 13: 15718.
- Tomczyk T, Orzechowska B. Vesicular stomatitis virus (VSV) as a vaccine vector for immunization against viral infections. *Postep Hig Med Dosw*. 2013; 67: 1345-1358.
- Chopra P, Yadavalli T, Palmieri F, Jongkees SAK, Unione L, Shukla D, Boons G-J. Synthetic heparanase inhibitors can prevent Herpes simplex viral spread. *Angew Chem Int*. 2023; 62: e202309838.
- Wróbel M, Małaczewska J, Kaczorek-Łukowska E. Antiviral effect of bovine lactoferrin against Enterovirus E. *Molecules*. 2022; 27: 5569.

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