



Short Communication

Effect of Cyclosporine A on the Non-Specific, Innate Antiviral Immunity of Mice

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Abstract. Different infections are the most common complication of immunosuppressive therapy. In this context, the effect of cyclosporine A (CsA) on the innate antiviral immunity of mice was studied. The presence of immunity was shown by infection of resident peritoneal cells (RPC) of BALB/c mice with herpes simplex virus type 1 (HSV-1) and vesicular stomatitis virus (VSV). While the cells infected immediately after isolation were resistant to the viruses, the cells cultured for several days before infection lost the immunity. The lack of activity to neutralize HSV-1 and VSV in the sera of the mice excluded a participation of specific antibodies in the resistance. To study the effect of CsA on the innate immunity, BALB/c mice were intraperitoneally (i.p.) injected with cyclosporine (20 or 100 µg/mouse, twice a day) for 3 days. The other group of animals was injected in the same way with PBS only. Then the peritoneal cells were isolated and infected with VSV immediately after cell isolation. The kinetics of viral replication in the control and CsA-treated groups was compared. While in the cells from the control group VSV did not multiply, in the cells from the CsA-treated mice the virus reached considerable titers. The cyclosporine effect on VSV replication was dose dependent and statistically significant. We conclude that innate antiviral immunity was suppressed in the cyclosporine-treated mice and that this mechanism may be involved in the high susceptibility of patients to viral infections during immunosuppressive therapy.

Key words: innate immunity; viral infections; cyclosporine.

Introduction

Different infections are the most common life-threatening complication of long-term immunosuppressive therapy. The potential sources of the infections are latent viruses and pathogens of community and hospital origin. Herpes simplex virus (HSV-1), cytomegalovirus (CMV) as well as hepatitis B and C virus infections are most common during the first month after transplanta-

tion⁴. Other viral infections Epstein-Barr virus (EBV), vesicular stomatitis virus (VSV), influenza virus, respiratory syncytial virus (RSV), adenoviruses occur later on. The consequences of viral infections for transplant patients are serious, since viruses may intensify the reactions of transplant rejection. In immunosuppressed transplant patients, an increased frequency of EBV-associated B cell lymphoproliferative disease is observed⁶. The effect of cyclosporine A (CsA) on the

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EBV-associated B cell proliferation *in vitro* was studied by TANNER and ALFIERI¹². They found that CsA increased not only the cell proliferation, but also the number of cells expressing the viral lytic cycle. As different viral, bacterial, fungal and parasitic infections are expressed early after transplantation, and the depression of immunity progresses in time and involves other pathogens, the possibility of innate immunity reduction by immunosuppressive drugs seems to be probable.

The presence of innate antiviral immunity was found in freshly explanted human and animal tissues and cells^{8-10, 13}. The innate non-specific immunity depended on endogenous cytokines such as the interferons IFN- α , IFN- β , IFN- γ and tumor necrosis factor type α (TNF- α). However, the contribution of other endogenous cytokines in the constitution of innate immunity cannot be excluded. Innate non-specific immunity against pathogens is present not only in mammals, but also in invertebrates and plants. Some elements used in the regulation of the immunity or the signaling pathway are very similar, and even identical in such different organisms. Contrary to specific immunity, characteristic only for vertebrates, non-specific immunity is MHC independent. Both kinds of immunity, specific and non-specific, differ from each other in the ways of pathogen recognition, cell engagement and effector functions. According to JANEWAY⁵, innate immunity plays an instructive role for adaptive immunity.

The aim of this study was to investigate if cyclosporine can activate viral replication by influencing the innate immunity.

Materials and Methods

BALB/c mice, female, 7–10 weeks old, conventional, were bred in the Animal Facility of the Institute of Immunology and Experimental Therapy, Wrocław, Poland.

Resident peritoneal cells (RPC) were washed out from the peritoneal cavity of the mice using 5 ml of Eagle's minimum essential medium (EMEM) supplemented with 10% heat inactivated calf serum (cs). A suspension of cells at a density of 10^6 cells/ml was used in the experiments.

Viruses: VSV (*Rhabdoviridae*), Indiana strain, was propagated and titrated in the murine cell line L₉₂₉; HSV-1 (*Herpesviridae*), McIntyre strain, was propagated and titrated in the human A₅₄₉ cell line.

Peritoneal cells from individual mice, at a density of 10^6 cells/ml, were infected with VSV or HSV-1 at

multiplicity of infection of 10. After virus adsorption (40 min, at room temperature) the cells were washed 4 times with 5 ml EMEM supplemented with 2% cs. A sample of the infected cells was kept at 4°C and used to control the starting levels of the viruses. The remaining infected cells were incubated at 37°C. Each day (1–5) after infection, samples of medium above the cells were collected and titrated: VSV in L₉₂₉ cells, HSV-1 in the A₅₄₉ cell line. The titers were expressed as the tissue culture infectious dose (TCID₅₀) from the cytopathic effect caused by the viruses in the cell line cultures.

Cyclosporine A (Sandoz, Pharma Ltd., Basle, Switzerland) was used in the experiments. Mice were injected intraperitoneally (i.p.) with doses of 20 or 100 μ g per mouse, twice a day, for 3 days.

A virus neutralization test was performed on 96-well plastic plates. Sera from individual mice were diluted from 1/4–1/8000. The viruses VSV or HSV-1 at a concentration of 15 TCID₅₀ were added to each well. After 2 h of incubation at room temperature, the plate contents were transferred by multichannel pipette to one day incubated tissue cultures: L₉₂₉ for VSV and A₅₄₉ for HSV-1. The plates were incubated at 37°C. The presence of a cytopathic effect was controlled after one or two days.

Statistics were performed using Student's *t*-test. The differences were considered statistically significant at $p < 0.05$.

Results

In the study, two viruses belonging to different taxonomic groups were used for the detection of natural innate immunity in murine RPC: HSV-1 and VSV. Freshly washed-out heterogeneous RPC from 12 individual BALB/c mice (non-provoked and non-stimulated) were infected with the viruses immediately after isolation ($t = 0$) or after one ($t = 24$) or two days ($t = 48$) incubation at 37°C. The kinetics of the viruses' replication was studied. The cells from all mice infected at time 0 ($t = 0$) were resistant to the both viruses. The resistance of the RPC was gradually diminished when cultured before virus infection. The time of culturing before infection, in which they develop sensitivity to viral infection, was individually differentiated and ranged from 1 to 5 days. Therefore, the individual cases are presented in Fig. 1.

In order to exclude a possible participation of adaptive antiviral immunity in the resistance, the presence of virus-neutralizing activities in sera of 12 non-in-

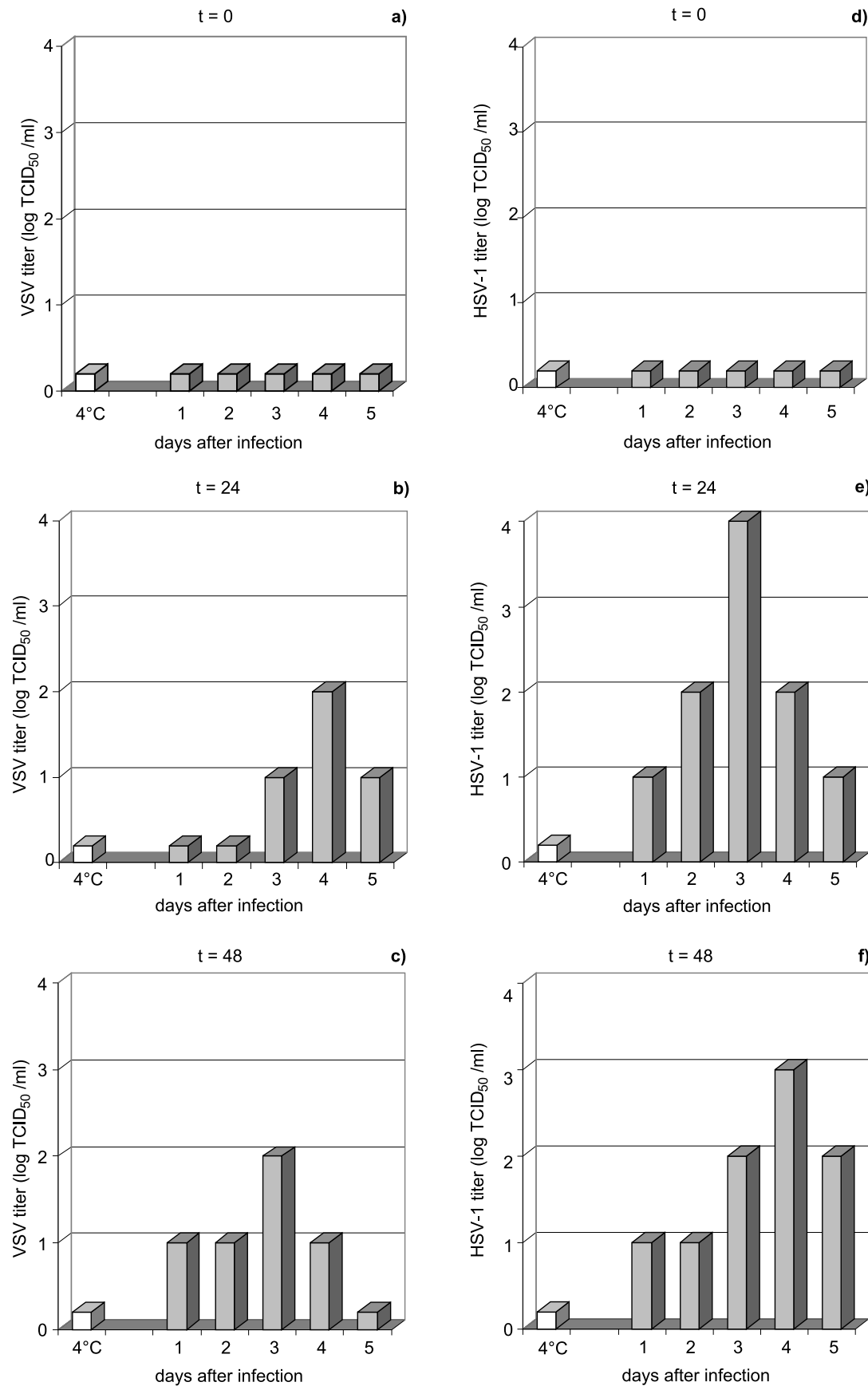


Fig. 1. Kinetics of VSV (a–c) and HSV-1 (d–f) replication in resident peritoneal cells (RPC) of BALB/c mice; dependence on the time culture of RPC before virus infection. There are presented the results performed on RPC from one individual mouse

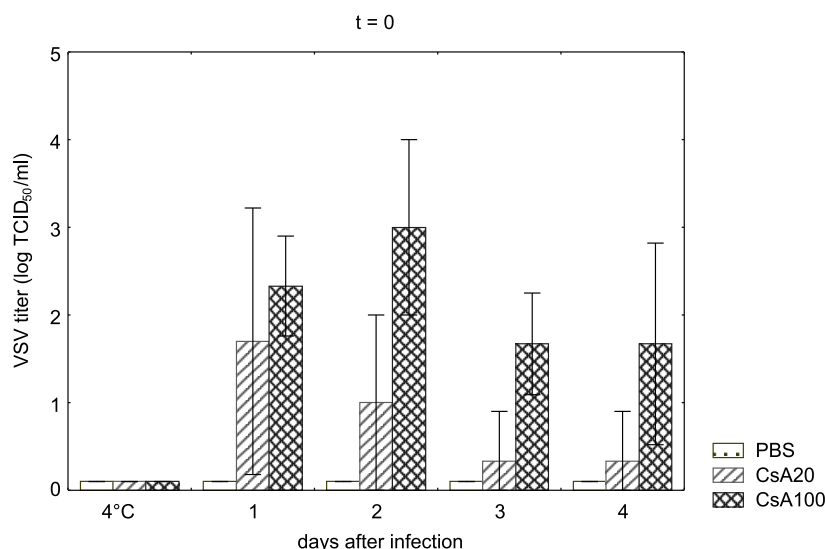


Fig. 2. Comparison of kinetics of VSV replication in RPC from CsA-treated and PBS-injected BALB/c mice. Mice were injected for 3 days with the dose of 20 or 100 µg of CsA in PBS. The control mice were injected in the same way with PBS only. Then the peritoneal cells were washed-out and infected with VSV immediately. There are presented mean titers and standard deviation. The control titers (4°C) of the viruses were below 10¹ TCID₅₀. The differences between titers of VSV in RPCs from CsA100- and PBS-treated mice are statistically significant: PBS/CsA100 – $p = 0.026388$, CsA20/CsA100 – $p = 0.036259$, PBS/CsA20 – $p = 0.113930$.

infected mice, bred under conventional conditions, was examined. None of the sera contained VSV- and HSV-1-neutralizing activity, which means that a possible participation of antiviral antibodies in the resistance of the RPC was excluded.

Then the influence of CsA on the natural innate antiviral immunity was studied. In this study, 27 BALB/c mice were used. They were divided into 3 groups: two groups were treated with different doses of CsA and the control group with PBS only. Cyclosporine in doses of 20 or 100 µg per mouse was given i.p., twice a day for 3 days. The control group of mice was injected in the same way with PBS. Then the cells were washed out from the peritoneal cavities of each mouse and immediately infected with VSV ($t = 0$). The kinetics of virus replication in these three groups was compared. The results are presented in Fig. 2. They show that CsA, given *in vivo*, reduced the innate antiviral immunity of peritoneal cells. The effect was statistically significant when CsA in a dose of 100 µg/mouse was used. However, a stimulatory effect on virus replication was not observed when cyclosporine was added *in vitro* to freshly isolated cells. The results are not presented here.

Discussion

In this study the presence of non-specific immunity, described as the resistance of freshly isolated resident murine peritoneal cells to VSV, was confirmed⁹. The resident peritoneal cells cultured *in vitro* one to several days before virus infection lost the immunity and acquired a sensitivity to viral infection. The non-specific

character of the immunity was confirmed by the resistance of the cells to infection with a DNA-containing herpes virus belonging to a different taxonomic group. Moreover, the participation of adaptive immunity was excluded because there were no specific antibodies against the viruses used in the experiments in the sera of the mice. The results of the study showed that in CsA-treated mice, innate non-specific antiviral immunity was significantly reduced. In contrast, CsA, added *in vitro* to freshly isolated RPC did not affect the innate immunity of these cells. The reason for this is unknown. Perhaps the time of CsA contact with the infected cells was too short. The mice were treated with CsA for 3 days, twice a day, before cell isolation and infection. Another explanation of the differences is that CsA given *in vivo* does not affect the innate immunity directly, but by other mediators induced by CsA.

In the light of the beneficial effect of cyclosporine in organ transplantation, the activation of different viruses in transplant patients during immunosuppressive therapy causes a serious problem. Cyclosporine prevents the development of adaptive immunity against the transplanted organs. It was considered that CsA acts mainly on specific antiviral immunity. That effect on antiviral humoral immunity was at first studied by some authors^{2, 3, 11}. They found that the production of antibodies against influenza A virus and VSV was not much affected by CsA. CHARAN et al.² noticed only an inhibition of IgG in primary VSV infection, but IgM antibody, switching IgM-IgG, secondary IgG and the memory cells were not affected by cyclosporine. SCHILTKNECHT and ADA¹¹, who studied humoral and cellular responses in mice to influenza A virus, found that the hemagglutination-inhibiting

antibodies in CsA-treated mice were delayed but reached almost control levels. According to literature data, the presence of antibodies against some viruses does not prevent viral activation in immunosuppressed patients. For instance, the degree of hepatitis C virus activation was similar in antibody-positive and -negative cyclosporine-treated transplant patients¹. The results of our study indicate that cyclosporine A affects the cytokine-dependent innate antiviral immunity. It is possible, however, that other elements of the non-specific antiviral immune system are affected by cyclosporine also. According to MIEDZHITOV and JANEWAY⁷, innate immunity plays an instructive role for the adaptive system. If this hypothesis is true in relation to antiviral immunity, reduced innate immunity may influence the level of the primary specific response.

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