ANTIVIRAL EFFECT OF PROLINE-RICH POLYPEPTIDE IN MURINE RESIDENT PERITONEAL CELLS

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Summary. – It is known that resident peritoneal (RP) cells from BALB/c female mice express a constitutive non-specific antiviral immunity which is progressively reduced during several days of cultivation *in vitro*. In this report, we have studied the effect of a proline-rich polypeptide (PRP) isolated from ovine colostrum on the kinetics of vesicular stomatitis virus (VSV) replication in freshly isolated and one-day cultured RP cells. The polypeptide was added to the cells immediately after virus adsorption or one day before or after viral infection. Independently on time of PRP addition, an inhibition of VSV replication (virus titres reduced by up to 4 log units) was observed. Occasionally, however, a weak stimulation of VSV replication by PRP (virus titres increased by 1–2 log units) was noticed in RP cells constitutively resistant to the infection.

Key words: murine peritoneal cells; proline-rich polypeptide; ovine colostrum; vesicular stomatitis virus; antiviral effect

Introduction

Human and animal tissues *ex vivo* express a constitutive, non-specific, antiviral immunity (Orzechowska and Błach-Olszewska, 1996; Paradowska *et al.*, 1996; Zaczyńska *et al.*, 1995). Also an innate non-specific immunity against bacterial and protozoan infections has been observed (Albright *et al.*, 1997; Chen *et al.*, 1992; Gosselin *et al.*, 1995; Tsai *et al.*, 1997). This kind of immunity against infective agents may be a primary form of constitutive defence mechanism. For the constitutive antiviral immunity, endogenous cytokines such as interferons (IFNs) and tumour necrosis factors (TNFs) are responsible (Orzechowska and Błach-Olszewska, 1996; Paradowska *et al.*, 1996). However, the participation of other cytokines and mediators, e.g. interleukins 6 (IL-6), 10 (IL-10), and 12 (IL-12), NO and O_2^-, could not be excluded. During *in vitro* cultivation of human and animal tissues previously resistant to viruses their constitutive immunity gradually decreases and their susceptibility to viral infection increases (Orzechowska and Błach-Olszewska, 1996; Paradowska *et al.*, 1996; Zaczyńska *et al.*, 1995). The constitutive defence is reduced sometimes also *in vivo*. Human or animal organisms with reduced immunity subjected to viral infections frequently develop diseases. Stimulation of the non-specific immunity with immunomodulating drugs seems to be helpful in preventing development of diseases.

To study the effect of an immunomodulatory substance on antiviral non-specific immunity of cells, PRP was used. PRP, a milk product isolated and purified for the first time by Janusz *et al.* (1974), is present in colostrum of sheep during first days after delivery and has a high proportion of proline residues. It has been characterised biochemically and biologically (Janusz and Lisowski, 1996; Janusz *et al.*, 1981). PRP acts both *in vivo* and *in vitro* and is not species-specific. It possesses a regulatory activity stimulating low or suppressing high, both humoral and cellular immune response (Janusz *et al.*, 1978; Lisowski *et al.*, 1988; Wiec-

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Abbreviations: CS = calf serum; IFN = interferon; IL = interleukin; MEM = Minimal Essential Medium; PRP = proline-rich polypeptide; RP = resident peritoneal; TNF = tumour necrosis factor; VSV = vesicular stomatitis virus

Table 1. Effect of PRP on VSV replication

Time of PRP administration	Number of experiments with			
	RP cells sensitive to infection (n=14)		RP cells resistant to infection (n=22)	
	VSV inhibition	No effect	VSV stimulation	No effect
24 hrs before infection (n=12)	6	1	1	4
Immediately after adsorption (n=12)	4	1	2	5
24 hrs after infection (n=12)	2	–	4	6

Summary of results of 36 experiments. n = number of experiments.

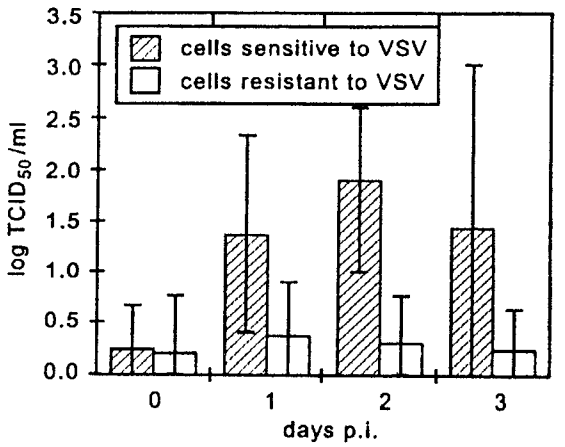

Fig. 1
Kinetics of VSV multiplication in RP cell cultures
One-day-old cell cultures were used for infection. Of total of 42 mice, 16 yielded sensitive cells and 26 yielded resistant cells to VSV infection.

zorek *et al.*, 1979). Recently, we have found that PRP at concentrations of 1 – 100 μg/ml induced IFN-β and TNF-α in RP cells *ex vivo* (Błach-Olszewska and Janusz, 1997). According to Inglot *et al.* (1996), PRP induces IFN-γ, IFN-α and TNF-α in human blood cultures and isolated leukocytes. PRP shows practically no toxicity as its LD_{50} for mice by oral or parenteral route is more than 1.25 g/kg (Inglot *et al.*, 1996). In view of its potential therapeutic use we decided to study its influence on virus replication in experimental system of murine RP cells and VSV.

Materials and Methods

Mice. Female BALB/c mice in age of 7 – 10 weeks obtained from the Inbred Mice Unit of the Institute of Immunology and Experimental Therapy, Wroclaw, were used.

RP cells were washed out from the peritoneal cavity of mice by using 5 ml of Eagle's Minimal Essential Medium (MEM) supplemented with 10% calf serum (CS). The obtained cell suspension (1 x 10^6 cells/ml) was used directly or after 1 day of cultivation at 37°C for experimental infection. Survival of the cells during the first four days of culture was very good and reached 97 – 100% (Orzechowska and Błach-Olszewska, 1996).

Virus. Indiana strain of VSVwas propagated and titrated in mouse L929 cells.

PRP was isolated from sheep colostral whey by the method described by Janusz *et al.* (1981). PRP in concentration of 10 μg/ml was used in cell culture experiments.

Experimental infection. Fresh RP cells or one-day-old cultures of RP cells were infected with VSV at multiplicity of 0.1 $TCID_{50}$ per cell. After virus adsorption at 37°C for 40 mins, the cultures were washed three times with fresh medium and further incubated at 37°C. PRP was added to the cultures at different times before or after infection. Samples of medium were taken from the cultures on days 0 – 3 post infection (p.i.) and were titrated for infectious virus (log $TCID_{50}$/ml).

Results and Discussion

In the first stage of the study, the constitutive antiviral immunity of RP cells in population of mice used for experiments was examined. To demonstrate the constitutive immunity of the cells, a VSV infection was used. Out of 44 mice used for the experiment, the virus multiplied in freshly isolated RP cells from 9 animals only. The rest of mice had RP cells constitutively resistant to the virus. The proportion of mice with RP cells sensitive to the virus infection increased when the cells were cultivated *in vitro* for one day before infection. Most of the mice, however, were still resistant to VSV (Fig. 1).

To study the effect of PRP on VSV replication, freshly isolated RP cells as well as one-day-old RP cell cultures were used. PRP was added to the RP cells from individual mice at a concentration of 10 μg/ml of culture medium immediately after virus adsorption (12 experiments), one day before (12 experiments) and one day after virus infection (12 experiments.). The kinetics of virus replication in PRP-treated and non-treated RP cell cul-

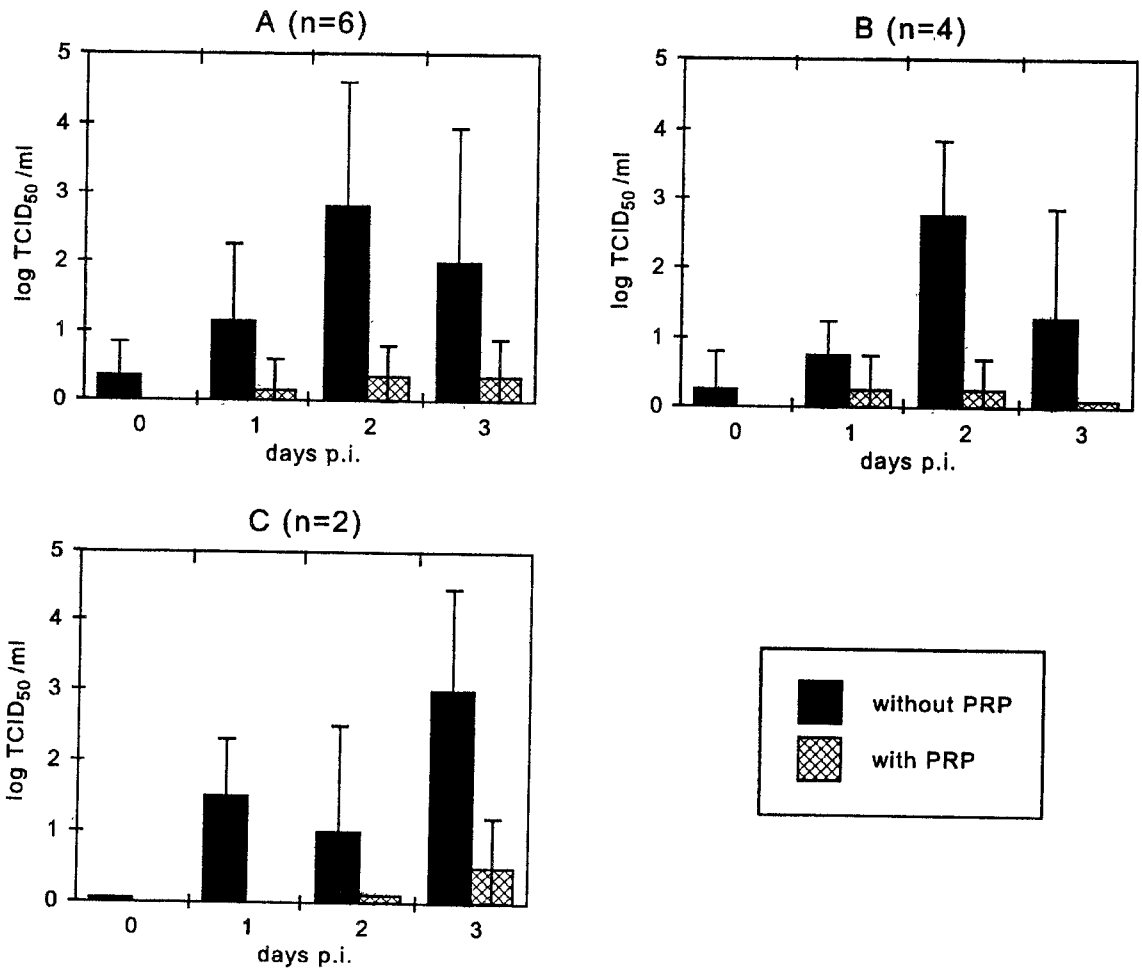

Fig. 2
Antiviral effect of PRP
PRP was added to cell cultures 24 hrs before infection (A), immediately after virus adsorption (B) and one day p.i. (C).

tures was compared (Table 1 and Fig. 2). Independently on time of PRP addition, an effect of inhibition of VSV replication was noticed in RP cells sensitive to the infection. In 7 out of 22 experiments with RP cells resistant to infection, a light stimulation of VSV titer by PRP was observed. This effect was rather marginal (virus titres reduced by 1–2 log units).

The obtained results indicate that PRP, a natural milk product, expresses a strong antiviral effect on murine RP cells. The antiviral effect is probably dependent on IFNs and TNF which are produced by PRP-treated RP cells. However, further experiments on the mechanism of the PRP effect are needed. The effect was independent on the time of PRP addition. This independence may be important for an eventual therapeutic use of PRP.

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