VESICULAR STOMATITIS VIRUS NUCLEOCAPSID PROTEIN PRODUCTION IN CELLS TREATED WITH SELECTED FAST PROTEIN LIQUID CHROMATOGRAPHY FRACTIONS OF TICK SALIVARY GLAND EXTRACTS

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Summary. – A salivary gland extract (SGE) prepared from 5-days-fed *Dermacentor reticulatus* female ticks was fractionated by fast protein liquid chromatography (FPLC). The effect of three FPLC fractions selected on the basis of anti-interleukin 8 (anti-IL-8) activity on vesicular stomatitis virus (VSV) nucleocapsid (N) protein formation in mouse L-cells was determined. Infected ^{14}C-labeled cells treated with the FPLC fractions were analyzed by two-dimensional (2D) electrophoresis. The yields of VSV N protein were evaluated by Imagemaster software analysis. Most noticeable was an increase in the N protein production after treatment with the fraction 39 corresponding to the major peak of the anti-IL-8 activity. The nature of the substance in SGE that was responsible for this effect remains unclear.

Key words: vesicular stomatitis virus; nucleocapsid protein; IL-8; tick salivary gland; Dermacentor reticulatus

Tick saliva contains many pharmacologically important components which had evolved to support a parasitic style of life of ticks (Ribeiro *et al.*, 1990; Ramachandra and Wikel, 1992; Wikel, 1996; Paesen *et al.*, 1999). Tick saliva also plays an important role in transmission of pathogens. The supported pathogen transmission, designated the saliva-activated transmission (SAT), has been originally described *in vivo* (Jones *et al.*, 1987; Labuda *et al.*, 1996). The possible explanation for SAT is that immunomodulatory activities of tick saliva offer better conditions for viruses in the feeding area (Fuchsberger *et al.*, 1995; Kubeš *et al.*, 2002).

In an earlier report we have described for the first time promotion of virus growth *in vitro* both at the level of virus growth and N protein production during virus infection in cells treated with SGE (Hajnická *et al.*, 1998). N protein seems to be an excellent marker for detecting extremely low levels of virus replication, and, particularly, for establishing the time of the start of promotion of virus replication by SGE. In another article (Kocáková *et al.*, 1999) we have described a quantitative evaluation of such a promotion. Interestingly, this effect has been demonstrated on VSV, although this virus is not known to be transmitted by ticks. VSV, an arthropod-borne virus, is a well known laboratory model virus used in many *in vitro* tests. By contrast, comparatively little is known about its *in vivo* survival

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Abbreviations: 2D = two-dimensional; FPLC = fast protein liquid chromatography; anti-IL-8 = anti-interleukin 8; N = nucleocapsid; PAGE = polyacrylamide gel electrophoresis; SGE = salivary gland extract; SAT = saliva-activated transmission; VSV = vesicular stomatitis virus

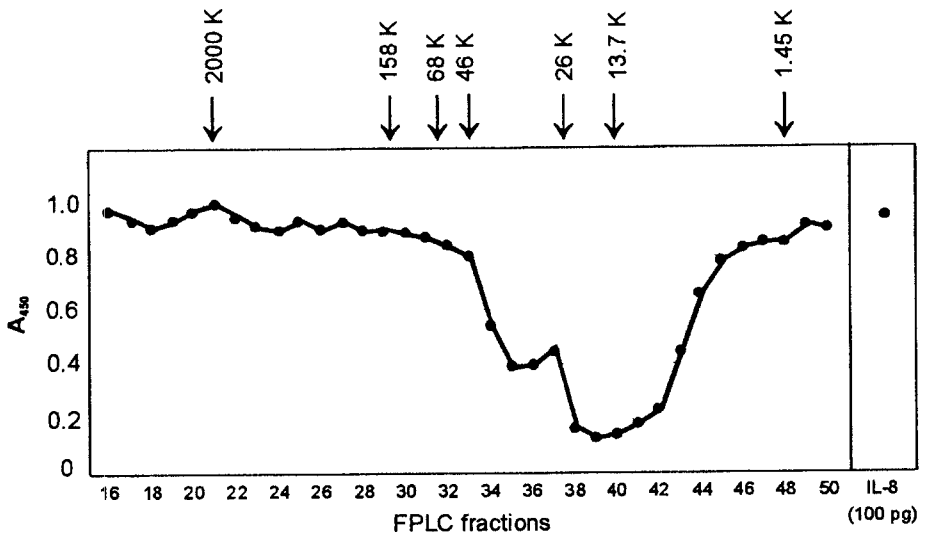

Fig. 1

FPLC profile of anti-IL-8 activity in SGE derived from 5-days-fed female *D. reticulatus* ticks as determined by ELISA

A reduction in A_{450} relative to the control indicates the anti-IL-8 activity. Arrows indicate the positions of protein size standards (from left to right): blue dextran (2000 K), aldolase (158 K), bovine serum albumin (68 K), ovalbumin (45 K), chymotripsinogen A (25 K), RNase A (13.7 K), and bacitracin (1.45 K).

strategy. Sandflies and black flies are responsible for transmission of VSV to susceptible hosts. Following our demonstration that the tick SGE promotes VSV replication, a non-viremic transmission and potentiation of infection for this virus by its natural arthropod vector has been described (Mead *et al.*, 2000; Limesand *et al.*, 2000). Because of difficulties in obtaining a sufficient amount of tick saliva for experimental studies, we have tested SGE. The component(s) in the tick SGE or saliva, responsible for promoting virus growth, is so far unknown. Candidate activities, the alpha interferon suppressing activity and the anti-IL-8 activity, both potentially beneficial for virus growth, have been reported by us earlier (Hajnická *et al.*, 2000, 2001).

Here we compare the virus-promoting activity *in vitro*, namely the activity promoting the VSV N protein formation in L cells, of three FPLC fractions of SGE representing the major peak, minor peak and background of the anti-IL-8 activity.

SGE was prepared from 5-days-fed female *D. reticulatus* ticks (4.7 mg of total protein from 83 ticks), separated by FPLC in native form at 5°C using a Superose 12 HR10/30 column (Pharmacia) and an equilibrium buffer consisting of 0.02 mol/l Tris-HCl pH 7.5 and 0.15 mol/l NaCl, a flow rate of 0.4 ml/min, and one fraction collected per min. Mixtures of 100 pg of IL-8 and 1 µl of the fractions were incubated at room temperature for 2 hrs and applied to ELISA plates. A colored product was formed in proportion to the IL-8 present in the mixture and its A_{450} was measured.

Fig. 1 demonstrates a typical profile of anti-IL-8 activity in *D. reticulatus* SGE fractionated by FPLC. A major peak was present in the fractions 38–41 and a minor one in fractions 35–36.

To determine whether this potent anti-chemokine activity corresponds to the virus growth promoting activity, fractions 39, 36, and 33 (representing the background) were subjected to testing. In this experiment monolayers of L-cells in 6-well plates were treated with 20 µl of the fractions 33, 36 and 39 per well, respectively, or were left untreated. After 3 hrs they were infected with 5 $TCID_{50}$ of VSV per well. One hr following infection, the cells were washed with PBS and 19 hrs incubated in the Leibowitz medium containing 1.5 MBq of ^{14}C-protein hydrolyzate (UVVVR, Prague). The cells were then harvested, solubilized in a buffer containing 9 mol/l urea and 4% Triton X-100, and subjected to 2D-

Table 1. Effect of pre-treatment of L cells with selected FPLC fractions on the level of VSV N protein

Pre-treatment of cells before infection	N protein	
	Volume[a]	Area[b]
None	37,549	1,189
Fraction 33	46,020	1,372
Fraction 36	47,685	1,431
Fraction 39	70,338	1,487

[a]Sum of intensities of each pixel in the spot.
[b]Size of a spot expressed as total number of pixels in the spot.

PAGE. (Hajnická *et al.*, 1998). The virus growth-promoting assay and 2D-PAGE were performed as described elsewhere (Kocáková *et al.*, 1999). From each pattern we show only an enlarged window with the area where N protein is present. The results are summarized in Fig. 2. The most apparent effect was observed with the fraction 39. This finding was verified by analyzing the gels by the Imagemaster software and by quantification of the spots corresponding to N protein. The results are presented in Table 1, in which the volume values are important.

Comparison of the three FPLC fractions of tick SGE for their capacity to influence the N protein production in VSV-infected L cells indicated that the fraction 39 (major peak of the anti-IL-8 acti-vity) was most active, while very little promotion activity was found in the fractions 36 (minor peak of the anti-IL-8 activity) and 33 (background).

It is not yet clear if this effect resulted from an inhibition of IL-8, an important chemokine. Another possibility is that the components with anti-IL-8 activity have also other, so far unknown activities ensuring better conditions for virus growth. Such an effect has been described for pro-&teoglycans, which are known to bind IL-8 (Mandl *et al.*, 2001). Still another possibility is that other components are present in the active fractions of tick SGE that are unrelated to the observed anti-IL-8 activity. Although further studies are obviously needed, the contribution to the identification of the virus growth promoting activity of the tick SGE made here may represent a step to this aim.

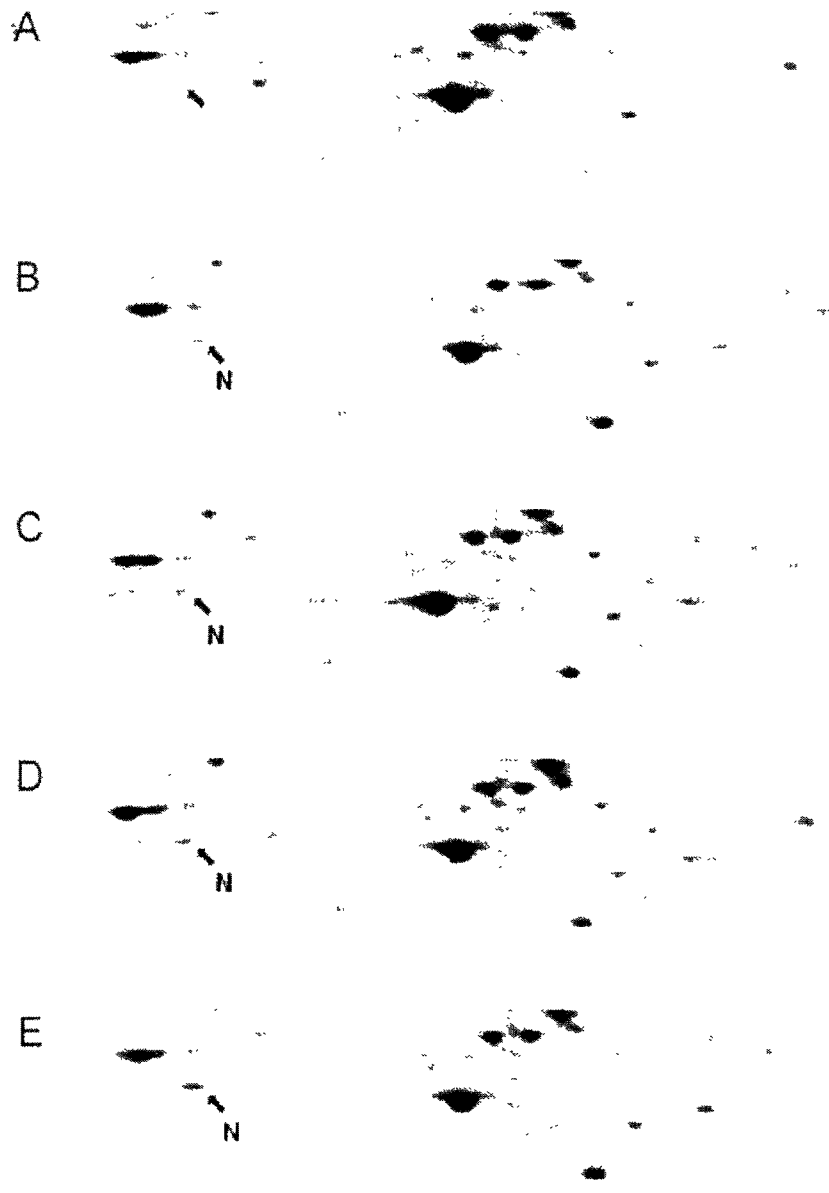

Fig. 2

Effect of selected FPLC fractions of tick SGE on VSV N protein production

Enlarged windows represent 2D-PAGE patterns of areas with VSV N protein: Untreated uninfected cells (A), untreated cells infected with VSV (B). Cells pretreated with FPLC fractions 33 (C), 36 (D) and 39 (E) prior to VSV infection. VSV N protein (N).

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