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# Peptide Cytokines in CNS and the Immune System

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## Summary

This study describes the effects of cytokine peptides released into the supernatant during an early allogeneic reaction (AR) of mouse spleen lymphocytes or brain cortex cells which differ in their major histocompatibility complex (MHC). The peptides were isolated by ultrafiltration, liquid chromatography and HPLC. We found that both peptides stimulated the cell surface $Na^+$,$K^+$-ATPase and $Ca^{2+}$-ATPase activities of quiescent spleen lymphocytes *in vitro* and mimicked early allogeneic cell interactions. Both brain and spleen AR peptides inhibited Concanavalin A-stimulated spleen lymphocyte proliferation, whereas $^3$H-TdR incorporation into DNA of the E7 neuroblastoma cell line was stimulated by these peptides. The peptide isolated from the supernatant of the allogeneic brain cell reaction inhibited phagocytosis in phorbol myristate-stimulated LA5-9/8 mouse macrophage cell line. Immunosuppressive activity of spleen AR peptide is supported by inhibition of spontaneous E rosette formation by lymphocytes. The immunosuppressive effect of isolated peptide cytokines on lectin-activated lymphocytes was comparable with the serum thymic factor (FTS, Lenfant *et al.* 1983). These changes demonstrate the pleiotropic cytokine actions mediated by plasma membrane of immune system and brain cells.

**Key words**
Peptide cytokine – Spleen lymphocyte – Brain cells – Cell activation – Proliferation

## Introduction

Cytokines are a group of secretory proteins that display diverse biological activities, namely in immune and inflammatory responses of the organism. It has become increasingly evident that cytokines also play a crucial role in bidirectional flow of information between the immune system and CNS. They take part in the maintenance of homeostasis of the organism and have important functions during embryogenesis, tissue repair, trauma infection or tumour growth. The cytokines are also implicated in the pathogenesis is of a number of pathological states of the brain such as Alzheimer disease, multiple sclerosis and AIDS dementia (Hopkins and Rothwell 1995, Benveniste *et al.* 1995). The term "cytokine" is now used for an increasing number of mediator families including interleukins, chemokines, tumour necrosis factors, interferons, colony stimulation factors, growth factors, neurotrophins and neuropoietins (Hopkins and Rothwell 1995, Rothwell and Hopkins 1995). Many cytokines are expressed in the brain *in situ* or in cells isolated from the CNS, mainly microglia and astrocytes, although neurones, perivascular and endothelial cells also seem to be involved. Receptors for IL-1, IL-2, IL-3, IL-6, TNF-$\alpha$ and many growth factors, have been identified in the brain tissue by radioligand binding, immunocytochemical techniques

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or expression of receptor mRNA (Hopkins and Rothwell 1995). There is now good evidence that some of the cytokines as well as peptide hormones and neurotransmitters and their receptors are common to the brain, immune and endocrine systems (Blalock 1994). In the immune system, all cytokines cooperate with neuropeptide signalling (e.g. vasoactive intestinal peptide, substance P, etc.) especially during T lymphocyte differentiation (Goetzl *et al.* 1995). Great attention is focused on cytokine "cross-talk" during a mixed lymphocyte reaction (MLR) as an *in vitro* model of graft *versus* host reaction. Cell signalling associated with allorecognition involves the interaction of cell surface located T-cell receptor and the MHC peptide complex, as well as formation of a cascade of second messengers induced *via* activation of phospholipase C or alternative pathways (Weber and Cantor 1994). Lectin lymphocyte activation is associated with changes in monovalent ion transport, membrane potential, $Na^+/H^+$ exchange and activation of ion-specific ATPases (Kaplan and Owens 1980, Brams and Claesson 1989). The study of alloantigen- or mitogen-lectin induced cell surface events in both the mixed lymphocyte reaction (MLR) and mixed brain cell reaction (MBR) showed H-2 alloantigen-dependent specific stimulation of $Na^+,K^+$-ATPase (EC 3.6.1.3), the non-specific activation of $Ca^{2+}$-ATPase (EC 3.6.1.3) and inhibition of ecto-5'-nucleotidase (EC 3.1.3.5) (Kovářů and Kovářů 1979, Kovářů 1980, Kozáková *et al.* 1996a, Kovářů *et al.* 1997b). These changes had a rapid time course, i.e. they appeared up to 2 h after the initiation of both MLR and MBR.

Our aim was to study the effects of peptide cytokines released in early MLR or MBR on various effector cells including those of the brain cortex and spleen, E7 neuroblastoma and LA5-9/8 macrophage cell lines. In these cells we determined activities of cell surface $Na^+,K^+$-ATPase and $Ca^{2+}$-ATPase and $^3$H-TdR incorporation into DNA. Modulation of proliferation was also assayed in Concanavalin A (Con A) stimulated lymphocytes. Finally, we compared the immunomodulatory effects of the MLR released cytokine and several regulatory peptides, such as the serum thymic factor (FTS, SDIP, Lenfant *et al.* 1983), bradykinin and tuftsin, as a function of their concentrations.

## Material and Methods

*Animals*

Two-month-old male mice of two H-2 incompatible strains (A/Ola and C3H/Cbi/BOM) reared under specific pathogen-free conditions (Velaz Šumice Farm, Czech Republic) were used as a source of cells for the allogeneic reaction. For syngeneic reactions, cells from either strain were used.

*Preparation of spleen and brain cell suspensions*

The spleen lymphocytes were isolated according to Kovářů and Kovářů (1979) and stored at 4 °C in Eagle Minimal Essential Medium (MEM, Institute of Sera and Vaccines, Prague) with 1 % bovine serum albumin (Sigma) overnight and then transferred into isotonic media or an albumin-free MEM (Kovářů *et al.* 1997). The purity and viability of the isolated lymphocytes was more than 94 %. The brain cell suspensions were prepared from the forebrain cortex by mechanical sieving through nylon sieves in an isotonic saline medium with 7.5 % polyvinylpyrrolidone (Kovářů and Lodin 1975, Kovářů 1980) and immediately used for the enzyme assays.

*Allogeneic reaction*

The two-way allogeneic reaction was based on a 1:1 ratio of mixed cells (mouse lymphocytes or brain cortex cells from A/Ola and C3H/Cbi/BOM mice). Spleen lymphocytes ($1x10^7$ cells/ml in 100 ml MEM) were incubated at 37 °C for 4 h. Brain cortex cell suspensions were diluted to 7.5 mg protein/ml of isotonic saline medium (Kovářů 1980, Kovářů *et al.* 1997). Equal volumes of the brain cell suspension of inbred strains (4.5 ml + 4.5 ml) were mixed and incubated in an ice-cold bath for 30 min with 10 mM D-glucose (Serva). The samples were then incubated in a shaking water bath at 37 °C for 60 min. Cell suspensions were centrifuged at 30 000 x g for 1 h and the cell free supernatants stored at −25 °C.

*Isolation of peptides from allogeneic reaction supernatants*

Thawed cell-free supernatants were ultrafiltrated in two steps through Amicon YM-10 followed by UM-2 membranes (AMICON Co.) providing fractions with m.w. lower than 10 kDa and higher than 1 kDa. Brain or spleen peptides released in allogeneic reactions (BP-AR and SP-AR, respectively, Kovářů *et al.* 1997) were separated on Biogel P-2 (BioRad Labs, particle size 37−75 mm, Kovářů and Pospíšil 1980). In some experiments SP-AR was purified by HPLC (absorbance 215 nm, 0.32, 8 mV, 2 % methanol/min) in cooperation with Dr. M. Ryba, Institute of Organic Chemistry and Biochemistry of Academy of Sciences of the Czech Republic, Prague. Its molecular weight (1233 Da) was determined by matrix-assisted laser desorption mass analyser (LASERMAT, Finnigam MAT Co.). Unless indicate otherwise, both SP-AR and BP-AR were used at a final concentration 0.1 $\mu$g/ml.

*Determination of $Na^+K^+$-ATPase and $Ca^{2+}$-ATPase activities*

The enzyme activities were determined by spectrophotometric measurements of the released

inorganic phosphate (Kovářů and Lodin 1980, Kovářů and Kovářů 1979, Kovářů *et al.* 1997).

*Proliferation assay of mouse spleen lymphocytes*

Cells were transferred into an albumin-free MEM medium supplemented with 2 mM L-glutamine (Serva), 5 mM sodium pyruvate (Lachema, Brno), 5 mM KCl (Aristar), 10 mM HEPES, pH 7.3 (Serva), 100 units/ml penicillin, 100 mg/ml streptomycin (both from Institute of Sera and Vaccines, Prague), and 1 % bovine serum albumin (Serva) and kept overnight at 4 °C. The cells were then washed out into supplemented MEM medium without albumin and seeded ($5x10^5$ cells/well) in 96 flat-bottomed microwell plates. Cells were incubated with or without the 3 μg/ml Con A (Pharmacia-LKB) and with or without tested peptides SP-AR or BP-AR or FTS (Sigma), bradykinin (Sigma), tuftsin (Sigma) in a 5 % $CO_2$ humified atmosphere at 37 °C for 66 h (a similar procedure as Kozáková *et al.* 1996b). The cells were labelled with 10 μl $^3$H-thymidine (37 kBq, UVVVR, Czech Republic) 18 h before the end of cultivation. Incorporated activity was measured on GF/C filters (Whatman, Great Britain) in 8 ml toluene scintillation liquid SLT-31 (Spolana, Neratovice, Czech Republic) using beta-counter Rackbeta 1214 (LKB) and a computer program by Šiman (1992).

*E7 neuroblastoma cell line*

Cells of the mouse neuroblastoma cell line C 1300, clone E7, were cultured in Petri dishes in MEM medium supplemented with growth proteins (Baudyšová *et al.* 1984). After 18 h, the cells were replaced into the medium without protein and one of the tested peptides (BF-AR or SF-AR) were added 2 h later and incubated with them for the next 90 min at 37 °C. Then, the cells were gently scraped from Petri dishes and washed twice in isotonic saline medium (Kovářů *et al.* 1997). The cells were used for determination of $Na^+$,$K^+$-ATPase and $Ca^{2+}$-ATPase activities. Some cultures were labelled with $^3$H-TdR (56 kBq/mmol) for 8 h. Part of the cells was used for liquid scintillation counting (as above), the other part was used for autoradiography. The autoradiograms were prepared by Ilford Nuclear Emulsion K-2 from Carnoy fixed and ethanol washed specimens (9-day exposition, staining with 1 % toluidine blue).

*Determination of phagocyte activity of macrophages*

Macrophage cell line LA5-9/8 was activated with micrococcus (*Micrococcus luteus*, Worthington) opsonized with rabbit antibodies and harmshire complement 3 or triggered with phorbol-1,2-myristate-13-acetate (PMA, all chemicals from Sigma), BF-AR and interferon γ (1 U, INFγ). Macrophages ($10^4$ cells/tube) were cultured at 37 °C in a humified atmosphere with 5 % $CO_2$. Two days later, INFγ or BF-AR, or both INFγ and BF-AR, were added and the cells were cultured for additional 2 days. Luminol ($10^{-4}$ M, Sigma), opsonized *Micrococcus luteus* or PMA were added to samples immediately before the measurements. Chemiluminiscence was measured with Berthold-Biolumat LB 9505 apparatus in the Department of Molecular Biology of Professor R. Hamers (Vrije Universiteit, Rhode. St. Genessius) (for details see De Baetselier and Schram 1988).

*Protein determination*

Protein concentrations in brain cell homogenates were determined according to Lowry *et al.* (1951), by the method of Murphy and Kies (1960), and a modified biuret method (Kovářů and Lodin 1980) using bovine serum albumin (Serva) as a standard.

*Statistics*

The data are expressed as mean values ± S.D. The differences between experimental samples were evaluated by Student's t-test for unpaired values.

**Table 1.** Effect of SP-AR or BP-AR on $Na^+$,$K^+$-ATPase of mouse spleen lymphocytes

| | − Con A | | + Con A | |
|---|---|---|---|---|
| | s.a. | % | s.a. | % |
| Control | 0.092±0.008 | 100 | 0.153±0.018 | 100 |
| SP-AR | 0.125±0.011* | 136 | 0.102±0.016* | 67 |
| BP-AR | 0.099±0.012 | 108 | 0.105±0.013* | 69 |

*Con A concentration was 3 μg/ml., s.a. − specific activity in μmol $P_i$.mg protein$^{-1}$.h$^{-1}$, data are means ± S.D. of 6−9 measurements; * $P<0.05$.*

**Table 2**
Effect of SP-AR or BP-AR on $Na^+,K^+$-ATPase and $Ca^{2+}$-ATPase of mouse brain cortex cells

| | $Na^+,K^+$-ATPase | | | | $Ca^{2+}$-ATPase | | | |
|---|---|---|---|---|---|---|---|---|
| | Brain cortex cells | | E7 neuroblastoma cells | | Brain cortex cells | | E7 neuroblastoma cells | |
| | s.a. | % | s.a. | % | s.a. | % | s.a. | % |
| Control | 3.78±0.22 | 100 | 0.72±0.11 | 100 | 3.18±0.38 | 100 | 0.31±0.08 | 100 |
| SP-AR | 5.22±0.63* | 138 | 0.90±0.08 | 125 | 3.56±0.12 | 112 | 0.30±0.12 | 96 |
| BP-AR | 4.84±0.42* | 128 | 1.11±0.20 | 154 | 4.20±0.13* | 132 | 0.33±0.10 | 100 |

*s.a. – specific activity in μmol $P_i$.mg protein$^{-1}$.h$^{-1}$. Data on $Ca^{2+}$-ATPase in brain cortex cells are from the paper of Kozáková et al. (1990). Results are means ± S.D. of 6–9 measurements; * $P<0.05$.*

**Table 4**
Effect of BP-AR on phagocytosis of mouse LA5-9/8A macrophage cell line

| | PMA | | PMA + INFγ | | *Micrococcus* | | *Micrococcus* + INFγ | |
|---|---|---|---|---|---|---|---|---|
| | cpm | % | cpm | % | cpm | % | cpm | % |
| Control | 273±42 | 100 | 339±110 | 100 | 426±51 | 100 | 1108±104 | 100 |
| BP-AR | 60±20 | 22 | 105±33 | 31 | 285±29 | 67 | 824±82 | 74 |

*Results are means ± S.D. of 3 experiments in triplicate.*

## Results

*Effect of SP-AR and BP-AR on cell surface membrane enzymes*

We found that SP-AR stimulated $Na^+,K^+$-ATPase in quiescent lymphocytes while BP-AR was ineffective (Table 1). In Con A activated cells $Na^+,K^+$-ATPase activity was inhibited by both SP-AR or BP-AR.

Both SP-AR and BP-AR stimulated $Na^+,K^+$-ATPase activity in the brain cortex and E7 neuroblastoma cells (Table 2). The activity of $Ca^{2+}$-ATPase in brain cortex cells was markedly enhanced by BP-AR while no changes were observed in E7 neuroblastoma cells. SP-AR did not significantly affect $Ca^{2+}$-ATPase of brain cells and E7 neuroblastoma cells.

*Effect of SP-AR and BP-AR on spleen lymphocyte proliferation*

Incorporation of $^3$H-TdR in mouse spleen lymphocytes was stimulated by SP-AR while BP-AR was ineffective. In Con A stimulated lymphocytes SP-AR and BP-AR significantly decreased $^3$H-TdR (Table 3).

*Effect of SP-AR and BP-AR on E7 neuroblastoma cell proliferation*

In comparison to the controls, both SP-AR and BP-AR increased the incorporation of $^3$H-TdR into E7 neuroblastoma cells (Fig. 1). The stimulation index was 1.9 for BP-AR and 2.2 for SP-AR. This effect was similar to that found in spleen cells (Table 3). The autoradiographically determined labelling index increased to 69.1 % (for BP-AR) and 65.9 % (for SP-AR) compared to 54.1 % in the controls.

*Effect of BP-AR on phagocytosis in activated mouse LA5 9/8A macrophage cell line*

Phagocytosis of cells activated by PMA, PMA + INF$\gamma$, *Micrococcus luteus*, or *Micrococcus luteus* + INF$\gamma$ was inhibited by BP-AR, namely when PMA or PMA + INF$\gamma$ were used as activators (Table 4).

*Effect of SP-AR on spontaneous E rosette formation*

Immunomodulatory effects of SP-AR were tested using the spontaneous pig lymphocyte E rosette forming assay (Jarošková and Kovářů 1978). We observed that rosette formation was inhibited by SP-AR (25 to 100 ng/ml) by 25.6 to 15.7 %, respectively. The reference value for rosette forming inhibition with monoclonal anti-CD2 antibody (dilution 1:1000, gift from Dr. R. Binns from the Institute of Animal Physiology and Molecular Genetics, Babraham, U.K.) was 91.7 % (analyzed in cooperation with Dr. Z. Knotek, University of Veterinary and Pharmaceutical Sciences, Brno).

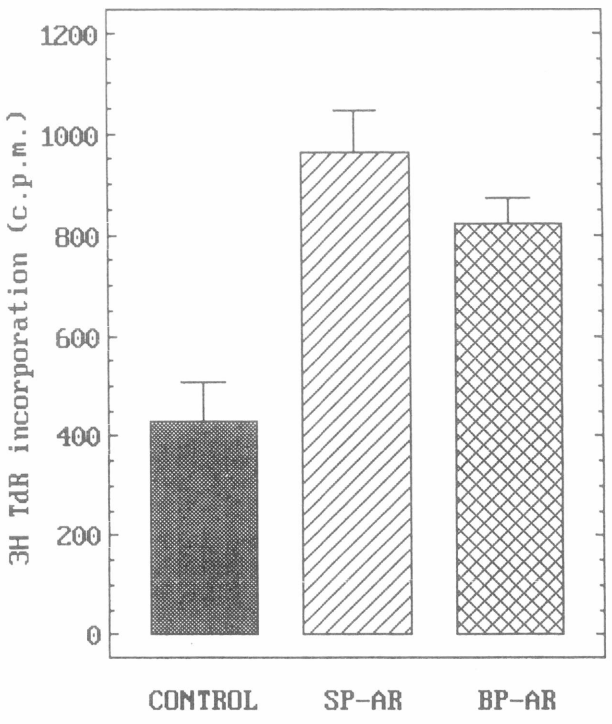


**Fig. 1.** *Effect of SP-AR and BP-AR on E7 neuroblastoma cell proliferation. Data are means ± S.D. of 3–5 measurements.*

*Comparison of the effect SP-AR, serum thymic factor (FTS), bradykinin, and tuftsin on spleen lymphocyte proliferation*

Both SP-AR and FTS activated $^3$H-TdR incorporation into non-stimulated cells but both peptides caused marked inhibition of Con A-stimulated proliferation (Table 5). Bradykinin did not significantly affect Con A-stimulated lymphocytes in concentration from $10^{-6}$ to $10^{-12}$ M (Table 6A), while tuftsin inhibited Con A-activated lymphocyte proliferation in all the tested concentrations (Table 6B).

**Table 3.** Effect of SP-AR and BP-AR on spleen lymphocyte proliferation

| | − Con A | | + Con A | |
|---|---|---|---|---|
| | cpm | SI | cpm | SI |
| Control | 1 320±255 | 1.0 | 12 157±907 | 1.0 |
| SP-AR | 2 561±249 | 1.9 | 1 927±250 | 0.2 |
| BP-AR | 1 346±315 | 1.0 | 2 297±137 | 0.2 |

*Con A concentration was 3 μg/ml, data are means ± S.D.of 3 experiments in triplicate, SI (stimulating index) = stimulation cells (cpm)/control cells (cpm).*

**Table 5.** Effect of SP-AR and FTS on [$^3$H]TdR incorporation of mouse spleen lymphocyte

| | | — Con A | | + Con A | |
|---|---|---|---|---|---|
| | | cpm | SI | cpm | SI |
| Control | | 1 320±255 | 1.0 | 12 157±907 | 1.0 |
| SP-AR | $10^{-6}$ M | 1 369±99 | 1.0 | 3 607±136 | 0.3 |
| | $10^{-7}$ M | 4 053±112 | 3.0 | 1 832±312 | 0.2 |
| | $10^{-8}$ M | 1 018±129 | 0.8 | 7 240±370 | 0.6 |
| FTS | $10^{-6}$ M | 1 669±208 | 1.3 | 5 575±788 | 0.5 |
| | $10^{-7}$ M | 1 201±188 | 0.9 | 4 573±1 220 | 0.4 |
| | $10^{-8}$ M | N.D. | | N.D. | |

*Con A concentration was 3 μg/ml, data are means ± S.D. of 3 experiments in triplicate, SI (stimulation index) = stimulated cells (cpm)/control cells (cpm).*

## Discussion

Our attention was focused on the release of peptide cytokines during the early allogeneic reaction (AR) into the supernatant. Release of these peptides could be linked to increased protease activity of interacting allogeneic cells (Kovářů 1992). Serine protease dipeptidyl peptidase IV (CD 26 lymphocyte marker) is involved in the lymphocyte activation mechanism (Brandt *et al.* 1995). Formerly, we studied the immunocytochemical localization of AR peptides in spleen tissue sections by the indirect immunofluorescence method with our rabbit antibodies against peptide cytokines coupled to rabbit IgG. In the spleen, antipeptide-AR antibody positivity was predominantly localized in the cytoplasm of cells of the germinal centres of lymphatic follicles i.e. suggesting presence of a preformed cytokine-AR peptide or a related amino acid sequence (Kovářů *et al.*, unpublished results).

It is generally accepted that cytokines affect the cell surface through their receptors (Ihle 1995, Rothwell and Hopkins 1995). Cytokine receptor occupancy is accompanied by nuclear signalling with activation pathways *via* small GTP-proteins (Ras) to a mitogen-activated protein (MAP) kinase cascade and tyrosine phosphorylation of transcription factors, signal transducers and activators of transcription (STATs, Karnitz and Abraham 1995).

In this study, we demonstrated that the modulatory ability of both BP-AR and SP-AR is dependent on cell type and proliferation activity as was shown by differences between quiescent and Con-A stimulated lymphocytes, or macrophages activated to phagocytosis. Thus, it can be suggested that the effects of cytokines can be realized through several cell surface structures.

**Table 6.** Effects of various concentrations of bradykinin or tuftsin proliferation of mouse spleen lymphocytes

| Bradykinin | − Con A cpm | SI | + Con A cpm | SI |
|---|---|---|---|---|
| Control | 1 320 ± 255 | 1.0 | 12 157 ± 907 | 1.0 |
| $10^{-6}$ M | 660 ± 94 | 0.5 | 15 561 ± 194 | 1.3 |
| $10^{-7}$ M | 1 280 ± 188 | 1.0 | 13 212 ± 163 | 1.0 |
| $10^{-8}$ M | 2 115 ± 204 | 1.6 | 10 421 ± 243 | 0.9 |
| $10^{-9}$ M | 1 850 ± 293 | 1.4 | 10 663 ± 286 | 0.9 |
| $10^{-10}$ M | 1 111 ± 227 | 0.8 | 13 329 ± 605 | 1.1 |
| $10^{-11}$ M | 1 718 ± 89 | 1.3 | 16 964 ± 930 | 1.4 |
| $10^{-12}$ M | 2 115 ± 413 | 1.6 | 14 540 ± 1 252 | 1.2 |

| Tuftsin | − Con A cpm | SI | + Con A cpm | SI |
|---|---|---|---|---|
| $10^{-6}$ M | 1 452 ± 226 | 1.1 | 8 388 ± 641 | 0.7 |
| $10^{-7}$ M | 1 636 ± 377 | 1.2 | 8 260 ± 916 | 0.7 |
| $10^{-8}$ M | 1 188 ± 94 | 0.9 | 6 924 ± 250 | 0.6 |
| $10^{-9}$ M | 1 976 ± 240 | 1.5 | 7 289 ± 676 | 0.6 |
| $10^{-10}$ M | 1 952 ± 379 | 1.5 | 6 317 ± 134 | 0.5 |
| $10^{-11}$ M | 962 ± 100 | 0.7 | 9 481 ± 1 496 | 0.8 |
| $10^{-12}$ M | 1 307 ± 285 | 1.0 | 7 531 ± 1 444 | 0.6 |

*Con A concentration was 3 μg/ml, data are means ± S.D. of 3 experiments in triplicate. SI (stimulation index) = stimulated cells (cpm)/control cells.*

Furthermore, $Na^+,K^+$-ATPase can play a role as a signal amplifier and modifier as demonstrated by mitogen lectin dose-dependent stimulation of $Na^+,K^+$-ATPase activity of lymphocytes correlating with $^3$H-TdR incorporation (Kovářů *et al.* 1982, Kovářů 1992). The modulatory role of $Na^+,K^+$-ATPase can participate in Con A-stimulated $^3$H-TdR incorporation, markedly inhibited by BP-AR or SP-AR peptides. Similarly, BP-AR induced inhibition of phagocytosis of the PMA-stimulated macrophage cell-line indicates cytokine peptide immunosuppressive effect on signalling via protein kinase C (PKC) because PMA is its direct activator. Multiple cytokine peptide effects observed by us are in agreement with the activation of various steps of cell signal transduction, e.g. PKC mediated phosphorylation connected with stimulation of $Na^+,K^+$-ATPase or ion channels, etc. (for review see Grinstein and Dixon 1989). It is of interest that the final *in vitro* effects of cytokine peptides on lymphocytes or macrophages indicated strong immunosuppressivity. The immunosuppressive activity of both brain and spleen peptide fractions was confirmed by the inhibitory SP-AR effect on pig lymphocytes by testing spontaneous E-rosette formation and inhibitory effect of the peptide fraction on allogeneic immunization of mice *in vivo* (Kovářů 1992).

Comparison of our peptides cytokines with some other biologically active peptides of the similar molecular weight, as represented by serum thymic factor (FTS, Glp-Ala-Lys-Ser-Gln-Gly-Ser-Asn, m.w. 857), bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Phe-Arg, m.w. 1060) and tuftsin (Thr-Lys-Pro-Arg, m.w. 501) revealed immunosuppressive effects of FTS and of both BP-AR and SP-AR peptides on Con A-stimulated lymphocyte proliferation. Earlier data showed that proline is present in peptides with stimulatory effects on lymphocyte proliferation (Wieczorek *et al.* 1991). Thus, peptides without proline, such as FTS, are immunosuppressive in the present as well as earlier study (Lenfant *et al.* 1983). The same may account for proliferation suppression caused by SP-AR and BP-AR peptides as well as for their other immunosuppressive effects on spontaneous E-rosette forming lymphocytes

and macrophage activation. The spleen or brain peptide cytokine activities presented here demonstrate that these molecules can act as physiological messengers in the immune-CNS crosstalk (Savino and Dardenne 1995).

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