

Effect of SXWS/WSXWS peptides on chemotaxis and adhesion of the macrophage-like cell line J774

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ABSTRACT

WSXWS motif is a conserved amino acid sequence which is present in type I cytokine receptors. This motif that can be found both in the ligand binding chains and signal transducer molecule of the receptors with different amino acids at the position 'X', plays a role in the receptor folding, ligand binding and signal transduction as well. Structural analysis proved that WSEWS motif of IL-6R is located in a highly accessible location in the protein. Structural properties and chemotaxis of a tetrapeptide library with SXWS sequence, where X was the 19 proteinogenic amino acids except cysteine were systematically studied earlier. It has been proved that C-terminal amidation and the identity of amino acid 'X' had a pronounced influence on the chemotactic properties, but less of the structure of the peptides. Here we present our findings on the effect of a tetra- and a pentapeptide library with the sequence of SXWS and WSXWS on the chemotaxis and adhesion of J774 murine macrophage cell line. We studied the effect of the presence/absence of N-terminal tryptophan and the different amino acids at the 'X' position on these physiological responses. Results indicated that amino acid 'X' had a marked influence on chemotaxis, adhesion as well as on proliferation induced by (W)SXWS peptides. Elongation of SXWS sequence with a tryptophan at the N terminus also altered pronouncedly all the physiological responses of the cells studied. A good correlation could be observed between the chemotaxis and the proliferation and physicochemical parameters of the amino acid 'X'.

INTRODUCTION

The WSXWS pentapeptide motif is a characteristic sequence of cytokine receptor family type I such as IL-2R, IL-4R, IL-6R, IL-7R and interferon receptors (Bazan *et al.*, 1990, Foxwell *et al.*, 1992). This highly conserved sequence has a great significance in the regulation of the immune system, since it is present both in the ligand binding chains of cytokine receptors type I e.g. IL-6R α chain and also in the gp130 protein, the common signal transducer molecule of the IL-6 receptor family such as oncostatin M, leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTf) (Kishimoto *et al.*, 1995). WSXWS motif plays a significant role in the correct folding of the proteins of these receptors (Hilton *et al.*, 1996) as well as it is required for the ligand binding and signal transduction in the same signaling pathway (Yawata *et al.*, 1993; Kishimoto *et al.*, 1995). In case of IL-6R structural analyses proved that WSEWS motif is located on a loop in the hinge region between two barrel-like fibronectin type domains, therefore they are accessible for the ligand (Yawata *et al.*, 1993). A variety of amino acids can occur at the position 'X'. WSEWS sequence can be found in the IL-6R α chain (Biró *et al.*, 1995), WSDWS motif is present in gp130 molecule (Hilton *et al.*, 1996, Kernebeck *et al.*, 1999), while erythropoietin receptor contains a WSAWS motif (Furmanek *et al.*, 2003). Meissner and coworkers found that WSKWS sequence was essential for the inhibition of cytokine signaling mediated by common γ chain of the soluble cytokine receptor in mice (Meissner *et al.*, 2001)

Structural properties of a tetrapeptide library with SXWS sequence, where X position was substituted with all proteinogenic amino acids except cysteine were studied earlier (Láng *et al.*, 2012). It has been established that most of the tetrapeptides (except X = Gly, Pro or Arg) have a non-flexible conformation stabilized by a characteristic H-bond pattern, from which the X residue emerges and has no significant influence on the conformation of the whole peptide. Chemotactic characteristics of synthetic peptides has been investigated on monocyte and lymphocyte cultures (Kőhidai *et al.*, 2002) and on the ciliated protozoan *Tetrahymena pyriformis* as well. Tri-, tetra-, penta- and hexapeptides containing EWS motif and a tetrapeptide library with 19 proteinogenic amino acids at the X position were also systematically examined. These results showed that some peptides like SEWS (Chemotaxis index=660%) had a pronounced positive chemotactic potential on *Tetrahymena pyriformis*, whereas shorter (EWS) and elongated (WSEWS) sequences rather elicited a negative chemotactic effect. Amidation of the C-terminus of the peptides and the quality of amino acid at position 'X' also greatly influenced the chemotactic properties of the peptides (Kőhidai *et al.*, 2003a, Illyés *et al.*, 2002).

Migration of monocytes and macrophages play an important role in the early steps of the immune response. Thus, we wanted to investigate, how a mammalian monocyte derived cell line J774 reacts to the peptides containing SXWS motif. J774 cells have been used for different cell physiological studies, e.g. signal transduction (Kügler *et al.*, 1997), phagocytosis, (Kügler *et al.*, 1997, Ralph and Nakoinz, 1975, Liang-Takasaki *et al.*, 1982) and chemotactic activity (McCloskey *et al.*, 1999, Zhou *et al.*, 2012) have been equally investigated.

In this communication we describe our new findings on the effect of a tetrapeptide (SXWS) and a pentapeptide (WSXWS) library on the chemotaxis and adhesion of J774 murine macrophage cell line. In order to clarify the correlation between the chemical structure (amino acid length, composition and sequence) of the peptides including the role of amino acid X, we studied the chemotaxis and adhesion of J774 cells elicited by the peptides in several aspects. Our objective was (i) to examine whether the elongation of SXWS peptides with a tryptophan on the N terminus can influence these physiological responses and (ii) to reveal whether

amino acid substitutions at the X position are able to alter ability of the peptide to elicit chemotaxis, adhesion and proliferation in J774 cells.

MATERIALS AND METHODS

Abbreviations

Abbreviations of amino acids and their derivatives follow the revised recommendation of the IUPAC-IUB Committee on Biochemical Nomenclature entitled “Nomenclature and Symbolism for Amino Acids and Peptides” (recommendations of 1983). All amino acids are L configuration unless otherwise stated. The other abbreviations in this paper are the following: DMF: *N,N*-dimethylformamide, DCM: dichloromethane, DIC: *N,N'*-diisopropylcarbodiimide, DMAP: 4-(dimethylamino)pyridine, HOBt: 1-hydroxybenzotriazole, TFA: trifluoroacetic acid, FBS: fetal bovine serum, DMSO: dimethyl sulfoxide, PBS: phosphate buffered saline, CF: 5(6)-carboxyfluorescein, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, f-MLF: formyl-methionyl-leucyl-phenylalanine

Materials

p-Alkoxybenzylalcohol resin (Wang-resin, 0.96 mmol/g) was obtained from Bachem, Bubendorf, Switzerland (Cat. No. D 1250; lot 504143). Fmoc-L-amino acids were purchased from Fluka, Buchs, Switzerland. Reagents (DIC, DMAP, HOBt, piperidine, TFA) and solvents (DMF and DCM) were Fluka products of analytical grade.

Peptide synthesis

Synthesis of the peptides with free C-terminal carboxylic group was performed on Wang resin by manual solid phase synthesis using Fmoc/^tBu strategy with a DIC/HOBt coupling protocol as described before (Illyés *et al.*, 2002). Briefly, the resin (0.96 mmol/g) was swollen in DMF. Coupling of the Fmoc-protected first amino acid to the resin was performed with DIC using DMAP as an acylating reagent. During the coupling cycles, first the resin was swollen with DMF, Fmoc deprotection was performed with piperidine/DMF (1:1, v/v; for 1, 9 and 1 min) followed by washing with DMF (5×1 min); 3 eq. of Fmoc-amino acids were pre-activated for 5 min in the presence of HOBt and DIC in DMF and coupled for 90 min. Resin was then washed with DMF (4×2 min) and DCM (2×2 min). The efficiency of the coupling was checked with ninhydrin test. Peptides were cleaved from the resin with the mixture of TFA (95%), ethanedithiol (2.5%) and water (2.5%) for 3 h. The mixture was then filtered, the filtrate was precipitated with ether, centrifuged in a sealed tube, then it was washed three times with ether, dissolved in water or AcOH, freeze dried and purified by RP-HPLC. The purity of the peptides was checked with analytical RP-HPLC and mass spectrometry.

Cell culturing

J774 murine macrophage cell-line was maintained in RPMI 1640 medium containing 10% FBS, 2 mM L-glutamine, and 0.16 mg/mL streptomycin at 37°C in 5% CO₂ atmosphere. Cells were harvested in logarithmic phase of growth.

Chemotaxis assay on J774 cells

Chemotactic ability of the cells was measured in a 96 well NeuroProbe[®] chamber. The peptides were dissolved in RPMI-1640 medium and were applied at 10^{-12} M, 10^{-10} M, 10^{-8} M and 10^{-6} M concentrations. Complement C5a (McCloskey *et al.*, 1999) was applied as positive control. A polycarbonate filter with 5 μ m pore diameter was placed between the inner chambers containing the peptide solution and the outer chambers, in which J774 cells were placed (10^5 cells/well). The chamber was incubated at 37°C for 3 h. The amount of the viable cells migrated to the peptide solution was determined by MTT assay. MTT (0.36 mg/mL) dissolved in 0.1 M PBS (pH 7.4) was added to each well and the inner chamber was incubated with MTT for 24 hours, then crystals were dissolved in DMSO and optical density was detected by ELISA reader (Labsystems Multiskan MS, Finland) at $\lambda=540$ nm and $\lambda=620$ nm as reference wavelength. Statistical analysis of data was performed using Student's *t* test of Origin8.6 at the 95% confidence level.

Measurement of the adhesion and proliferation of J774 cells

Adhesion and proliferation of J774 cells were monitored in real-time mode in xCELLigence SP system (Roche Applied Science, Indianapolis, USA) by measuring the change of electrical impedance in a microelectrode array containing 96-well E-plate (ACEA Biosciences, Ind., San Diego, USA). At first, impedance of culture medium was recorded as a baseline and absolute control. After 1 hour pre-incubation, 10^4 cells were plated into each well with the solution of the peptides dissolved in culture medium ($c=100$ μ g/mL). Adhesion and cell proliferation was monitored for 72 hours at 37°C in 5% CO₂ atmosphere. Based on the impedance change cell index data were calculated by the RTCA 1.2 software of xCELLigence system by the following formula

$$\text{Cell Index} = (Z_i - Z_0) / 15 \quad (1),$$

where Z_0 is electrical impedance at 0 time point and Z_i is electrical impedance at i time point.

Each data point represents the average of three identical values of parallel samples. Besides the variation of Cell Index compared to the baseline at different time point (delta Cell Index, Δ CI), the slope of the curve was also estimated by the RTCA 1.2 software. During the evaluation of results the following parameters were considered following normalization to the average of negative control wells: i. normalized slope of the early part of the impedance curve (about the first 3 h post seeding) representing the effect of treatment on the early adhesion phase; ii. normalized standard deviation value of parallel measurements at time point that was chosen as end point in slope estimation (range fitting study iii. normalized Δ CI at 24 h of treatment; iv. normalized slope of the impedance curve between 30 h and 72 h representing the effect of treatment on the cell proliferation.

RESULTS AND DISCUSSION

Chemotaxis of J774 cells

Chemotaxis of J774 cells was measured in a 96 well NeuroProbe[®] chamber at 10^{-12} M, 10^{-10} M, 10^{-8} M and 10^{-6} M concentrations at 37°C for 3 h with complement C5a as positive control (McCloskey *et al.*, 1999). The number of the viable cells migrated was determined by MTT assay. The chemotactic activity of the peptides is summarized in Table 1. SXWS peptides

(where X= A, F, G, M, S) could elicit a positive chemotaxis on J774 cells. SMWS proved to be the most effective chemoattractant at 10^{-6} M (chtx. idx.= $250\pm 55\%$). SXWS peptides, where X=D, K, P, T, Y induced a negative chemotactic response. In case of some peptides we could observe a diverse effect depending on the peptide concentration. SQWS was chemoattractant at 10^{-10} M and it acted as a chemorepellent at 10^{-6} M concentration. Similarly, SRWS was repellent at $c=10^{-12}$ M and it was neutral at 10^{-8} M concentration. We were looking for correlations between chemotactic activity of the peptides and some physicochemical properties of the corresponding 'X' amino acids (Table 2). We found that in case of peptides with SXWS sequence, a lower hydrophobicity could be observed for the 'X' amino acids in the chemorepellent peptides. In case of WSXWS sequence we found that repellent peptides had a higher average pK_a of the side chains of 'X' amino acids, compared to the neutral peptides. The other difference was, similarly to the SXWS peptides, the lower average hydrophobicity of the amino acids 'X' in the repellent peptides. On the other hand, we observed that if tetrapeptides were elongated with a tryptophan at the N terminus, most of the peptides lost their chemotactic activity, or showed an altered chemotactic effect (Figure 1). According to the different chemotactic response we could distinguish the following five groups: group I. where SXWS was attractant and WSXWS was neutral (X=A, F, G, M, S); group II. SXWS was attractant, WSXWS was repellent (X=Q at $c=10^{-10}$ M); group III. SXWS was repellent, WSXWS was neutral (X=D, K, Q at $c=10^{-6}$ M, R at $c=10^{-12}$ M, Y); group IV. SXWS was neutral, while WSXWS was repellent (X=I, N, R at $c=10^{-8}$ M, W); and group V. SXWS was repellent, WSXWS was attractant (X=T). We could not detect the change of chemotactic potential from neutral to attractant, or did not find any SXWS-WSXWS peptide pairs, where both peptides proved to be chemoattractant. The change of the chemotactic potential of the peptides is shown in Table 3 and Figure 2. We could distinguish two SXWS-WSXWS peptide pairs, which influenced the chemotaxis of J774 cells into the same direction (X= P, Y). In case of some peptides we could observe a similar effect in J774 cells compared to *Tetrahymena pyriformis*: SXWS peptides, where X was A, M, Q and S were attractant, while SKWS, SYWS and WSYWS peptides were repellent in both cell types.

Chemotactic range fitting

Chemotactic range fitting is a phenomenon which describes the relationship between the chemotactic activity and the range of the effectivity (Köhida *et al.*, 2003b). According to data of literature chemoattractant ligands can take their effect in a wide concentration range, whereas the concentration range of the effect in case of neutral or chemorepellent ligands is usually narrower. Our results show a good correlation with former observations on *Tetrahymena pyriformis* (Köhida *et al.*, 2003b; Láng *et al.*, 2012). The most effective chemoattractant tetrapeptide (SMWS) elicited an effect in the widest range (100-250%) and other chemoattractant tetrapeptides such as SQWS (100-141%), SAWS (59-134%) and SSWS (96-141%) elicited an effect also in a relatively wide range. Neutral and repellent SXWS tetrapeptides as well as WSXWS pentapeptides, including the only attractant one (WSTWS) induced an effect in a much narrower range (Figure 3).

Adhesion and proliferation

Adhesion and proliferation of J774 cells were studied in xCELLigence SP system by measuring the change of electrical impedance on fibronectin coated surface of a 96-well E-plate. Cell adhesion was followed for 72 h. Change of relative cell index (ΔCI) was depicted in the function of time. Curves of two peptide pairs, where X=N (SNWS/WSNWS) or M (SMWS/WSMWS) are shown on Figure 4. Early phase of adhesion was characterized by the normalized slope of the first 2 h from the addition of the cells; normalized ΔCI at 24 h and normalized slope of treatment between 30 h and 72 h was calculated for describing cell

proliferation.

Early phase of adhesion (2-4 h)

At the first two hours, six SXWS tetrapeptides (X= G, H, N, P, R and V) as well as three WSXWS pentapeptides (X= G, R at $c=10^{-12}$ M and S) induced a decreased attachment to the surface compared to the untreated control. A faster adhesion was elicited only by a few WSXWS peptides (X= D, E, L, M), but none of the SXWS tetrapeptides (Figure 5A).

Long term proliferation of J774 cells elicited by (W)SXWS peptides

According to the slope of the adhesion curves from 3h to 72h, three tetrapeptides (X=E, K, Q) and five pentapeptides (X= E, Q, R at $c=10^{-12}$ M, W, Y) proved to be proliferative to J774 cells (10-20%). A slight inhibitory effect compared to the control could be observed in case of four SXWS tetrapeptides (X=F, L, M, V). No WSXWS pentapeptides inhibited the long term proliferation of J774 cells (Figure 5B). When analyzing ΔCI at 24 h we found slight differences between the effect of the tetra- and pentapeptides (when X = F, G, H, I, R, S, V, W, Y), but in all cases cell indexes of WSXWS peptides were higher than that of SXWS peptides (Figure 6A). When comparing solvent exposed area (SEA) of amino acids at the X position in the effective and ineffective WSXWS peptides we can observe that in most cases SEA is inversely proportional to the efficacy of proliferation (Figure 6B). Calculating the average of SEA of 'X' amino acids of the effective and ineffective WSXWS pentapeptides, we can see a similar phenomenon: the average SEA of amino acid 'X' in case of the effective pentapeptides is relatively low (2.18=68%) compared to the average of SEA of the 19 proteinogenic amino acids except cysteine (3.21=100%). By contrast, the average SEA values in case of ineffective peptides are relatively high (4.95=154%) compared to the average of the 19 proteinogenic amino acids except cysteine (Table 4).

Chemotactic vs. adhesive peptides?

The dominance of the physiological responses of J774 cells was different for each peptide. Most of the peptides (SXWS, where X = A, D, F, G, K, M, Q, S, T, Y; and WSXWS, where X=I, P, Q, T, W, Y) elicited an either positive or negative chemotactic response and did not influence significantly the adhesion of the cells. In case of four WSXWS peptides (X= D, E, L, M) enhanced adhesion proved to be the dominant physiological response. Except some peptides, which did not elicit either chemotaxis or adhesion, we found two tetrapeptides (X= P and R at $c=10^{-12}$ M) and a pentapeptide (WSRWS at $c=10^{-8}$ M) that affected similarly chemotaxis and adhesion. These molecules induced a negative chemotaxis and also inhibited the adhesion of J774 cells at 3h. Besides, a group of 7 peptides (SHWS, SNWS, SRWS at $c=10^{-8}$ M, SVWS, WSGWS, WSRWS at $c=10^{-12}$ M and WSSWS) proved to be neutral in chemotaxis studies, but inhibited the early adhesion of J774 cells. Adhesive, chemotactic and proliferative effects of the peptides are summarized in Table 5.

CONCLUSION

Chemotaxis, cellular adhesion and proliferation are essential physiological responses of the cells. Small peptides, like f-MLF often induce a chemotactic response either in the ciliate protozoan *Tetrahymena* (Köhida *et al.*, 2003c) or in organisms at the higher rank of phylogeny like monocytes (Gouwy *et al.*, 2009).

Our findings suggest that (W)SXWS peptides had a significant influence on the chemotaxis, adhesion and proliferation of J774 monocyte-macrophage cell line. We found that the identity of amino acid 'X' had a marked influence on the effect of the peptides in case of all physiological responses studied. Elongation of SXWS sequence with a tryptophan at the N

terminus also altered pronouncedly the characteristics of the physiological reactions of the cells. A good correlation was demonstrated between the chemotactic effect and certain physicochemical parameters (e.g. pK_a of the side chain) of the amino acid 'X'. Also a correlation was established between low hydrophathy index of amino acids and negative chemotaxis of the tetra- as well as pentapeptides. On the other hand, the low SEA values of pentapeptides and its proliferation feature was in harmony.

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TABLES

Peptide sequence	Chemotaxis index [% of control] ± SD			
	10 ⁻¹² M	10 ⁻¹⁰ M	10 ⁻⁸ M	10 ⁻⁶ M
SAWS	87±35	90±50	129±28	134±17*
SDWS	128±37	77±19	57±11*	61±23*
SEWS	96±1	98±7	94±4	93±3
SFWS	113±13	108±8	113±3*	99±6
SGWS	98±9	102±8	102±6	125±7*
SHWS	102±6	104±3	106±8	109±5
SIWS	102±4	102±11	99±6	102±5
SKWS	91±15	84±12*	90±15	96±15
SLWS	91±3	88±10	97±4	94±3
SMWS	125±46	107±30	130±48	250±55*
SNWS	100±8	106±5	100±8	102±9
SPWS	93±11	85±14	85±9*	80±12*
SQWS	101±28	142±29*	94±36	75±7*
SRWS	85±8*	92±7	94±8	91±7
SSWS	96±10	98±22	96±10	141±14*
STWS	99±10	95±5	92±3	88±5*
SVWS	95±19	99±14	100±17	90±11
SWWS	88±21	87±9	102±12	99±15
SYWS	77±6*	79±15*	85±7*	84±14*
WSAWS	98±3	100±4	103±10	109±11
WSDWS	94±6	98±4	100±2	100±4
WSEWS	95±11	106±8	109±9	105±3
WSFWS	100±12	102±12	107±9	100±8
WSGWS	97±15	111±6	105±8	87±14
WSHWS	106±10	104±11	104±12	101±11
WSIWS	88±3*	91±6	92±6	82±6*
WSKWS	100±10	104±10	100±12	102±8
WSLWS	101±9	95±12	100±11	100±13
WSMWS	92±4	96±3	100±4	106±8
WSNWS	74±14*	91±20	83±14*	91±19
WSPWS	86±14*	87±11*	84±5*	84±12*
WSQWS	83±23	81±18*	83±15*	91±14
WSRWS	97±14	87±15	87±9*	97±9
WSSWS	90±16	106±11	105±13	95±9
WSTWS	100±6	102±5	104±8	115±6*
WSVWS	102±19	105±16	107±12	106±10
WSWWS	78±13*	87±12	91±18	97±18
WSYWS	103±7	95±8	84±6*	97±6
	10 ⁻¹⁰ M	10 ⁻⁹ M	10 ⁻⁸ M	10 ⁻⁷ M
C5a	106±85	102±10	134±11*	149±11*

*Table 1 Chemotaxis of J774 cells induced by SXWS and WSXWS peptides. Chemotaxis was determined in a 96 well NeuroProbe® chamber at 37°C for 3 h. The amount of the migrated cells was determined by MTT assay, chemotaxis index is expressed in the percentage of the negative control. Statistical analysis of data was performed using Student's t test at the 95% confidence level; *: p>0.05*

<i>Sequence</i>	<i>Chemotactic activity</i>	<i>Amino acid X</i>	<i>average pK_a (^αCOOH)</i>	<i>average pK_a (^αNH₂)</i>	<i>average pK_a (side chain)</i>	<i>average SEA</i>	<i>average hydrophathy</i>
SXWS	chemoattractant	A,F,G,M,Q,S	2.32±0.15	9.40±0.34	-	0.56±0.16	0.3±2.33
	neutral	E,H,I,L,N,V,W	2.17±0.22	9.44±0.35	5.09±1.44	0.59±0.22	0.20±3.82
	chemorepellent	D,K,P,R,T,Y	2.11±0.08*	9.52±0.69	8.15±3.73	0.68±0.16	-2.20±1.41*
WSXWS	chemoattractant	T	2.09	9.1	-	0.71	-0.7
	neutral	A,D,E,F,G,H,K,L,M,S,V	2.23±0.20	9.47±0.33	6.14±3.10	0.56±0.19	-0.07±3.12
	chemorepellent	I,N,P,Q,R,W,Y	2.16±0.15	9.40±0.61	11.28±1.70	0.63±0.18	-1.54±2.98

*Table 2 Chemotactic activity of the tetra- and pentapeptides and physicochemical properties of the corresponding amino acids at the 'X' position. Statistical analysis of data was performed using Student's t test; *: p<0.05. SEA values for each amino acid were obtained from JENA library of biological macromolecules (Bordo and Argos, 1991). Hydrophathy indexes were obtained from Kyte and Doolittle (Kyte and Doolittle, 1982)*

<i>Amino acid</i> 'X'	<i>Chemotactic potential</i>		<i>Effective concentration</i>
	SXWS	WSXWS	lg c [M]
A	chemoattractant	neutral	-6
D	chemorepellent	neutral	-8, -6
E	neutral	neutral	-12, -10, -8, -6
F	chemoattractant	neutral	-8
G	chemoattractant	neutral	-6
H	neutral	neutral	-12, -10, -8, -6
I	neutral	chemorepellent	-12, -6
K	chemorepellent	neutral	-10
L	neutral	neutral	-12, -10, -8, -6
M	chemoattractant	neutral	-6
N	neutral	chemorepellent	-12, -8
P	chemorepellent	chemorepellent	-8, -6
Q	chemoattractant	chemorepellent	-10
Q	chemorepellent	neutral	-6
R	chemorepellent	neutral	-12
R	neutral	chemorepellent	-8
S	chemoattractant	neutral	-6
T	chemorepellent	chemoattractant	-6
V	neutral	neutral	-12, -10, -8, -6
W	neutral	chemorepellent	-12
Y	chemorepellent	neutral	-12, -10, -6
Y	chemorepellent	chemorepellent	-8

Table 3 Change of the chemotactic potential of SXWS peptides elongated with a tryptophane at the N terminus. X is any proteinogenic amino acid except cysteine.

	<i>SEA – 30Å (%)</i>	<i>Buried (%)</i>	<i>SEA – 10-30 Å (%)</i>	<i>SEA of Accessible/Buried</i>
Means of 19 proteinogenic amino acids except cystein	64.21	23.73	12.05	76.26/23.73 = 3.21 (100%)
Means of amino acids F, G, H, I, R, S, V, W, Y (peaks induced by the corresponding 9 WSXWS ligands at 24h)	56.44	31.44	12.11	68.55/31.44 = 2.18 (67.91%)
Means of amino acids A, D, E, K, L, M, N, P, Q, T (proved to be neutral in the 10 WSXWS ligands at 24h)	71.2	16.8	12.00	83.2/16.8 = 4.95 (154.20%)

Table 4 Correlation between solvent exposed area (SEA) of amino acid 'X' and proliferation of the peptides at 24h

<i>Amino acid</i>	<i>SXWS</i>			<i>WSXWS</i>		
	Chemotaxis	Adhesion	Proliferation	Chemotaxis	Adhesion	Proliferation
A	+	∅	∅	∅	∅	∅
D	-	∅	∅	∅	+	∅
E	∅	∅	+	∅	+	+
F	+	∅	-	∅	∅	∅
G	+	-	∅	∅	-	∅
H	∅	-	∅	∅	∅	∅
I	∅	∅	∅	-	∅	+
K	-	∅	+	∅	∅	∅
L	∅	∅	-	∅	+	∅
M	+	∅	-	∅	+	∅
N	∅	-	∅	∅	∅	∅
P	-	-	∅	-	∅	∅
Q	+	∅	+	-	∅	+
R	∅	-	∅	-	-	∅
S	+	∅	∅	∅	-	∅
T	-	∅	∅	+	∅	∅
V	∅	-	-	∅	∅	∅
W	∅	∅	∅	-	∅	+
Y	-	∅	∅	-	∅	+

Table 5 Chemotaxis, adhesion and proliferation of J774 cells. +: positive effect, -: negative effect, ∅: no effect

FIGURE LEGENDS

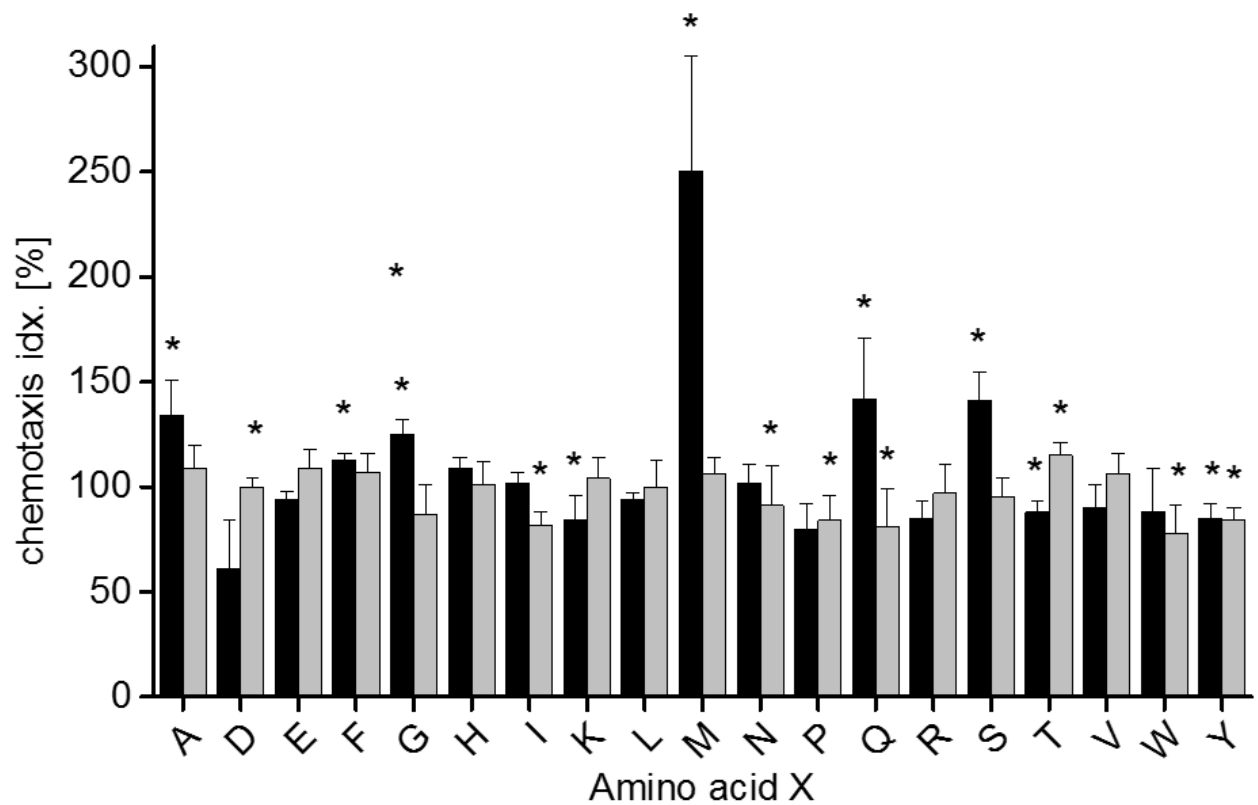


Figure 1 Chemotaxis of J774 cells induced by SXWS and WSXWS peptides at the optimal concentrations in case of SXWS sequence. Chemotaxis index is expressed in the percentage of the negative control. Statistical analysis of data was performed using Student's t test; *: p<0.05

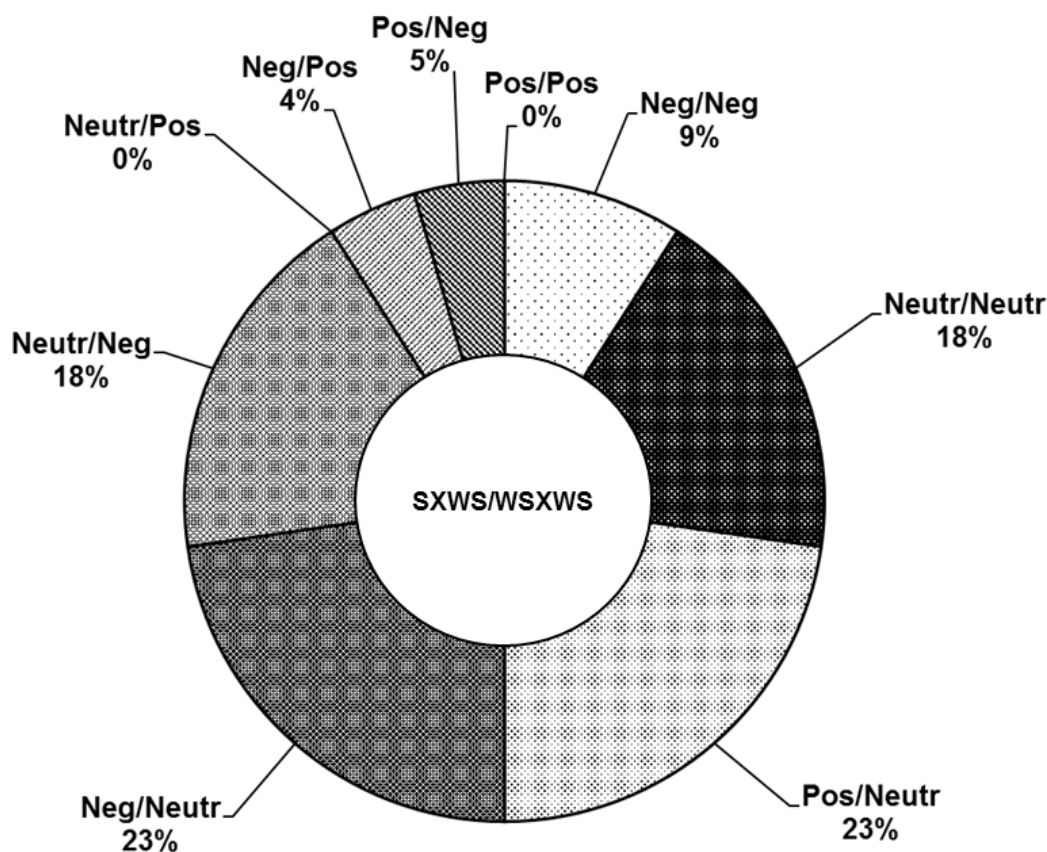


Figure 2 Distribution of the different groups of SXWS/WSXWS peptide pairs according to the change of their chemotactic activity. Pos: both SXWS and WSXWS were attractant, Neg: both SXWS and WSXWS were repellent, Neutr: both SXWS and WSXWS were neutral; Pos/Neutr: SXWS was attractant, WSXWS was neutral, Pos/Neg: SXWS was attractant, WSXWS was repellent, Neg/Neutr: SXWS was repellent, WSXWS was neutral, Neutr/Neg: SXWS was neutral, WSXWS was repellent, Neutr/Pos: SXWS was neutral, WSXWS was attractant, Neg/Pos: SXWS was repellent, WSXWS was attractant.

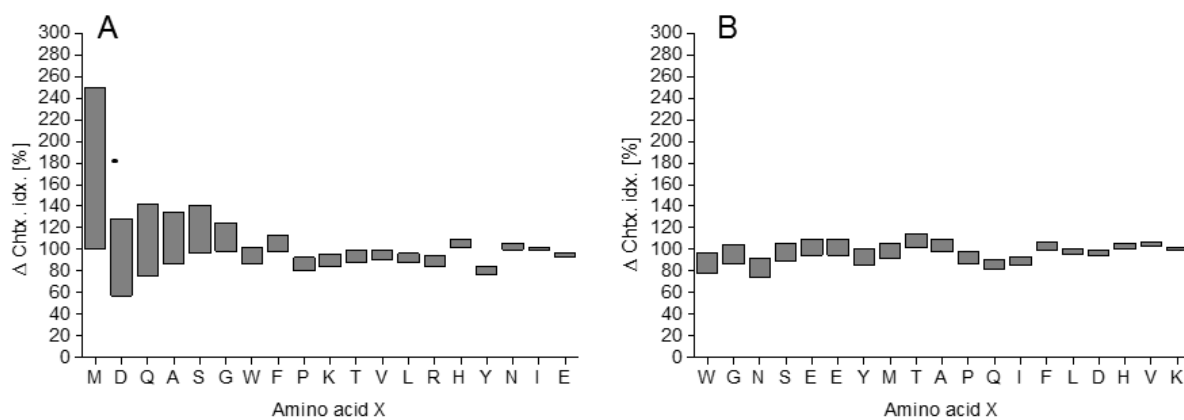


Figure 3 Chemotactic range fitting of SXWS and WSXWS peptides in J774 cells.

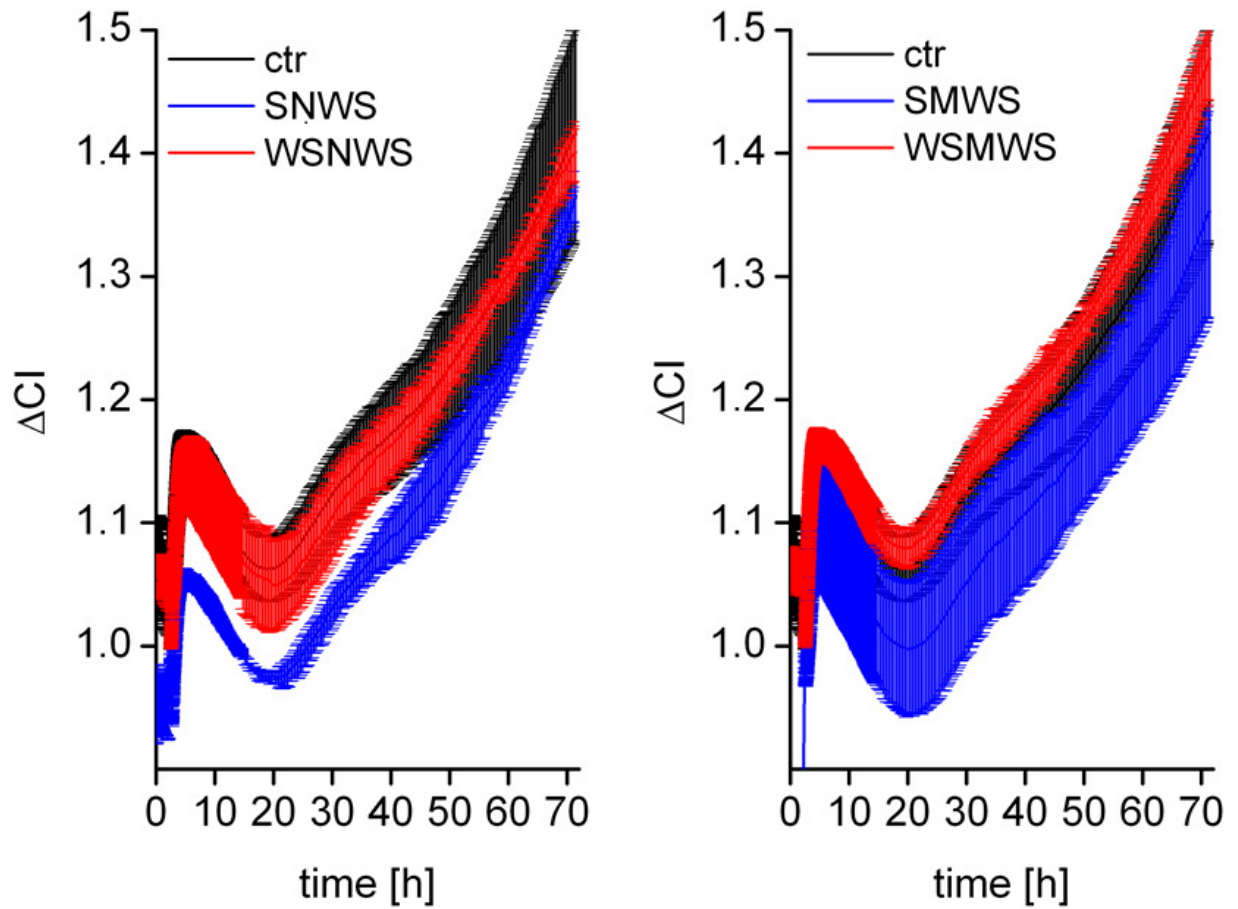


Figure 4 Curves of relative cell index (ΔCI) \pm SD of SNWS/WSNWS and SMWS/WSMWS peptide pairs in the function of time. Early adhesion can be characterized by the normalized slope of the first 2 hours from the addition of the cells, while cell proliferation is characterized by normalized ΔCI at 24 h and normalized slope of treatment between 30 h and 72 h.

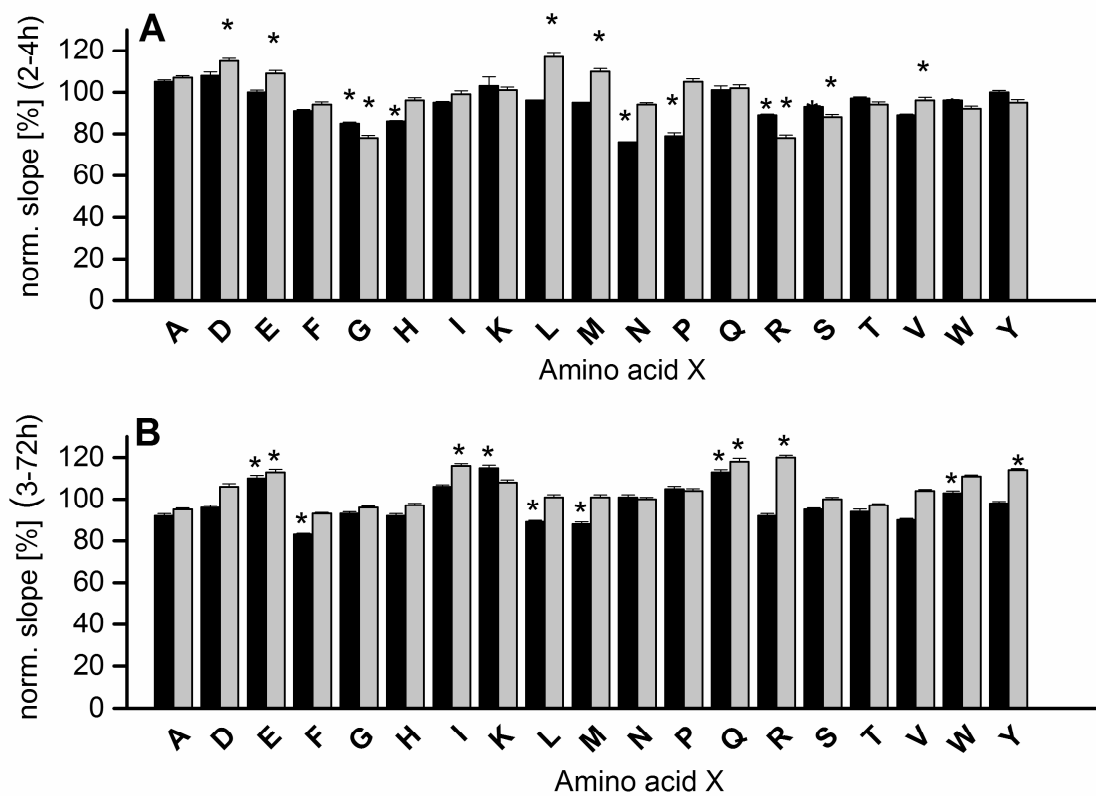


Figure 5 Adhesion (A) and proliferation (B) of J774 cells elicited by SXWS/WSXWS peptides (black/grey columns, respectively). Early adhesion profile of the cells was calculated from the normalized slopes of the curves in the first 2 hours from the addition of the cells. Long term proliferation was calculated from normalized slope of the curves between 30 h and 72 h. Statistical analysis of data was performed using Student's t test; * : $p < 0.05$

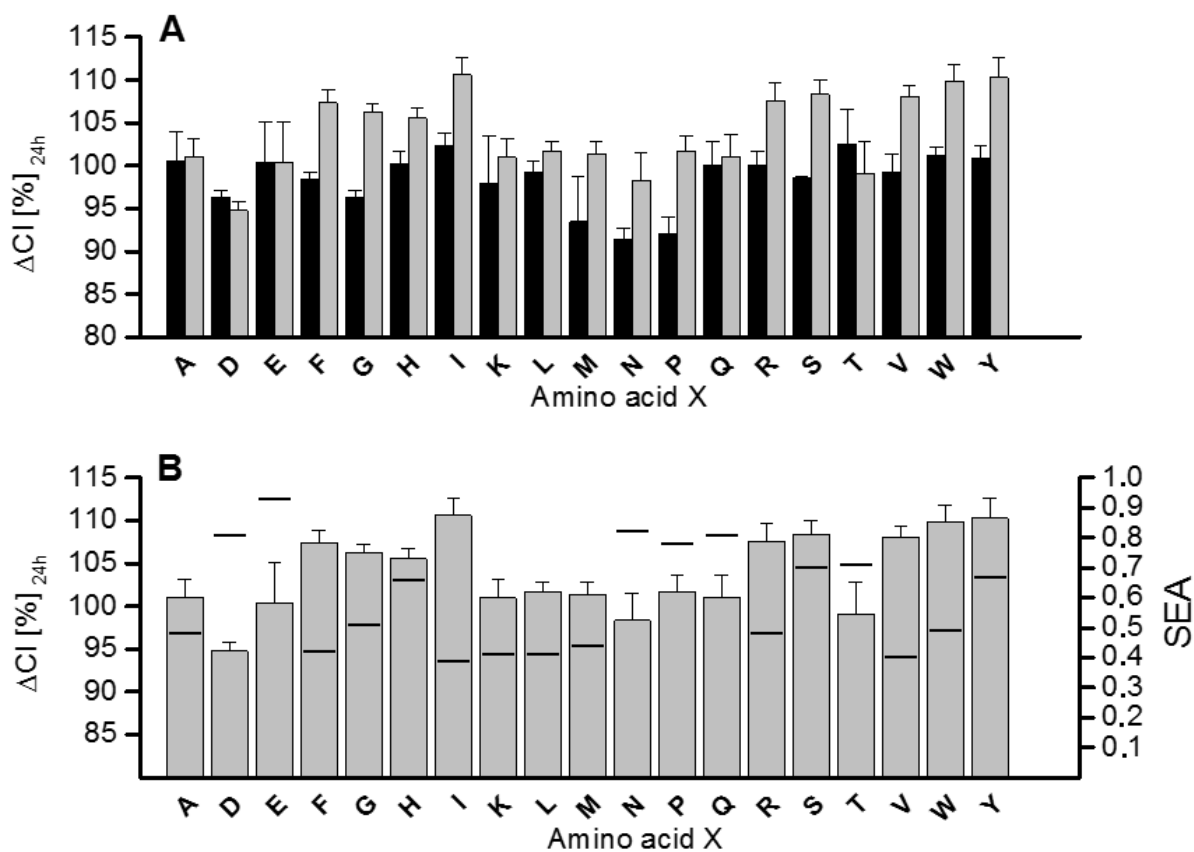


Figure 6 Proliferation of J774 cells 24 hours following the treatment with (W)SXWS peptides (A) Black columns represent SXWS and grey columns represent WSXWS peptides. Correlation between 24 h proliferation (columns) and solvent exposed area (SEA) (bars) of amino acids at the 'X' position (B). Proliferation was characterized by normalized ΔCI at 24 h.