Volume 317, number 1,2, 93–95 FEBS 12073 February 1993

Augmentation of human natural killer cells by splenopentin analogs

A. Rastogi[a], V.K. Singh[b], S. Biswas[b], W. Haq[a], K.B. Mathur[a] and S.S. Agarwal[b]

[a]*Division of Biopolymers, Central Drug Research Institute, Chattar Manzil Palace, Post Box No. 173, Lucknow 226001, India* and [b]*Department of Immunology, Sanjay Ghandi Post-Graduate Institute of Medical Sciences, Post Box No 375, Lucknow 226 001, India*

Received 14 September 1992; revised version received 3 December 1992

Splenopentin, Arg-Lys-Glu-Val-Tyr (SP-5) and its synthetic analogs; Arg-D-Lys-Glu-Val-Tyr (pentapeptide **1**), Lys-Lys-Glu-Val-Tyr (**2**), D-Lys-Lys-Glu-Val-Tyr (**3**), Arg-Lys-Gly-Val-Tyr (**4**), and Arg-Lys-Gln-Val-Tyr (**5**) have been examined for augmentation of human natural killer (NK) cell activity and human T-cell transformation response. Pentapeptides **2** and **3** were found to significantly augment the in vitro human NK cell activity. However, none of them had any effect on lymphocyte proliferative responses.

Splenopentin; Thymopoietin; NK cell cytotoxicity; T-cell transformation; Immunomodulator

1. INTRODUCTION

Immunomodulatory activity of thymopentin (TP-5) and its synthetic analogs has been well established and some of them are presently being evaluated clinically [1–4]. According to a report from Audhya et al. [5], a closely related pentapeptide called splenopentin (SP-5), the amino acid sequence of which differs from that of TP-5 only at position 3 due to the presence of a Glu residue in place of Asp, exhibits a particularly interesting immunopharmacological profile. Unlike TP-5, splenopentin (Arg-Lys-Glu-Val-Tyr) does not affect neuromuscular transmission and induces phenotypic differentiation of both T- and B-cell precursors. Recently, stimulation of the recruitment of epidermal Langerhans cells by SP-5 has also been demonstrated [6]. In view of these important findings, we undertook the synthesis of some novel analogs of SP-5: Arg-D-Lys-Glu-Val-Tyr (pentapeptide **1**), Lys-Lys-Glu-Val-Tyr (**2**), D-Lys-Lys-Glu-Val-Tyr (**3**), Arg-Lys-Gly-Val-Tyr (**4**) and Arg-Lys-Gln-Val-Tyr (**5**) for studying their immunopotentiating activity. In the case of analogs **1–3**, the amino acid Glu has been retained at position 3 and modifications have been introduced at positions 1 and 2. However, in analogs **4** and **5** Glu at position 3 has been replaced by Gly and Gln residues, respectively, with a view to investigate the role of Glu side chain for the biological activity of SP-5. Immunostimulating activity of the new analogs **1–5** has been examined for augmentation of human NK cell activity and human T-cell transformation response. We have found that analogs **2** and **3** significantly augment human NK cells.

Correspondence address V.K. Singh, Department of Immunology, Sanjay Gandhi Post-Graduate Institute of Medical Sciences, Post Box No 375, Lucknow 226 001, India. Fax: (91) (522) 51993.

Abbreviations: FCS, foetal calf serum; NK cells, natural killer cells; PHA, phytohaemagglutinin.

2. MATERIALS AND METHODS

2.1. *Synthesis of pentapeptides*

SP-5 and its analogs **1–5** were synthesized by the solution phase procedure using the 2 + 3 fragment condensation approach. Deblocking of the protected pentapeptides Boc-Arg(NO_2)-Lys(Z)-Glu(OBzl)-Val-Tyr-OBzl, Z-Arg(NO_2)-D-Lys(Z)-Glu(OBzl)-Val-Tyr-OBzl, Z-Lys(Z)-Lys(Z)-Glu(OBzl)-Val-Tyr-OBzl, Z-D-Lys(Z)-Lys(Z)-Glu-(OBzl)-Val-Tyr-OBzl, Z-Arg(NO_2)-Lys(Z)-Gly-Val-Tyr-OBzl and Z-Arg(NO_2)-Lys(Z)-Gln-Val-Tyr-OBzl afforded SP-5 and the analogs **1–5** in a pure state. They were characterized as hydrochloride or acetate salts with the help of elemental analysis, TLC, HPLC, NMR and FAB-MS.

2.2. *NK cell cytotoxicity assay*

NK cell activity was evaluated by a standard ^{51}Cr release assay [7]. Non-adherent cells were collected by plastic adherence and stimulated for 18 h with human recombinant interferon gamma (100 U/ml, G1105, Genentech Inc, San Francisco, USA) and/or different peptides (at 10^{-6}, 10^{-5} and 10^{-4} molar concentration) for NK cell augmentation. An erythroleukemic cell line K562, labelled with ^{51}Cr (sodium chromate in saline; spec. act. 50–150 mCi/mg BARC, Bombay, India) was used as target. Target cells were incubated with effectors at E/T ratio of 50:1 for 4 h. Each set was taken in triplicate. The supernatant was collected and counted in multigamma counter. Data is presented as percent cytotoxicity (7).

2.3. *Lymphocyte proliferation assay*

Mononuclear cells were separated from peripheral blood of healthy donors as described above. Triplicate cultures were set up in 96-well flat-bottom plates, each containing 0.2 ml of cell suspension (2×10^5 cells) with or without stimulants. The stimulants included phytohaemagglutinin (PHA; 5 μg/ml, Sigma, USA), and various synthetic peptides mentioned above. The cultures were incubated for a total of 3 days and were pulsed with 0.5 μCi [^{3}H]thymidine (6 Ci/mmol, BARC, Bombay, India) per well during the last 18 h of incubation. Thymidine uptake was determined by liquid scintillation counting.

Table I

Effect of SP-5 and its synthetic congeners on in vitro human NK cell activity

Sr. No.	Peptide	Concentration	% cytotoxicity			% increase* (mean ± S.D.)
			I	II	III	
1	Control		50.8	38.2	45.3	
	IFN		91.6	80.7	82.3	90 ± 17
	Arg-Lys-Glu-Val-Tyr (SP-5)					
		10^{-4} M	55.1	42.3	47.3	7 ± 3
		10^{-5} M	53.7	35.2	39.5	5 ± 0
		10^{-6} M	54.6	39.6	43.2	5 ± 2
		10^{-4} M + IFN	97.7	72.3	80.9	6 ± 0
		10^{-5} M + IFN	90.6	78.6	88.6	7 ± 0
		10^{-6} M + IFN	94.4	83.2	85.3	3 ± 0
2	Control		41.6	52.3	46.3	
	IFN		77.7	78.2	66.3	59 ± 23
	Arg-D-Lys-Glu-Val-Tyr (1)					
		10^{-4} M	48.5	45.6	52.3	**
		10^{-5} M	51.1	47.3	39.4	**
		10^{-6} M	48.2	40.2	45.2	**
		10^{-4} M + IFN	77.6	80.6	69.9	4 ± 1
		10^{-5} M + IFN	77.6	86.2	73.2	4 ± 3
		10^{-6} M + IFN	76.6	80.1	75.7	4 ± 3
3	Control		51.3	46.2	42.1	
	IFN		78.1	80.6	68.5	62 ± 11
	Lys-Lys-Glu-Val-Tyr (2)					
		10^{-4} M	60.6	60.2	61.7	31 ± 14
		10^{-5} M	53.2	66.1	60.8	30 ± 23
		10^{-6} M	53.8	60.2	53.6	20 ± 14
		10^{-4} M + IFN	76.3	80.1	70.3	2 ± 0
		10^{-5} M + IFN	77.4	73.5	77.2	12 ± 0
		10^{-6} M + IFN	79.1	75.6	70.5	1 ± 0.7
4	Control		48.5	52.3	47.0	
	IFN		85.0	87.0	73.0	65 ± 10
	D-Lys-Lys-Glu-Val-Tyr (3)					
		10^{-4} M	66.2	62.3	53.7	23 ± 11
		10^{-5} M	69.4	67.2	55.9	29 ± 12
		10^{-6} M	66.1	62.0	55.8	24 ± 10
		10^{-4} M + IFN	85.4	81.5	76.2	2 ± 2
		10^{-5} M + IFN	80.1	89.2	74.5	2 ± 0
		10^{-6} M + IFN	75.6	72.3	73.8	1 ± 0
5	Control		41.7	38.7	49.2	
	IFN		72.5	60.5	82.3	65 ± 8
	Arg-Lys-Gly-Val-Tyr (4)					
		10^{-4} M	43.0	42.3	46.3	6 ± 3
		10^{-5} M	39.95	40.1	40.8	3 ± 0
		10^{-6} M	40.9	35.6	48.1	0
		10^{-4} M + IFN	77.5	65.9	85.3	5 ± 2
		10^{-5} M + IFN	74.9	62.1	79.3	2 ± 0
		10^{-6} M + IFN	72.2	60.3	86.2	4 ± 0
6	Control		40.0	46.2	51.3	
	IFN		71.3	80.6	78.0	65 ± 11
	Arg-Lys-Gln-Val-Tyr (5)					
		10^{-4} M	37.0	44.2	50.1	0
		10^{-5} M	44.5	46.1	39.8	**
		10^{-6} M	46.3	40.3	nd	**
		10^{-4} M + IFN	79.3	nd	45.2	**
		10^{-5} M + IFN	73.4	72.3	62.5	2 ± 0
		10^{-6} M + IFN	72.8	75.1	63.8	2 ± 0

IFN = 100 U/ml of recombinant human gamma interferon was used, nd = not done.

* % increase on cytotoxicity was calculated in relation to control or interferon. Peptide or interferon was compared with control and peptide plus interferon was compared with interferon only. For this purpose control or interferon cytotoxicity was considered 100%.

** Did not show a consistent increase in all 3 experiments.

3. RESULTS

3.1. *Augmentation of human NK cells*

SP-5 and its synthetic congeners (**1–5**) were tested in vitro for augmentation of human NK cells. Results of three different experiments are presented in Table I. It shows that two analogs of SP-5 viz., Lys-Lys-Glu-Val-Tyr (**2**) and D-Lys-Lys-Glu-Val-Tyr (**3**), show significant in vitro augmentation of human NK cells. However, the degree of activation of NK cell activity by these peptides was considerably lower than that by human interferon gamma. The peptides neither potentiated nor inhibited the augmentation of NK cell activity induced by gamma interferon.

3.2. *Human lymphocyte transformation test*

SP-5 and its analogs were also tested for their ability to stimulate or inhibit human lymphocyte transformation. It was observed that neither SP-5, nor its analogs either stimulate T-cell proliferation by themselves or inhibit the PHA induced [^{3}H]thymidine uptake (data not presented).

4. DISCUSSION

NK cells are a discrete subset of lymphocytes which are capable of killing tumor and virus infected cells in a non-MHC restricted manner. As compared to T cells, the NK cells do not need any antigenic exposure for their activation [8]. Most of the large granular lymphocytes are considered to be NK cells. These are $CD3^-$ and TCR^- but express CD16 and CD56 surface markers [9–12]. These cells mainly circulate in the peripheral blood. Interferons are known to augment NK cells, both in vivo as well as in vitro [12–15]. Augmentation of NK cells by small synthetic peptides such as analogs of muramyl dipeptide, thymopoietin fragments and endorphins has also been reported [16–19]. SP-5 is known to induce both T- and B-cell precursors [5]. Our results show that splenopentin by itself does not have any effect on NK cells but substitution of Arg at position 1 in SP-5 by Lys (**2**) and D-Lys (**3**) results in the appearance of significant natural killer cell activity (in vitro). Replacement of Lys at position 2 by D-Lys (analog **1**) and Glu at position 3 by Gly and Gln residues (analogs **4** and **5**) renders them inactive. This indicates the importance of Lys and Glu residues at positions 2 and 3 for the NK cell activity of this series of peptides. To the best of our knowledge this is the first report showing in vitro augmentation of human NK cells by analogs of SP-5. The magnitude of augmentation by the two analogs of SP-5 was considerably lower than interferon gamma, but is quite comparable to other peptides reported in the literature [16–18]. If these in vitro results are substantiated by in vivo studies, these compounds may provide a new tool for stimulation of innate host resistance, particularly against cancer and viral infections.

Acknowledgements: Financial assistance from CSIR, DST and DBT, Govt. of India, New Delhi is acknowledged.

REFERENCES

[1] Scheid, M.P., Goldstein, G. and Boyse, E.A. (1978) J. Exp. Med. 147, 1727–1743.
[2] Georgiev, V.S. (1991) Med. Res. Rev. 11, 81–119.
[3] Hadden, J.W. (1991) Trends Pharmacol. Sci. 12, 107–111.
[4] Kessler, H., Kutscher, B., Obermaier, R. and Muellner, H. (1985) Ger. Patent 3, 401, 545: (1985) Chem. Abstr. 103, 54465u.
[5] Audhya, T., Scheid, M.P. and Goldstein, G. (1984) Proc. Natl. Acad. Sci. USA 81, 2847–2849.
[6] Gruner, S., Diezel, W., Strunk, D., Zwirner, A., Soennichsen, N. and Anhalt, G.J. (1990) Arch. Dermatology Res., 281, 526–529.
[7] Pedersen, B.K. and Kharazmi, A. (1987) Infect. Immun. 55, 986–972.
[8] Herberman, R.B. (1981) Clin. Immunol. Rev. 1, 1.
[9] Zarling, J.M. and Kung, P.C. (1980) Nature 288, 394–396.
[10] Lanier, L.L., Le, A.M., Phillips, J.H., Warner, N.L. and Babcock, G.F. (1983) J. Immunol. 131, 1789–1796.
[11] Hercend, T., Griffin, J.D., Bensussan, A., Schmidt, R.E., Edson, M.A., Brennan, A., Murray, C., Daley, J.F., Schlossaman, S.F. and Ritz, J.J. (1985) J. Clin. Invest. 75, 932–943.
[12] Herberman, R.B., Djew, J.Y., Kay, H.D., Ortaldo, J.R., Riccardi, C., Bonnard, G.D., Holden, H.T., Fagnani, R., Santoni, A. and Pucceti, P. (1979) Immunol. Rev. 44, 43–70.
[13] Gidlund, M., Orn, A., Wigzell, H., Senik, A. and Gresser, I. (1978) Nature 272, 759–761.
[14] Herberman, R.B., Ortaldo, J.R. and Bonnard, G.D. (1979) Nature 277, 221–223.
[15] Trinchieri, G. and Santoli, D.J. (1978) Exp. Med. 147, 1314–1333.
[16] Sharma, S.D., Tsai, V., Krahenbuhl, J.L. and Remington, J.S. (1981) Cell. Immunol. 62, 101–109.
[17] Legarrec, Y., Morin, A. and Chedid, L. (1985) Int. J. Immunopharmacol. 7, 343.
[18] Kay, N., Allen, J. and Morley, J.E. (1984) Life Sci. 35, 53–59.
[19] Florilli, M., Sirianni, M.C., Pandolfi, F., Quiniti, I., Tosti, U., Aiuti, F. and Goldstein, G. (1981) Clin. Exp. Immunol. 45, 344–351.