non suggesting that humoral factors could indeed take part in the development of this immunity[11].

At the 4th day of our experiment, all recipients of immune serum developed rather good IgG titres (1/80, table 2) against *I. ricinus*, indicating perhaps involvement of circulating antibodies of that immunoglobulin class. Circulating homocytotropic specific antibodies seemed to be more diluted. In fact the titres were only 1/1 or 1/3 in contrast to 1/81 in the transferred immune serum. These differences in quantities of circulating antibodies may be due to the different physiological destination of IgG and homocytotropic antibodies, the latter fixing normally to receptors of basophils or tissue mast cells. Indeed, the skin of all treated rabbits proved to be sensitized to *I. ricinus* allergens (table 2). Studies are under way to determine the role which this immediate hypersensitivity may play in the host's acquired resistance to *I. ricinus*.

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Structure specificity of some immunoadjuvant synthetic glycopeptides

K. Mašek, M. Zaoral, J. Ježek and V. Krchňák

Institute of Pharmacology, Czechoslovak Academy of Sciences, Albertov 4, 12000 Praha 2, and Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Praha (Czechoslovakia), 21 December 1978

Summary. The immunoadjuvant activity of muramyl dipeptide seems to be critically dependent on the type of substitution of the γ-carboxyl group of the D-isoglutamine residue. Moreover the nonapeptide L-Ala-D-isoGlu-L-Lys-D-Ala-$(Gly)_5$-OME also shows a definite effect.

The identification of N-acetyl muramyl L-alanyl-D-isoglutamine as the minimal structure required for immunoadjuvant activity[1] opened the way for a systematic study of structure-activity relationships. Previous studies have established that the adjuvant activity is critically dependent on the presence of the D-configuration of glutamic acid residues, and on the amidation of α-carboxyl group of this amino acid[2-5]. The main objective of the present work was to extend the above mentioned original findings.

Material and methods. Compounds listed in the table were prepared as described elsewhere[6,7]: The peptides both by liquid and solid-phase peptide synthesis, the glycopeptides and glycoamino acid by acylation of the corresponding peptides or aminoacid derivative with 1-α-0-benzyl-4,6-0-benzylidenemuramic acid. The cleavage of the protecting groups was effected by hydrogen bromide in acetic acid[8] (peptides) or by sodium in liquid ammonia (glycopeptides)[6]. The immunoadjuvant activity was assayed on female albino guinea-pigs injected in the left hind footpad with 0.2 ml of water-in-oil emulsion (Drakeol, Arlacel and saline 4:1:5) containing 2.5 mg of crystalline ovalbumin and 200 μg of synthetic peptides or glycopeptides, or 1 mg of

Induction of delayed hypersensitivity to ovalbumin in guinea-pigs by synthetic peptides and glycopeptides

Compound tested	Dose (μg)	Skin response (24 h)
Freund's incomplete adjuvant (FIA)	-	7.5 ± 0.8
Freund's complete adjuvant (FCA)	1000	13.6 ± 0.9*
FIA + L-Ala-D-Glu-NH_2 ┌ Lys(Ac)-D-Ala-NH_2	200	7.6 ± 1.5
FIA + L-Ala-D-Glu-NH_2 ┌ Lys(Ac)-D-Ala-$(Gly)_5$-OME	200	7.3 ± 1.0
FIA + Ala-D-Glu-NH_2	200	11.5 ± 1.1*
FIA + NAM-Ala-NH_2	200	5.3 ± 0.5
FIA + NAM-Ala-D-Glu-NH_2	200	12.7 ± 1.5*
Freund's incomplete adjuvant	-	4.5 ± 0.4
Freund's complete adjuvant	1000	12.6 ± 1.5*
FIA + NAM-Ala-D-Glu-NH_2 | NH_2	200	11.2 ± 0.5*
FIA + NAM-Ala-D-Glu-NH_2 ┌ Lys(Ac)-D-Ala-NH_2	200	11.4 ± 1.2*
FIA + NAM-Ala-D-Glu-NH_2 | $NH-(CH_2)_3-CH_3$	200	13.3 ± 0.9*
FIA + NAM-Ala-D-Glu-NH_2 | $NH-(CH_2)_{17}-CH_3$	200	6.3 ± 1.6
FIA + NAM-Ala-D-Glu-NH_2	200	9.1 ± 1.1*

Values represent the means from 6–10 animals ± fiducial limits and are expressed in diameter of redness (mm). * Statistically significant results; $p < 0.05$. NAM = N-acetylated muramic acid. Unless stated otherwise, all optically active amino acids are of L-configuration.

Mycobacterium tuberculosis. 3 weeks later the skin reactions to 10 µg of ovalbumin were read.

Results and discussion. From the data presented in the table it is clear that the dipeptide (L-Ala-D-Glu-NH_2), and also the tetrapeptide (L-Ala-D-isoGlu-L-Lys-D-Ala) have no effect on delayed hypersensitivity in our tests. These results are in line with those published by Ellouz et al.[1] and Kotani et al.[3] who also reported no immunoadjuvant activity for these compounds. They suggested that N-acetyl muramic acid should be present to observe the adjuvant effect. Our results with the nonapeptide (L-Ala-D-isoGlu-L-Lys-D-Ala-$(Gly)_5$-OME), however, do not support the idea of a critical role of N-acetyl muramic acid. It is noteworthy that Nauciel et al.[9] originally reached the conclusion that the tetrapeptide subunit is already effective. However, they used the natural product in their experiments and azobenzenearsenate-N-acetyl tyrosine was administered instead of ovalbumin. Our present results would suggest that some longer peptides might be as effective as glycopeptides with a short peptide chain. This point certainly deserves more experimental work. From the table it is also evident that NAM-L-Ala-NH_2 showed no adjuvant effect and this is in agreement with results published by others[1-3]. Previous work also suggested that the amidation of the γ-carboxyl group instead of the α-carboxyl group of the glutamic acid residue abolished the adjuvant effect of muramyl dipeptide[3] in inducing delayed-type hypersensitivity. Also the derivatives where both α and γ-carboxyls of glutamic acid are esterified have lost most of their activity when administered in a water-in-oil emulsion[5]. The amidation of the γ-carboxyl group, in addition to the amidation of the α-carboxyl group of glutamic acid residue in our experimental design (NAM-L-Ala-D-isoGlu-NH_2), did not markedly affect the adjuvant potency. This is in agreement with results published by others[10]. On the other hand, the amidation of the γ-carboxyl of the D-iso-glutamine residue by butyl or stearyl groups, which gave muramyl dipeptide derivates with an increased hydrophobicity, clearly decreased the intensity of response of muramyl dipeptide. Kotani et al.[11] working with 6-0-stearoyl derivatives of N-acetyl muramyl dipeptide have recently noted that this compound is also adjuvant in water-in-oil emulsion, however, again less than the original compound. This, however, seems not to be the case when biodegradable liposomes are substituted for mineral oils. We have recently reported that Freund's incomplete adjuvant may be replaced by phosphatidyl choline-cholesterol liposomes. Muramyl dipeptide in aqueous suspension did not show any effect[12]. Stearyl amide of N-acetyl muramyl alanyl-D-isoglutamine injected in liposomes seems to be more effective in the induction of delayed hypersensitivity than muramyl dipeptide. The explanation for this finding might be that the introduction of a stearyl group increases the binding of muramyl dipeptide within the liposome. No experimental evidences to support this hypothesis, however, are at the present time available. Several papers published recently by the Chedid group[5,13] reported that in case of humoral immunity a potentiation can be achieved already with the compound in aqueous solution.

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Colony stimulating activity in acute and chronic endotoxinemia in man

W. Hinterberger, L. Fridrich, P. Ferenci and W. Paukovits

1st Department of Medicine, Department of Gastroenterology and Hepatology, and Institute of Cancer Research, University of Vienna, Lazarettgasse 14, A-1090 Wien (Austria), 12 December 1978

Summary. Blood granulocyte-macrophage colony stimulating activity (GM CSF) was measured in 6 normal individuals challenged with low-dose endotoxin and in 63 unselected patients with nonhaematological disorders. 5/63 patients were febrile and 5 other patients showed detectable endotoxin levels, as measured by the Limulus assay. CSA levels showed a rapid increase in normal individuals following endotoxin administration, but were in the normal range in patients with chronic endotoxinemia or in those with febrile disorders. Thus, unlike acute endotoxinemia, chronic endotoxinemia is not associated with elevated activity that promotes growth of myeloid commited stem cells. In addition, fever per se did not coincide with elevated blood CSA levels.

Granulocyte-macrophage colony stimulating activity (CSA) is rapidly elevated in the blood of experimental animals and man upon administration of endotoxin[1,2]. Mice, exposed to repeated administration of endotoxin, became nonresponsive[3]. Abnormally low CSA levels in germ-free animals led to the assumption that endotoxin releasing bacteria are necessary for maintaining a certain CSA level[4]. Since most data were obtained in experimental animals and usually refer to acute endotoxinemia, we compared the effects of acute and chronic endotoxinemia on blood CSA levels in man.

Material and methods. The effect of acute endotoxinemia on blood CSA levels was tested in 6 normal individuals, who gave their informed consent to the administration of low-dose endotoxin (Pyrifer, step 1, Aristopharm). Blood from 63 unselected, nonhaematological patients was simultaneously assayed for CSA levels and the presence of endotoxin. Blood CSA levels were assayed in a monolayer