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New Conjugates of Tuftsin and Muramyl Dipeptide as Stimulators of Human Monocyte-Derived Dendritic Cells

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Abstract: Muramyl dipeptide (MDP) and tuftsin are known biologically active compound displaying a significant influence on various cell populations of innate immune response. MDP, as a fragment of bacterial cell wall, stimulates not only macrophages and monocytes, but also dendritic cells. In contrast, little is known about tuftsin influence on these cells. Therefore it seemed vital to access whether tuftsin or its derivatives conjugated with MDP could influence the activity of this subpopulation of antigen presenting cells (APC). Immature dendritic cells (iDCs) were derived from human monocytes through eight-day tissue culture supplemented with hrIL-4 and hrGM-CSF. On the day 9 DCs were stimulated with newly synthesized conjugates of tuftsin and muramyl dipeptide. The influence of the examined compounds on the activity and maturity of monocyte-derived DCs was estimated by flow cytometry analysis. The flow cytometry analysis revealed that tuftsin and some of its analogues do stimulate maturation and activity of DCs but to a lesser extend in comparison to MDP. The obtained results suggest further development of the experiments concerning the influence of MDP and tuftsin analogues on the activity of dendritic cells.

Keywords: Tuftsin, muramyl dipeptide, dendritic cells.

INTRODUCTION

Muramyl dipeptide (MDP) and tuftsin are known biologically active compounds displaying a significant influence on various populations of cells of innate immune response [1, 2]. Both immunomodulators can significantly influence the activity of innate immune cells, including monocytes, macrophages and granulocytes. Additionally, we proved both *in vitro* and *in vivo*; their conjugates increase the reactivity of innate immune response in order to fight the infection [3-5]. Stimulation of the above mentioned innate immune cells triggers the cascade of adaptive immunity, but it is the indirect effect of the MDP and tuftsin. Having investigated various biological activity of these immunomodualtors and their derivatives, we have learned that little is known about their influence on dendritic cells (DCs). Of course, MDP, as a fragment of bacterial cell wall, should exert the effect on maturation and activity of DCs and it has already been proven by various scientists [6]. Unfortunately, there is no information about tuftsin as a stimulator of DCs. Hence, we found it vital to perform experiments helping to evaluate this tetrapeptide activity in terms of maturation and activation of DCs. It is intriguing if both tuftsin and MDP, able to stimulate various subpopulations of antigen presenting cells (APC), can also influence dendritic cells activity. Moreover, DCs are now being extensively investigated as potential therapeutic elements in treatment of diverse immunological disorders, including autoimmune deficiencies or post-transplant therapy. Newly synthesized conjugates of MDP and tuftsin were dedicated for this study in order to find out whether tuftsin can influence the activity of DCs and whether MDP and tuftsin in conjugates will act synergistically stimulating DCs.

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MATERIALS AND METHODS

Examined Compounds

MDP (muramyl dipeptide) and nor-MDP (nor-muramyl dipeptide) conjugates were modified at the C-terminus of the peptide residue by the formation of an amide bond between the isoglutamine carboxylic group and the amine group of the retro-tuftsin (Arg-Pro-Lys(Y)-Thr-OMe, Y = Gly, Val) (Table **1**). General procedure for the preparation of compounds **19-22** was described in details previously [7, 8]. Qualitative amino acid analyses of the hydrolyzates of the compounds were performed by TLC. Detection by: UV and ninhydrin. The mass spectrometry analysis was carried out on a MALDI MS (a Biflex III MALDI-TOF spectrometer, Bruker Daltonics, Germany). Below we present analytical characteristics of the examined compounds:

nor-Mur(NAc)-Pro-D-Glu(Arg-Pro-Lys(Ala)-Thr-OMe)-NH$_2$ **19**. Yield 30%; amino acid analysis (6 M, 110°C, 20 h): Ala, Arg, Glu, Lys, Pro, Thr; MALDI-TOF calcd. for $C_{45}H_{77}N_{13}O_{17}$, 1072.2, found 1073.1 $(M+H)^+$.

nor-Mur(NAc)-Pro-D-Glu(Arg-Pro-Lys(Gly)-Thr-OMe)-NH$_2$ **20**. Yield 32%; amino acid analysis (6 M, 110°C, 20 h): Arg, Glu, Gly, Lys, Pro, Thr; MALDI-TOF calcd. for $C_{44}H_{75}N_{13}O_{17}$, 1058.1, found 1058.4 $(M+H)^+$.

Mur(NAc)-Pro-D-Glu(Arg-Pro-Lys(Val)-Thr-OMe)-NH$_2$ **21**. Yield 35%; amino acid analysis (6 M, 110°C, 20 h): Arg, Glu, Lys, Pro, Thr, Val; MALDI-TOF calcd. for $C_{48}H_{83}N_{13}O_{17}$, 1114.2, found 1114.7 $(M+H)^+$.

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