Recent Developments in the Synthesis and Biological Activity of Muramylpeptides

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Abstract: Derivatives of muramyl dipeptide (MDP) are considered as immunostimulants and adjuvants in the immunotherapy of cancer and infections. The interest in these compounds is mainly related to a high variety of their structure and biological properties. Here, we describe the synthesis and biological activity of several recently developed classes of MDP analogues. We also report potential of these analogues in the treatment of cancer and infectious diseases in experimental systems and cancer patients.

Keywords: Muramyl dipeptide, MDP, MDP analogues, adjuvant, synthesis, cancer immunotherapy, innate immunity.

INTRODUCTION

N-Acetylmuramyl-L-alanyl-D-isoglutamine (**1**) (muramyl dipeptide, MDP, Fig. (**1**)), which is a fragment of peptidoglycan (PGN), has been identified as the smallest bacterial cell wall content with immunogenic capabilities [1, 2]. MDP has been known since the mid-1970s to be the key component of complete Freund's adjuvant for mediating adjuvant activity to co-injected antigens [3]. The mechanism of PGN action on the immune system has been recently clarified in details by Fujimoto *et al.* [4] who examined the effects of chemically synthesized defined peptidoglycan partial structures (mono-, di-, tetra- and octasaccharide fragments of peptidoglycan). In general, the most robust biological activities of the analogues covered the induction of proinflammatory cytokines from human monocytes induced, the most probably, through TLR and NOD2 - dependent signaling pathways. Vigorous response of innate immunity towards MDP analogues can be therefore utilized in the immunotherapeutic strategies as, regardless of the structure, the analogues trigger nonspecific resistance against bacterial, viral or parasitic infections. The same capabilities can be also profitable in the immunity against tumors. Numerous reports on muramyl peptides in immunotherapy have been published [5-11]. Some muramyl dipeptide derivatives, like murabutide (Mur*N*Ac-L-Ala-D-isoGln-*n*-Bn ester) (**2**) or MTP-PE (**3**) and lipopeptides, like pimelautide (**4**), are under clinical trials [12-16] as immunomodulators and adjuvants. It seems that the combination of MDP derivatives with drugs would allow utilizing both strong immunogenicity of the derivatives and the synergistic effects superb to the separate administration of MDP derivatives and particular drugs. The advantage of such a maneuver would be not only enhanced activity of the drugs but also adjuvant effect of the stimulation of the immune system [9]. In this review we would like to present new MDP derivatives, which have been obtained since 2002.

1. INFLUENCE OF MDP DERIVATIVES ON THE IMMUNE SYSTEM

MDP, a synthetic bioactive glycopeptide of microbiological origin, stimulates various functions of macrophages (such as phagocytosis, pinocytosis, motility, chemotaxis, bactericidal and antitumour activity) and monocytes. It is believed that MDP, an analogue of the bacterial wall fragment, acts *via* LPS receptor CD14 and *via* TLR receptors. It is a commonly known fact that the activation of the above-mentioned receptors stimulates a wide range of the elements of immune system. The effects of MDP and its analogues on macrophages include increased cytokine production (TNF, IL-1, IL-6, IL-8, Th2-cytokines), nitric oxide (NO) secretion, C-reactive protein, activation of cytotoxicity, and upregulation of adhesion molecules (CD11a,b,c/CD18, CD54) [17-21].

MDP and other muramylpeptides were considered as ligands for various receptors, such as the 5-HT receptor, CD14 and the TLR receptors. Interaction between MDP and 5-HT receptors has been postulated as the administration of MDP could affect sleep and animal behavior. Nevertheless, although confirmed, MDP agonist activity towards 5-HT receptors seems to be weak and majority of the effects are indirect [22]. Recently, the cytoplasmic NOD family of proteins, which seems to play an important role in intracellular immune defense, has been identified as a target for MDP derivatives [11]. Two intracellular proteins of the NOD family, NOD1 and NOD2, were discovered as receptors for MDP or other muramylpeptides and desmuramylpeptides (DMPs) [11]. Girardin *et al.* [10] described that MDP and its analogues are NOD2 ligands. Only recently, Marina-Garcia *et al.* [23] performed studies which provided evidence for a clathrin- and dynamin-dependent endocytosis that mediates MDP uptake and NOD2 activation. Not much is known about the details of MDP-NOD2 interactions. In fact, some authors question direct interaction of NOD2 and MDP [24]. Studies with MDP isomers revealed that the first amino acid of MDP (L-alanine) is the most important in the immunomodulatory activity of this compound [25]. Other groups reported that the interaction between NOD2 and MDP required an intact Mur*N*Ac sugar group substituted to a peptidic chain of MDP. Interestingly, NOD2 was capable of recognition of longer peptide moieties, if the third amio acid is lysine or ornithine. In NOD2 sensing, not the length of the third amino acid's side chain but chemical properties of α-carboxyl group have been found important for the interaction [10]. In addition, a synergism between MDP and LPS was observed. Combination of these two compounds increased significantly the ability of macrophages to produce cytokines [26]. Nevertheless, the effect might be limited to some structural forms of MDP analogues, as there are reports that NOD2 activation by MDP downregulates responses to TLR stimulation [27]. Immune system is equipped with intracellular receptors sensing MDP and other bacterial products. CARD-NOD2 monomers exposed to MDP dimerizes and interact with Rip2. Rip-2 activates NEMO and MAPK kinases pathways leading to the activation of transcriptional factors NFκB and probably AP1/Jun. These transcriptional factors in the nucleus switch on the transcription of various proinflammatory cytokines and chemokines, such as pro-IL-1β, pro-IL-18, IL-8, IL-6, TNF-α, MIP2, CCL2. At least some of those factors, for example very important pro-IL-1β, pro-IL-18, are produced as pro-proteins and must be transformed in order to be secreted from the cells in the active form. This transformation is mediated *via* caspase 1, which activation is also stimulated by bacterial products. After a challenge with bacteria-derived moieties, monomeric forms of different NLRPs interact with pro-caspase 1, oligomerize and create multi-protein structures known as inflammosomes. Inflammosome is capable of releasing active caspase 1, which processes pro-cytokines into active forms. Oligomerized NLPRs activates also production of IFNs type I.

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