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Comparative study of structurally related peptidoglycan monomer and muramyl dipeptide on humoral IgG immune response to ovalbumin in mouse

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ABSTRACT

Structurally related peptidoglycan monomer (PGM) and muramyl dipeptide (MDP) differ in several aspects of biological activity but have in common immunostimulating properties. Comparative study of the effects of these adjuvants on humoral IgG immune response specific for protein antigen ovalbumin (OVA) was carried out in two inbred mouse strains, CBA and NIH/OlaHsd, and their ability to modulate the bias of immune response towards Th1/Th2 was evaluated. MDP had better adjuvant activity at some points than PGM, whereas both adjuvants stimulated Th2-biased immune response specific for OVA. In comparison to Complete Freund's adjuvant (CFA), as a golden standard of adjuvant action, both PGM and MDP exhibited considerably lower activity. Addition of PGM to Incomplete Freund's adjuvant (IFA) on humoral immune response was studied also, and the effect of such adjuvant formulation was compared to the effect of CFA. While CFA induced the switch towards Th1-biased immune response, the addition of PGM into IFA did have no impact on modulating the immune response towards more pronounced Th2-type of immune response, defined by IFA itself.

1. Introduction

Adjuvants are the class of compounds that are essential for the improvement of efficacy of the majority of vaccines. Numerous studies have been carried out to elucidate effects and mechanisms of different adjuvants, especially those aimed for human studies. Adjuvants can be classified according to their component sources, physicochemical properties and depending on immunological events they induce [1–3]. Bacterial-derived adjuvants as highly conserved structural motifs are classified as pathogen associated molecular patterns (PAMP). Such structures are sensed by specific pattern-recognition receptors (PRR) on innate immune cells [2,3].

Muramyl dipeptide (MDP, MurNAc-L-Ala-D-*iso*Gln) is the minimum essential structure of *Mycobacterium tuberculosis* peptidoglycan that can activate an inflammatory response and adjuvant activity [4]. A variety of MDP analogs and derivatives have also been synthesized and their adjuvant activities have been examined [5–12]. MDP and its derivates are now known to be PAMPs [13]. According to classification of adjuvants given by Cox and Coulter, MDP and its derivatives are also distinct class within non-particulate adjuvants [1].

Peptidoglycan monomer (PGM) is a natural compound obtained by simple biotechnological procedure from *Brevibacterium divaricatum* cell wall peptidoglycan with well defined chemical structure (GlcNAc-MurNAc-L-Ala-D-*iso*Gln-*meso*Dpm(εNH_2)-D-Ala-D-Ala) [14,15]. PGM is an adjuvant active compound that by its origin, chemical structure, physicochemical properties and proposed mechanism of action should belong to the group of adjuvants to which MDP and its derivatives belong, although it is not obtained by MDP's chemical derivatisation. Immunostimulating properties of PGM have been well documented in several studies [16–19]. Since MDP's structure represents a portion of integral PGM's molecule, we found it reasonable to compare the effects of PGM to the effects of MDP. Although both compounds exhibit immunostimulating properties, they differ in several aspects of biological activity. For example, MDP is pyrogenic and somnogenic and PGM is not [20–24].

Therefore, the aim of this work was to carry out a comparative study of adjuvant activity of MDP and PGM on humoral immune response in a well defined mouse model. The effects of both compounds on induction of humoral IgG immune response specific for protein antigen ovalbumin (OVA) were investigated and their ability to modulate the bias of immune response towards Th1/Th2 was evaluated. Experiments were carried out in two inbred mice strains with variable histocompatibility specificity, CBA (H-2^k) and NIH/OlaHsd (H-2^q) mice, according to recommendations for adjuvants assessment [25].

The second aim of the study was to compare the adjuvant effects of both PGM and MDP to the effect of Complete Freund's adjuvant (CFA). CFA is a potent adjuvant which consists of killed mycobacteria in oil emulsion. Because of its toxicity and reactogenicity, CFA has been abandoned for human use but, in some animal models, it is still used as a golden standard of adjuvant action [26]. It was found that the addition of MDP to the Incomplete Freund's adjuvant (IFA) results in a

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formulation with strong adjuvant properties comparable to CFA [27]. IFA is the same oil adjuvant as CFA, but without mycobacterial cells. Instead of MDP, we had emulsified PGM into IFA, tested adjuvant activity of such formulation in mice and compared it to the activity of CFA.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA), Tween 20, Tris(hydroxymethyl) aminomethane, monoclonal anti-chicken egg albumin (clone OVA-14 mouse IgG1 isotype), *o*-phenylenediamine dihydrochloride (OPD) and avidin–peroxidase were from Sigma, USA. Horseradish peroxidase conjugated goat anti-mouse IgG (HRP-anti-mouse IgG) was from Cappel, USA. Biotin-conjugated rat anti-mouse IgG1 and anti-mouse IgG2a monoclonal antibodies and streptavidin–peroxidase were purchased from PharMingen, Becton Dickinson (USA). Chemicals for buffers and solutions were from Kemika, Croatia.

2.2. Antigen and adjuvants

Ovalbumin (OVA) was from Serva, Germany. It was free of endotoxin, checked by rabbit's pyrogenicity test *in vivo* according to Ph. Eur. 2002:2.6.8. MDP (Fig. 1A) was purchased from Sigma, USA. PGM (Fig. 1B) was produced in Pliva, Croatia and obtained by lysozyme digestion of uncrosslinked peptidoglycan chains isolated from the culture fluid of penicillin treated *B. divaricatum* [14]. Its chemical stability and purity was checked using HPLC analysis [28]. PGM was apyrogenic (250 μg/kg) as tested *in vivo* in rabbits according to Ph. Eur. 2002:2.6.8. CFA and IFA were from Sigma.

2.3. Experimental mice

Inbred mice of CBA strain were obtained from the Animal Facility of the Institute "Ruđer Bošković", Zagreb, Croatia. NIH/OlaHsd inbred mice were raised at the Institute of Immunology, Croatia. All mice used were females from 2 to 2.5 months old. During the experimental period animals were housed in the Animal Facility of the Institute of Immunology. Commercial food and water were provided *ad libitum*. All animal work was performed according to the Croatian Law on Animal Welfare (NN 135/06).

2.4. Immunizations

Experimental groups of five mice were immunized and boosted two times subcutaneously (*s.c.*) into the tail base at 21-day intervals. The doses used per mouse were: OVA 10 μg, PGM 200 μg and MDP 100 μg or 200 μg, respectively. Prior to each immunization, PGM or MDP was dissolved in saline and then admixed to OVA solution. CFA and IFA were mixed with the antigen solution in equal ratios (v/v). CFA/IFA-immunized mice received CFA in the first immunization and IFA in all subsequent injections.

The injection volume in all experimental groups was 0.1 ml per mouse. Mice were anaesthetized prior blood collection from axillary's plexus on the 7th day after each booster. Individual sera from each animal were decomplemented at 56 °C for 30 min and then stored at −20 °C until tested.

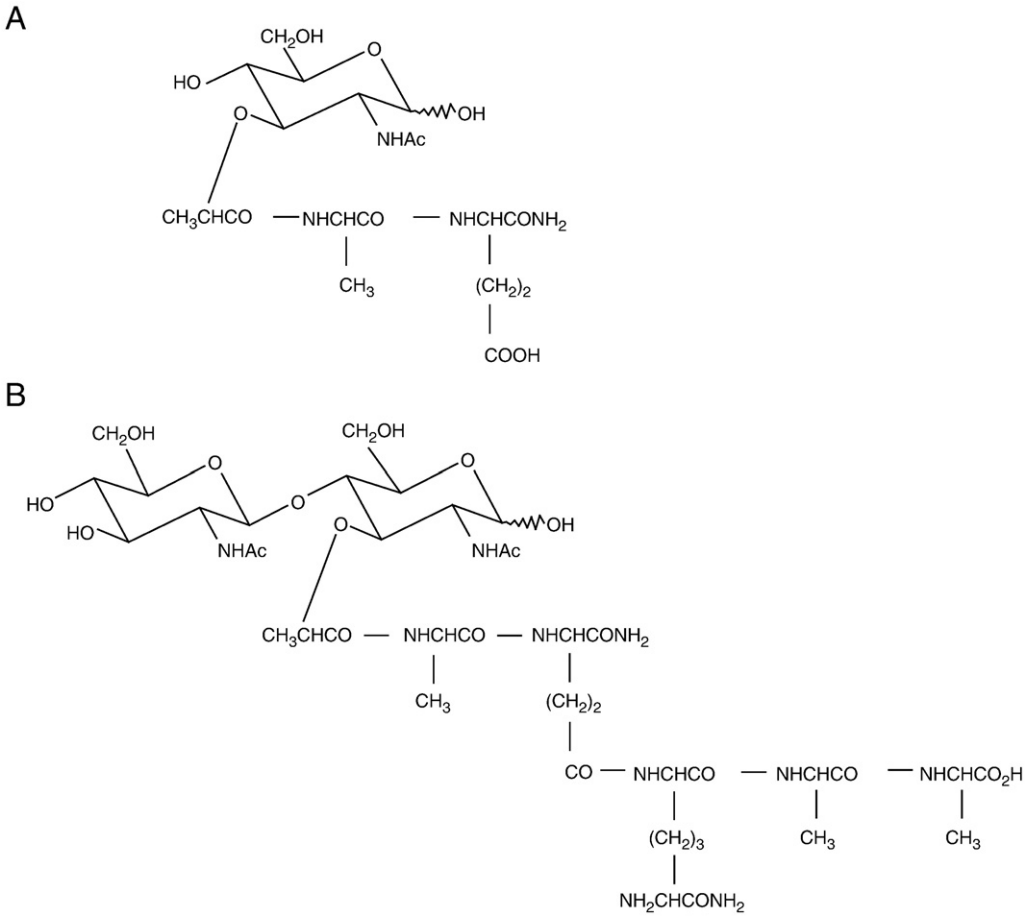

Fig. 1. The chemical structure of MDP (A) and PGM (B).