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COMMENTARY

PAST, PRESENT AND FUTURE OF THE SYNTHETIC IMMUNOADJUVANT MDP AND ITS ANALOGS

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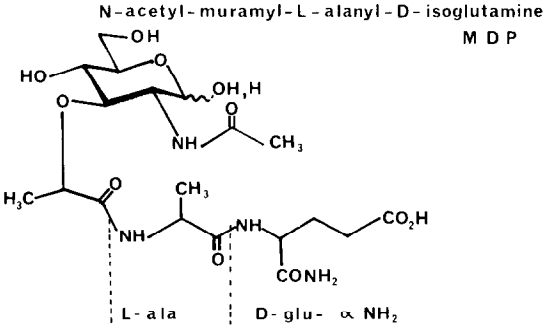
PAST AND PRESENT

In 1947, Jules Freund [1] wrote the prophetic sentence "It would be of great interest to identify the fraction or fractions which exert the adjuvant effect of mycobacteria and to know whether the effect could be produced without sensitization to tuberculin".

This has been achieved in 1974 [2, 3]: the minimal adjuvant active structure of bacterial peptidoglycans is *N*-acetyl muramyl-L-alanyl-D-isoglutamine (hereafter referred to as MDP, for muramyl dipeptide), as confirmed by Japanese authors [4]. Moreover,



although it was possible to induce delayed hypersensitivity to a variety of antigens by administering MDP in a water-in-oil emulsion, no sensitization to tuberculin was observed. Indeed, MDP does not elicit positive skin tests, even in guinea pigs which have been previously sensitized by this glycopeptide administered in Freund's complete adjuvant (FCA) [5]. Nevertheless MDP can fully replace mycobacteria in Freund's adjuvant since it can elicit positive responses towards an auto-antigen [6]. Encephalomyelitis can be induced by administering a synthetic encephalitogen antigen either with FCA or with Freund's incomplete adjuvant (FIA) containing minute amounts of MDP. In all previous studies with FCA in guinea pigs, a synthetic tryptophan nonapeptide had been shown to be the smallest active antigen. However, auto-immune encephalomyelitis was also induced by an analogous tryptophan heptapeptide administered with MDP in FIA [7]. These experiments are remarkable because they show a maximum amplification of the immune response under the influence of a synthetic antigen and of a synthetic adjuvant.

The kinetics of adjuvant action of MDP in a water-in-oil emulsion and FCA have been compared and were found in favor of MDP (higher activity, longer duration) [8]. MDP has, in comparison to FCA, some other advantages: as stated previously it is not immunogenic; moreover it is devoid of toxicity in mice and does not produce several of the side effects observed after administration of mycobacterial cells (lymphoid hyperplasia, increased susceptibility to endotoxins, adjuvant polyarthritis) [9].

One might wonder why only acid fast organisms such as mycobacteria or Nocardiae can be used in Freund's adjuvant since the structure of MDP is found in most bacterial cell walls and even in blue algae [10]. The reason for this is probably that in acid fast organisms the active MDP-like structure is more accessible to the cells of the immune system of the host and/or that in other bacterial species there may exist conflicting immunostimulating agents. Thus, for example, in gram-negative organisms, the peptidoglycan is covered with lipopolysaccharides (LPS) which are potent adjuvants of the humoral antibodies and may therefore commit the immune response differently.

The discovery of simple, well-defined synthetic molecules, such as MDP, has opened a new era in immunology; one of the first, obvious, developments was a study of the relation between structure and biological activity [11–13]. The principal results of such studies are the following:

(1) an *N*-acyl-muramyl structure is essential for full activity; shortening the lactyl side chain of MDP gives "nor-MDP", which is less adjuvant; opening of the pyranose ring by reduction with $NaBH_4$ gives an inactive "muramicitoldipeptide".

(2) in the peptide moiety L-Ala can be replaced by L-Ser, but not by Gly, without decreasing adjuvant

Abbreviations: MDP: muramyl-dipeptide, i.e. *N*-acetyl-muramyl-L-alanyl-D-isoglutamine; MDPA: muramyl-dipeptide acid, i.e. *N*-acetyl-muramyl-L-alanyl-D-glutamic acid; FCA: Freund's complete adjuvant; FIA Freund's incomplete adjuvant; LPS: lipopolysaccharide.

activity. Replacement of L-Ala by D-Ala gives a compound which is an anti-adjuvant [11] and immunosuppressor [12].

(3) D-Glu-α amide in MDP can not be replaced by D-Asp-α amide, nor by nor-leucine, nor by γ-amino-butyric acid without total loss of activity.

(4) The two carboxyl functions of D-Glu can be substituted in various manners (as amides, or as esters, or even as mono-methyl-amide), but the dimethyl-amide is inactive. The γ-carboxyl-group of D-Glu can be linked to L-Lys, or L-Lys-D-Ala, as in natural peptidoglycans, but replacement of the α-amide by Gly gives an inactive molecule. This last observation fits in very nicely with the fact that the peptidoglycan of *Micrococcus lysodeiktikus*, which contains just such a moiety, has no adjuvant activity [14].

A change in sterochemistry: i.e. replacement of D-Glu by L-Glu leads here again to an inactive, or even immunosuppressive, compound [12].

An unexpected development was the discovery that, contrary to other mycobacterial fractions and even to the large molecular weight WSA (Water soluble adjuvant) described earlier [15, 16], MDP is active in an aqueous medium; the use of the so objectionable paraffin oil in Freund's adjuvant is no more necessary. Moreover antigen and adjuvant can be injected by different routes and MDP was found to be active even after oral administration.

A further interesting finding was that MDP and some of its analogs stimulate non-specific resistance of mice against infections by virulent bacteria, such as *Klebsiella pneumoniae* [17]. This opens new possibilities of application of such compounds even against antibiotic resistent strains [18].

Although MDP can elicit strong biological responses, *in vivo* studies using ^{14}C labelled MDP have shown that it is eliminated very rapidly. Thus less than 1 hr after an intravenous injection, more than 90 per cent of the material is recovered unchanged in the urine [Parant, Petit and Yapo, unpublished results].

The mechanism of action of MDP has been studied in various systems, usually involving the humoral antibody response, the synthetic adjuvant being administered in saline [19–21]. The participation of *T* cells in enhancing the antibody response by MDP has been established [22] and more recently, this cell population has been identified as being helper *T* carrier-specific lymphocytes [23].

In vitro experiments have shown that MDP could enhance the immune response to sheep red blood cells and also induce a polyclonal activation, increasing the non-specific antibody response [19]. Moreover, MDP acts as a mitogen when incubated with spleen cells [24]; there is a good correlation between the stimulation *in vitro* and the adjuvant activity *in vivo* of several synthetic analogs [25]. The influence of MDP and several analogous synthetic glycopeptides on the viability *in vitro* of mouse spleen cells and immune response to sheep erythrocytes was also recently reported [26]. Furthermore, MDP administered in saline activates macrophages, enabling them to inhibit the growth of tumor target cells [27]. Oppenheim and Mizel have observed that MDP induces in the supernatant of mouse peritoneal exsudate cells and human peripheral mononuclear cells a mitogenic activity likely to be similar to LAF (personal communication).

One of the less desirable properties of MDP and several of its adjuvant active analogs is their pyrogenicity in rabbits [19, 28, 29]. MDP incubated with rabbit or human granulocytes or monocytes induces the production of endogenous pyrogens [30]. Adjuvant activity and pyrogenicity seem, however, to be two independent properties, since some strongly adjuvant active analogs are ten times less pyrogenic than MDP [28].

THE FUTURE

The synthetic glycopeptides can be considered as valuable laboratory reagents for production of sera or as interesting biological probes for a variety of experimental studies.

Applications of MDP and its analogs may also be foreseen as adjuvants for vaccines; preliminary experiments have already shown that MDP can increase the efficiency of *viral vaccines*, such as influenza subunit vaccine [31, 32].

The general tendency being now in favor of the use of purified vaccines, there will be a need for adjuvants such as MDP since the purified antigens are usually less immunogenic. In many cases enhancement of the immune response should also have the merit of reducing the amount of purified antigens which are usually difficult to produce in large quantities.

Adjuvants have been recommended in several fields of *parasitic diseases* and particularly in the case of a *Plasmodium* vaccine which has a high priority since Malaria represents one of the most serious health problems [33]. Killed vaccines with FCA have indeed been shown to extend the length of immunity and to protect monkeys very effectively against a *P. knowlesi* challenge.

Adjuvants could also be added to immunogens required as anti-fertility vaccine. This approach is being explored using female baboons immunized with a synthetic triacontapeptide corresponding to the sequence of a specific region of human βHCG. Such a modified peptide coupled to a carrier and administered in FCA has already been shown to induce circulating antibodies which can control fertility [34].

Although FCA has been proposed for human use [35], there is a general consensus that less toxic adjuvants must be found for clinical application. Indeed utilization of FCA other than in experimental procedures has been restricted by the toxic reactivity of mycobacteria and the use of the non-metabolizable oil component.

In all such studies substitution of FCA by synthetic molecules active in saline would clearly represent a very considerable advantage.

Potential utilization of these synthetic glycopeptides also exists in the following fields:

(1) *Stimulation of non-specific resistance* against infections: it is becoming more and more obvious that there is a need to restore the immune status in several cases of chemotherapy. Thus it is generally recognized that intercurrent infections with often fatal outcome constitute one of the greatest hazards for cancer patients.

(2) *Immunosuppression*: the properties of the D-D stereoisomer of MDP have still to be examined in various situations and might possibly lead to useful applications.

(3) *Antitumor agents*: in recent years, thanks to the pioneering work of Mathé and others several clinical investigators have been very active in this field [36], using either whole microorganisms or complex bacterial fractions. Current attempts to use chemically well-defined substances are justified and timely as they may lead to less toxic preparations with the required therapeutic effects. The use of synthetic molecules in cancer immunotherapy should be considered either for the stimulation of non-specific immunity or for increasing the effects of immunization by tumour antigens.

Recently Yamamura *et al.* [37] have shown that allogenic cell-mediated cytotoxicity was enhanced by MDP *in vitro* but not *in vivo* whereas a mycoloyl derivative of MDP was active *in vivo*. Administered *in vivo* to C57Bl/6J mice, 6-0-mycoloyl MDP was found to be as effective as BCG cell walls for the generation of cell-mediated cytotoxic effector cells against P815-X_2 mastocytoma. However, it was less active as adjuvant than BCG cell walls or MDP in enhancing circulating antibodies to a *T*-independent antigen (DNP-Ficoll) and on the generation of helper function of carrier-primed *T* cells. It was also inactive as a mitogen on murine spleen cells. Although MDP induces delayed hypersensitivity *in vivo* only when administered in FIA, preliminary observations show that some of its analogs may be capable of enhancing cell-mediated immunity *in vivo* if administered in an aqueous medium.

(4) *Various chemical conjugations* of synthetic adjuvants may also be a promising field for future immunological manipulations. Such molecules may be less easily excreted and perhaps less pyrogenic and more active. For instance, it was recently observed that following cross-linking of a β-D-ρ-aminophenyl glycoside of MDP with glutaraldehyde several biological activities were enhanced [38]. It has also been possible to conjugate MDP on carriers and to render it under certain conditions immunogenic (Reichert *et al.*, unpublished results).

It should also be possible to conjugate adjuvant glycopeptides directly to synthetic antigens such as certain modified peptides which are now available.

To summarize, the experimental evidence already accumulated in a short period of time with immunoregulating synthetic molecules augurs favorably for their potential biological and therapeutic value.*

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*MDP is now produced by Laboratoires Choay, Paris, and commercialized as "MDP-Pasteur" by Institut Pasteur Production, 28, rue du Dr. Roux, 75015 Paris, France.

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