Pure & Appl. Chem., Vol. 59, No. 3, pp. 449–454, 1987.
Printed in Great Britain.

Muramyl-peptides

Pierre Lefrancier[1] and Edgar Lederer[2]

[1] Institut Choay, 10 rue Morel, F-92120 Montrouge
[2] Laboratoire de Biochimie, CNRS, F-91190 Gif/Yvette

Abstract - Muramylpeptides are constituents of bacterial cell wall peptidoglycans. The simplest synthetic, biologically active analogue is N-acetyl-muramyl-L-alanyl-D-isoglutamine (MDP). Several hundred derivatives of MDP have been synthesized for the study of their immunomodulating, somnogenic, pyrogenic and other activities. Structure-activity relationships will be discussed as well as synergistic effects with other immunomodulators and the perspective of combined chemo- and immunotherapy.

N-acetyl-muramyl-L-alanyl-D-isoglutamine (MDP, for muramyl-dipeptide) is the smallest immuno-adjuvant active peptidoglycan derivative capable of replacing whole mycobacteria in Freund's complete adjuvant. Its glycodipeptide structure is an excellent object for chemical transformations, in an endeavour to obtain improved (and hopefully patentable !) molecules. The main biological activities of MDP and its derivatives, extensively reported in several reviews (1-8), are :

- adjuvant activity, i.e. stimulation of the production of antibodies when injected with an antigen in saline, induction of delayed type hypersensitivity against an antigen, when injected simultaneously in FIA ;
- stimulation of non-specific resistance against bacterial, viral and parasite infections and against tumors ;
- somnogenic activity, i.e. increase of the duration of slow wave sleep.

MDP also induces other, mostly undesirable, pharmacological responses, such as : transient leucopenia, sensitization to endotoxin, induction of arthritis, of granulomas, of uveitis, contraction of the ileum, bone resorption and pyrogenicity.

Here, we define some structure-activity relationships concerning immunomodulation. Due to restriction of space, we can only mention recent significant work.

MODIFICATIONS OF THE MURAMYL-MOIETY

Adjuvant activity

a. Increase of antibody production with compounds administered in saline

The anomeric hydroxyl in C-1 can be eliminated or substituted by an α- or β- methyl glycoside without loss of activity.

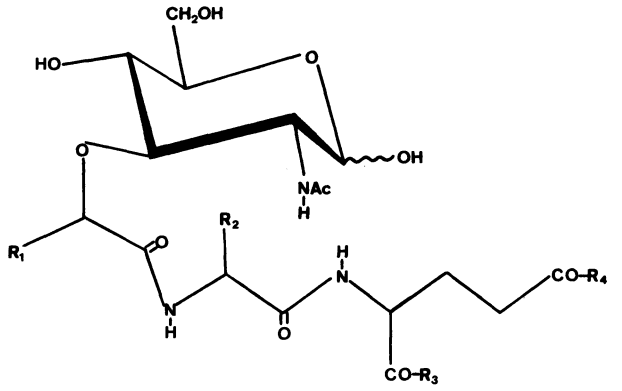
Modifications of the D-lactyl residue in C-3 give two interesting compounds : Nor-MDP and 3'n-propyl-MDP (scheme 1) (9), both having reduced toxicity : they are good candidates as adjuvants for vaccines. Nor-MDP in particular has been used in the adjuvant 65 with a malaria antigen (10) and emulsified in squalene Arlacel in a WHO sponsored antifertility vaccine (11-12).

The primary hydroxyl in C-6 can be replaced by an amino group or by an acylamido moiety without loss of activity.

b. Induction of delayed hypersensitivity with compounds administered in FIA

The cyclic osidic structure is essential for activity : only D-gluco-, D-manno- and D-galacto-MDP derivatives are active. However, some "desmuramyl-peptides", i.e. acyl-D-glu-meso-DAP-peptides, are also strong immunomodulators (see previous reviews).

Scheme 1



	R_1	R_2	R_3	R_4
MDP	CH_3	CH_3	NH_2	OH
Nor MDP	H	CH_3	NH_2	OH
3n-propyl MDP	nC_4H_8	CH_3	NH_2	OH
MDP [Thr][1]	CH_3	$CH(CH_3)OH$	NH_2	OH
MDP [αAbu]	CH_3	$(CH_2)_2H$	NH_2	OH
Murabutide	CH_3	CH_3	OnC_4H_9	NH_2
Muramethide	CH_3	CH_3	OCH_3	NH_2

Hydrosoluble, immunoactive MDP derivatives

At C-1, the anomeric hydroxyl can be replaced by a thiol or substituted by an α- or β-benzyl-glycoside. Lipophilic 1-O-acyl-MDP and 1-S-acyl-MDP derivatives are fully active.

At C-2, the acetamido group can be replaced by an hydroxyl, amino, methylamino or N-methylacetamido group. The introduction of a lipophilic acylamido group potentiates the activity.

At C-3, modifications of the lactyl moiety give the previously mentioned compounds, nor-MDP and 3n'-propyl-MDP (scheme 1).

At C-6, the hydroxyl can be replaced by a thiol or a non acylated amino function. In contrast to lipophilic 6-S-acyl-MDP derivatives, lipophilic 6-O-acyl-MDP derivatives are potent compounds.

Stimulation of non-specific resistance

At C-2, the acetamido group is necessary, but can be replaced by an other acylamido group.

Nor-MDP (scheme 1), administered in oil emulsion or in liposomes, augments non specific resistance to fungal, bacterial, or viral infection and activates macrophages to a tumoricidal state (13-18).

Lipophilic 6-O-acyl-MDP derivatives

The lipophilic 6-O-acyl-MDP derivatives are less good adjuvants for humoral response than MDP, but induce cellular specific response and increase more strongly non specific resistance, especially when encapsulated in liposomes.

The pharmacological profile depends on the structure of the hydrocarbon chain, which can be linear (L), α-branched (B), or α-branched β-hydroxylated (BH). L-18-MDP (6-O-stearoyl-MDP) is a potent anti-infectious compound, whereas B-30-MDP (6-O-[2-tetradecylhexadecanoyl]-MDP) is mainly an efficient adjuvant for the specific immune response (5). BH-48 (6-O-[3-hydroxy-2-docosylhexadecanoyl]-MDP) is an antitumor agent like various 6-O-mycoloyl-MDP-derivatives.

Quinonyl-MDP-66 (6-O-[10 (5,6-dimethoxy-3-methyl-1,4-benzoquinone-2yl)-decanoyl]-MDP-[Val]^1-OCH_3) increases humoral and cellular specific immune response and stimulates antitumor activity (19). Phospholipid residues have also been coupled to C-6 of MDP.

Disaccharide-dipeptides

Bacterial peptidoglycans contain a repeating disaccharide unit, N-acetyl-glucosaminyl β-(1->4)-N-acyl-muramic acid. Several types of disaccharide-dipeptides, and more complex muramyl-glycopeptides have been synthesized, sometimes improving the activity (see previous reviews).

MODIFICATIONS OF THE L-ALANYL RESIDUE

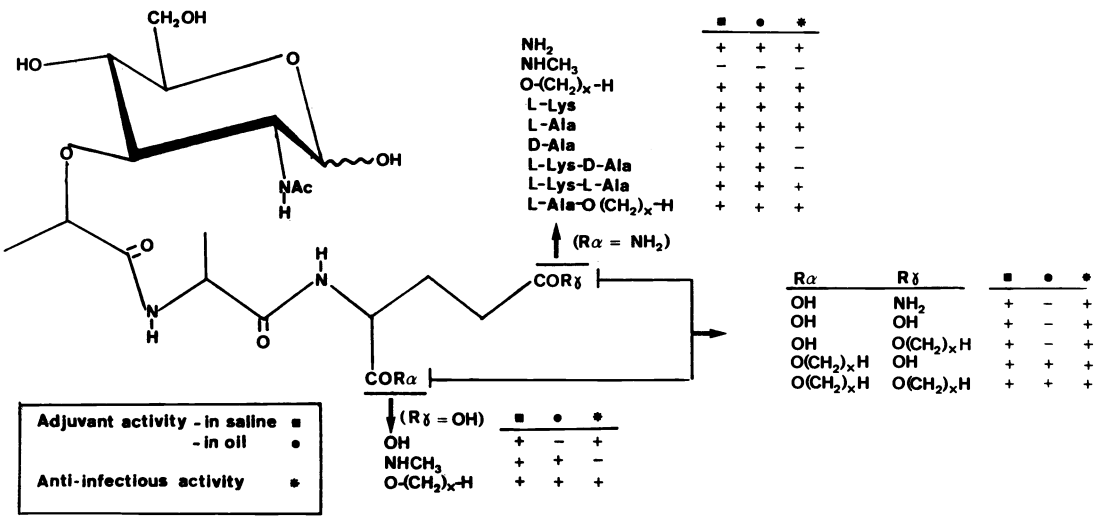
Replacement of L-alanine by D-alanine leads to an inactive compound. In some particular situations, however, MDP [D-Ala]^1 was found active. MDP analogues with an L-amino acid (or glycine) replacing the L-alanyl residue, administered in water or in FIA are all more or less adjuvant active. Among these, only MDP [α-Abu]^1 (scheme 1) is anti-bacterial, when injected in saline and activates alveolar macrophages when encapsulated in liposomes (19-21). Its 6-O-stearoyl derivative encapsulated in liposomes eliminates pulmonary metastases (22) and acts synergistically with the antimonial drug glucantime against visceral leishmaniasis and with amphotericin against murine candidiasis (23-25).

MDP [Thr]^1 (scheme 1) when injected in a pluronic polyol formulation increases strongly humoral and cellular immune responses to various antigens. Its low toxicity and relative inactivity for stimulating non specific resistance make it a good candidate as adjuvant for vaccines (26).

MODIFICATIONS OF THE D-GLUTAMIC ACID RESIDUE

The following structural elements are essential for biological activity of MDP derivatives : (1) The D-configuration. (2) Two methylene groups between two carboxyl groups. (3) These can be free or substituted and are essential for stimulating non-specific resistance. Recently, several D-Norleucine derivatives (MDP [D-Nle]^2-OR ; OR = alkyl ester) have been shown to be good adjuvants, however they do not stimulate non-specific resistance (P. Lefrancier et al., unpublished). (4) In scheme 2, various substitutions of the two carboxyl groups are reported. Most of them are compatible with adjuvant activity, in contrast with the non specific immune response. The latter strongly depends on the length of the alkyl ester chain.

Scheme 2



Modifications of D-Glu functionalities

Muramethide and Murabutide (scheme 1) are good candidates for therapeutic purposes. Murabutide is as effective as MDP for adjuvant activity and has also non-specific immunostimulant activity, it is non pyrogenic and devoid of other untoward biological properties (27). It is now in clinical experimentation as adjuvant of vaccines (28).

The conjugation of the γ-carboxyl of the D-glu residue with a lipophilic group, either directly or via an amino acid, has led to several interesting derivatives.

MDP-Lys-(L-18) (N$^{\alpha}$MDP-N$^{\varepsilon}$stearoyl-L-Lys) is strongly active for stimulating non-specific resistance against bacteria, or viruses in normal or immunodepressed hosts (29-34).

MTP-glyc-myc (MDP-L-Ala-glycerolmycolate) is a good adjuvant of humoral and cellular immune responses, when administered in FIA, in liposomes or in saline suspension and strongly stimulates non-specific resistance against bacterial infections.

MDP-GDP (1-MDP-2,3-dipalmitoyl-sn-glycerol) (35), MTP-Chol (MDP-L-Ala-cholesterylester) (36), when encapsulated in liposomes have been shown to activate macrophages to a cytostatic or cytotoxic state against tumor cells.

MTP-PE (MDP-L-Ala [1',2'-dipalmitoyl-sn-glycerol-3'-hydroxyphosphoryl-oxyethanolamine) has been studied extensively. When administered in liposomes, it can eradicate pulmonary metastases after repeated injections (37-38). It also makes human monocytes tumoricidal (39). MTP-PE has prophylactic activity against viral infections, even after intranasal application (40).

MDP prolongs slow-wave sleep by intracerebroventricular injection into rabbits (41). A shift of the amido group from the α-position of the D-glutamyl residue to the γ-position leads to inactive compounds. Thus, Murabutide is not active as sleep factor. It is suggested that specific mammalian amide synthesizing or hydrolysing enzymes may exist in the body that control the somnogenic activity of muramyl-peptides.

Synergism of MDP with other immunoactive compounds

MDP and its derivatives have been shown to act synergistically with several other immunomodulators (for references, see 42). Thus, liposome encapsulated MDP potentiates the activity of macrophage activating factor and of recombinant mouse and human inferferon-γ for activation of macrophages. MDP also acts synergistically with endotoxin. A suboptimal concentration of LPS is necessary for *in vitro* cytotoxic activation of rat alveolar macrophages by MDP. MDP also acts synergistically, in emulsion with TDM (trehalose dimycolate) for antitumor activity (43,44) and for protection of mice against *M. tuberculosis* and influenza virus infection (45).

Combined chemo- and immunotherapy

Several papers report the synergistic effect of MDP, 6-O-stearoyl MDP, MTP-PE, etc... with antibiotics (adriamycin, amphotericin, chloramphenicol, cephaloridin, etc...) (for references, see 42, 46, 47). An impressive anti-parasitic effect has been obtained against visceral leishmaniasis in mice and hamsters by the combined use of glucantime (an antimonial drug) and a lipophilic MDP derivative encapsulated in liposomes (24).

PERSPECTIVES

We have seen that slight structural variations of MDP can lead to molecules with improved properties ; also, new ways of more efficient and inocuous administration are developed. Thus, undesirable side effect can be excluded, or at least minimized. It is known, also, that activation of the monocyte-macrophage system proceeds by several steps and thus needs the combined use of several immunomodulators such as MDP + lymphokines, MDP + TDM, MDP + LPS, etc. This synergy opens new perspectives for veterinary and clinical applications. We have also mentioned the impressive results obtained by combining chemotherapy with immunotherapy.

Much work lies still ahead, but it is most probable that the coming decade will see the use of various immunomodulators in clinical and veterinary practice. Amongst these, muramyl peptides are certainly amongst the best candidates.

REFERENCES

1. DUKOR P., TARCSAY L. and BASHANG G., Annu. Rep. Med. Chem., **14**, 146 (1979).
2. PARANT M., Springer Semin. Immunopathol., **2**, 101 (1979).
3. LEFRANCIER P. and LEDERER E., Prog. Chem. Org. Nat. Prod., **40**, 1 (1981).
4. ADAM A., PETIT J.F., LEFRANCIER P. and LEDERER E., Mol. Cell. Biochem., **41**, 27 (1981).
5. KOTANI S. et al., in Bacteria and Cancer (Jeljaszewicz J., Pulverer G., Roszkowski N. Eds), 1982, p. 67, Academic Press.
6. LECLERC C. and CHEDID L. in Lymphokines, Vol. **7**, Pick E. Ed., 1982, p. 1, Academic Press, N.Y.
7. ADAM A. and LEDERER E., Medicinal Res. Rev., **4**, 111 (1984).
8. ADAM A., Synthetic Adjuvants. Modern concepts in Immunology, Vol. **1**, 239 pp. (1985). Wiley and sons, N.-Y.
9. BYARS N.E., Infect. Immun., **44**, 344 (1984).
10. MITCHELL G.H., RICHARDS W.H.G., VOLER A., DIETRICH F.M. and DUKOR P., Bull. World Health Organisation, **57**, 189 (1979).
11. STEVENS V.C., CINADER B., POWELL J.E., LEE A.C. and KOH S.W., Am. J. Reproduct. Immun., **1**, 315 (1981).
12. NASH H.A., CHANG C.C. and TSONG Y.Y., J. Reproduct. Immun., **7**, 151 (1985).
13. MOROZUMI P.A., BRUMMER E. and STEVENS D.A., Mycopathologia, **81**, 35 (1983).
14. FRASER-SMITH E.B. and MATTHEWS T.R., Infect. Immun., **34**, 676 (1981).
15. FRASER-SMITH E.B., WATERS R.V. and MATTHEWS T.R., Infect. Immun., **35**, 105 (1982).
16. ACEVEDO H.F., RAIKOW R.B., ACEVEDO H.O., DELGADO T.F. and PARDO M., Antimicrobial Agents and Chemother., **28**, 589 (1985).
17. SONE S., LOPEZ-BERESTEIN G. and FIDLER I.J., J. National Cancer Institute, **74**, 583 (1985).
18. FIDLER I.J., SONE S., FOGLER W.E., SMITH D., BRAUN D.G., TARCSAY L., GISLER R.H. and SCHROIT A.J., J. Biol. Resp. Mod., **1**, 43 (1982).
19. KOBAYASHI S., FUKUDA T., YUKIMESA H., FUJINO M., AZUMA I. and YAMAMURA Y., Bull. Chem. Soc. Jpn, **57**, 3182 (1984).
20. FRASER-SMITH E.B., WATERS R.V. and MATTHEWS T.R., Infect. Immun., **35**, 105 (1982).
21. JACOBS R.F., WILSON C.B., SMITH A.L. and HAAS J.E., Am. Rev. Resp. Dis., **128**, 862 (1983).
22. FRASER-SMITH E.B., EPSTEIN D.A., LARSEN M.A. and MATTHEWS T.R., Infect. Immun., **39**, 172 (1983).
23. LOPEZ-BERESTEIN G., MILAS L., HUNTER N., MEHTA K., HERSH E.M., KURAHARA C.G., VAN DER PAS V.M. and EPPSTEIN D.A., Clin. and Exp. Metastasis, **2**, 127 (1984).
24. ADINOLFI L.E., BONVENTRE P.F., VAN DER PAS M.V. and EPPSTEIN D.A., Infect. Immun., **48**, 409 (1985).
25. MEHTA R.T., LOPEZ-BERESTEIN G., HOPFER R.L., MEHTA K., WHITE R.A. and JULIANO R.L., Antimicrobial Agents and Chemother., **28**, 511 (1985).
26. ALLISON A.C., BYARS N.E. and WATERS R.V. in Advances in Carriers and Adjuvants for Veterinary Biologics (Nervig R.M. et al. Eds), 1986, p. 91, The IOWA University Press.
27. CHEDID L., PARANT M., AUDIBERT F., RIVEAU G., PARANT F., LEDERER E., CHOAY J. and LEFRANCIER P., Infect. Immun., **35**, 417 (1982).
28. TELZAK E., WOLFF S., DINARELLO C.A., CONLON T., CHOAY J., MORIN A., BAHR G. and CHEDID L., J. Infec. Dis., **153**, 628 (1986).
29. KOTANI S., TAKADA H., TSUJIMOTO M., OGAWA T., MORI Y., SHIBA T., KUSUMOTO S., INAGE M. and KASAI N., Bact. Endotoxin, 111 (1984).
30. ENDO N., OKUDA T., OSADA Y. and ZEN-YOJI H., Infect. Immun., **42**, 618 (1983).
31. ONOZUKA K., SAITO-TAKI T. and NAKANO M., Microbiol. Immunol., **28**, 1211 (1984).

32. OTANI T., KATAMI K., UNE T., OSADA Y. and OGAWA H., Microbiol. Immunol., 28, 1077 (1984).
33. IKEDA S., NEGISHI T. and NISHIMURA C., Antiviral Res., 5, 207 (1985).
34. ISHIRA C., HAMADA N., YAMAMOTO K., IIDA J., AZUMA I. and YAMAMURA Y., Vaccine, 3, 370 (1985).
35. PHILLIPS N.C., MORAS M.L., CHEDID L., LEFRANCIER P. and BERNARD J.M., Cancer Res., 45, 128 (1985).
36. PHILLIPS N.C., MORAS M.L., CHEDID L., PETIT J.F., TENU J.P., LEDERER E., BERNARD J.M. and LEFRANCIER P., J. Biol. Resp. Modif., 4, 464 (1985).
37. FIDLER I.J., FOGLER W.E., TARCSAY L., SCHUMANN G., BRAUN D.G. and SCHROIT A.J. in Advances in Immunopharmacology 2 (Eds. J.W. Hadden et al.), 1983, p. 235, Pergamon Press.
38. KEY M.E., TALMADGE J.E., FOGLER W.E., BUCANA C., FIDLER I.J., J. Natl. Cancer Inst., 69, 1189 (1982).
39. KLEINERMANN E., ERICKSON K.L., SCHROIT A.J., FOGLER W.E. and FIDLER I.J., Cancer Res., 43, 2010 (1983).
40. BROWNBILL A.F., BRAUN D.G., DUKOR P. and SCHUMANN G., Cancer Immunol. Immunother., 20, 11 (1985).
41. KRUEGER J.M., PAPPENHEIMER J.R. and KARNOVSKY M.L., Proc. Natl. Acad. Sci. USA, 79, 6102 (1982).
42. LEDERER E., in Present Status of non toxic concepts in Cancer, Proc. Int. Symp. Nonnweiler-Trier, 1986, Karger, Basle, in press.
43. YARKONI E., LEDERER E. and RAPP H.J., Infect. Immun. 32, 273 (1981).
44. RIBI E., CANTRELL J., FELDNER T., MYERS K., PETERSON J., Microbiology, 1986, in press.
45. MASIHI K.N., BREHMER W., AZUMA I., LANGE W. and MULLER F., Infect. Immun., 43, 233 (1986).
46. PARANT M., in the Reticuloendothelial System, 1986, Plenum Press N.Y., in press.
47. PARANT M. and CHEDID L., in Antimicrobial Agents and Immunity, 1986, Plenum Press N.Y., in press.