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Perspective

Synthetic Immunostimulants Derived from the Bacterial Cell Wall

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In recent years an increasing number of natural and synthetic compounds have been shown to have interesting immunomodulating activities. Amongst the *natural* compounds are $\beta(1\rightarrow3)$-glucans,[1] the tetrapeptide tuftsin,[2] thymic peptides,[3] the protease inhibitor bestatine,[4] proteins such as the interferons,[5] liposoluble vitamins (the ubiquinones,[6] retinoic acid and derivatives[7]), the polyene antibiotic amphotericine,[8] and last, but not least, bacterial glycolipids and peptidoglycan derivatives, which we shall discuss in detail.

In a special category of *synthetic immunostimulants* derived from natural compounds one finds synthetic polynucleotides,[9] lysolecithin analogues,[10] and dehydrodipeptides.[11]

Some synthetic compounds having no apparent structural relation with natural products should be mentioned here: the phenylimidothiazole, levamisole and its analogues,[12] the cyanaziridines imexon and azimexon,[13] the fluorenone derivative tilorone,[14] the diphenylamine derivative CCA,[15] a pyran copolymer,[16] and the aliphatic dialkyldiamine CP 20961.[17] Some of these (e.g., tilorone, the pyran copolymer, and the polynucleotides) are known to be inducers of interferon. Other new synthetic compounds will certainly come in the near future.

The impressive variety of compounds mentioned above is the reflection of the still more impressive complexity of the immune system and the large number of different types of immune cells and of their interactions. Any compound inhibiting or stimulating one of these may have a profound effect on the overall result, which is measured as immunostimulation or immunodepression, or more generally speaking as immunomodulation. For an excellent recent summary on "immunostimulation", see Dukor et al.[18]

Here we shall focus our attention on two categories of compounds derived from the bacterial cell wall:[19a] (a) synthetic peptidoglycan derivatives, such as MDP[20a] (muramyldipeptide), and (b) trehalose esters (cord factor) and their lower, synthetic analogues.

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(20) (a) Abbreviations used are: FCA, Freund's complete adjuvant; FIA, Freund's incomplete adjuvant; LPS, lipopolysaccharide; MDP, muramyldipeptide (*N*-acetylmuramyl-L-alanyl-D-isoglutamine); MPP, muramylpentapeptide (MurNAc-L-Ala-D-isoGln-*meso*-DAP-D-Ala-D-Ala); MurNAc, *N*-acetylmuramic acid; Myc, mycolate; P_3, trehalose dimycolate (cord factor); TDM, trehalose dimycolate (cord factor). (b) Freund's complete adjuvant (FCA, mycobacterial cells in a water in oil emulsion containing the antigen in the water phase) is widely used for producing high titers of antibodies. Freund's incomplete adjuvant (FIA) does not contain the mycobacterial cells.

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Table I. Examples of Dissociation between Various Biologic Activities of MDP and Derivatives[a]

compd	adjuvant effect: W/O DH[b]	adjuvant effect: saline Ab[c]	anti-infectious effect[d]	mitogenic effect[e]	pyrogenic effect[f]	LAL test[g]
AcMur-L-Ala-D-Glu-NH_2 (MDP)	+	+	+	+	++	+
AcMur-L-Ala-D-Glu(OMe)-OMe	+	+	+	+	–[b]	+
AcMur-L-Ala-D-Glu	–	+	+	+	–	–
AcMur-L-Ala-D-Glu(NH_2)-OMe	+	+	–	+	–[b]	+
AcMur-L-Ala-D-Glu(NH_2)	–	+	+	+	–	++
AcMur-L-Ala-D-Glu(L-Lys-D-Ala)-NH_2	+	+	–	+	++	–
p-aminophenyl-AcMur-L-Ala-D-Glu-NH_2	+	–	–	–	–	–
(*p*-aminophenyl-AcMur-L-Ala-D-Glu-NH_2)$_n$	+	±	++	++	+	++

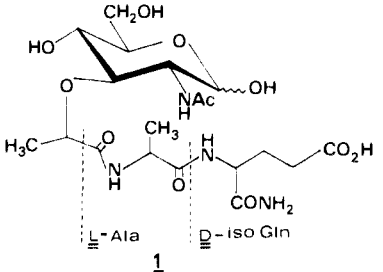
[a] See ref 19c. [b] Delayed hypersensitivity in guinea pig after administration in a water in oil emulsion. [c] Antibody titer in mice after injection with antigen in saline. [d] Protective activity in mice infected with *Klebsiella pneumoniae*. [e] [^{3}H]-Thymidine uptake in mouse spleen cell cultures. [f] Pyrogenicity in the rabbit: (–) nonpyrogenic at 1 mg/kg; (+) pyrogenic at 1 mg/kg; (++) pyrogenic at 100 μg/kg. [g] *Limulus* amoebocyte lysate test: (–) negative at 100 μg/mL; (+) positive at 100 μg/mL; (++) positive at 10 μg/mL. [h] Nonpyrogenic at 3 mg/kg.

Some of these compounds may be candidates for important antibacterial, anticancer, and antiparasitic applications. The future will tell whether they shall emerge as widely used drugs in veterinary and clinical medicine or whether they are only interesting reagents for experimental studies.

We shall discuss the structure–activity aspects of these substances rather than the detailed immunological events they influence or the methods and test systems used. The latter have been summarized in recent reviews.[19b,c]

Muramyldipeptide (MDP) and Derivatives

Muramic acid, the 3-*O*-D-lactyl ether of D-glucosamine, is a typical bacterial cell-wall constituent.[19a] In 1974 it was shown that a simple dipeptide derivative of muramic acid, *N*-acetylmuramyl-L-alanyl-D-isoglutamine (1; MDP), is the



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minimal adjuvant-active structure capable of replacing whole mycobacterial cells in complete Freund's adjuvant[20b] for increasing levels of humoral antibodies against a given antigen and for inducing delayed hypersensitivity.[21] Chedid and his colleagues have found that it is also active in saline (without oil) and even when given by the oral route[22] and that MDP also stimulates nonspecific resistance to bacterial infections[23] (even to antibiotic-resistant strains[24a]) and also in neonatal mice where endotoxin (LPS) is inactive.[24b] MDP is also mitogenic for splenocytes.[25-27]

The first enthusiastic period following the discovery of the strong immunoadjuvant activity of such a simple synthetic glycodipeptide as MDP was soon followed by disappointment because of some quite unexpected untoward effects, such as pyrogenicity,[28-31] a transitory leukopenia,[28] thrombocytolysis,[31] and possible sensitization to endotoxin (in guinea pigs).[32]

Thus, it became all the more interesting to modify the structure of MDP, hopefully to obtain compounds with fewer side effects.

Modification of the Structure of MDP

Several hundred analogues and derivatives of MDP have been prepared. For reviews, see ref 18 and 33. The following is a summary of some structure–activity relationships for adjuvant activity.

The Peptide Moiety. The first amino acid should have the L configuration; *N*-acetylmuramyl-D-alanyl-D-isoglutamine (MDP-D,D) is not only inactive, but under certain experimental conditions, it is antagonistic to MDP[34] and immunosuppressive.[35] L-Alanine can be replaced by a number of L-amino acids (or glycine); the L-serine and L-valine analogues seem to be more active than MDP itself.[18] The D-glutamic acid residue is essential; the D-aspartic analogue is inactive, as is the L-glutamic analogue.[28,34] The functionality of D-Glu is important. For example, the α-amido group is not essential for activity, but its *N*-methyl derivative is inactive; the α-methyl ester and the α,γ-dimethyl ester are fully active, whereas the *N*,*N*-dimethylamide is inactive. The various biological effects of MDP and its derivatives can be dissociated,

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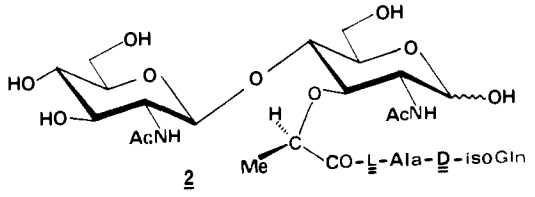
depending on chemical structure (see Table I). Some simple modifications lead to biologically active nonpyrogenic molecules, such as MurNAc-*N*-Me-L-Ala-D-isoGln or MurNAc-L-Ala-D-Gln-butyl ester.[42] The synthetic methods for a variety of modifications have been reported.[18,19,33,36-41]

The Carbohydrate Moiety. Several modifications of the MurNAc moiety have mostly led to inactive molecules,[34] but the manno isomer is as active as MDP.[39] nor-MDP (in which the methyl group of the muramyl side chain is replaced by H) is less active than MDP[34] but is also less toxic;[18] it is frequently mentioned in the patent literature.

Disaccharide Peptides. Several groups have recently described disaccharide peptides, some of which seem to be more active than MDP. Ovchinnikov et al.[43] have enzymatically prepared the disaccharide of the cell wall of *Lactobacilli* and coupled it to the dipeptide L-alanyl-D-isoglutamine; this disaccharide dipeptide (DS-DP) **2** is as



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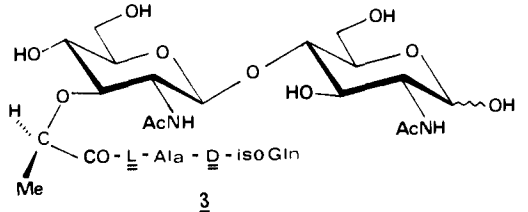

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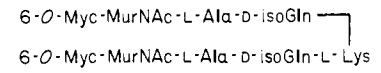
active as MDP against Sarcoma 180 and three to seven times more active than MDP in tests of adjuvancy.[44] This has been also prepared by total synthesis.[41a,45] Durette et al.[46] synthesized the isomeric *O*-(*N*-acetyl-β-muramyl-L-alanyl-D-isoglutamine)-(1→4)-*N*-acetyl-D-glucosamine (**3**), the repeating disaccharide dipeptide unit obtained by endo-*N*-acetylglucosaminidase lysis of bacterial cell walls.

It is quite possible that still larger peptidoglycan fragments, such as the tetra- to hexasaccharides of di- to pentapeptides, might have greater activity in certain tests (this is the case, for example, for arthritogenicity[47]) but, as they are much more difficult to synthesize, it seems preferable to use larger doses of MDP to obtain, hopefully, the same effect.

Lipophilic Derivatives. Of great interest are lipophilic derivatives of MDP, since Japanese authors[47-49] showed that 6-*O*-mycolyl-MDP, 6-*O*-nocardomycolyl-MDP, and 6-*O*-corynomycolyl-MDP have strong antitumor activity and are only weakly pyrogenic.

The French groups has described another category of lipophilic MDP derivatives, bearing the lipid moiety at the end of the peptide chain.[51] The most lipophilic compound prepared was MDP-L-Ala-glycerol mycolate (MurNAc-L-Ala-D-isoGln-L-Ala-$OCH_2CH(OH)CH_2O$-Myc). It strongly stimulates nonspecific resistance against bacterial infections.[52]

Azuma et al.[53] have prepared analogous compounds such as MurNAc-L-Ala-D-isoGln-L-Lys(Myc), MurNAc-L-Ala-D-isoGln-$NHCH_2CH_2O$-Myc, and even dimycolyl derivatives of MDP. The latter includes compounds such as 6-*O*-Myc-MurNAc-L-Ala-D-isoGln-L-Lys(Myc) and



The introduction of the second mycolyl group does not seem to have any pharmacological advantage. Various MDP esters of acids derived from ubiquinones have also been prepared and some of these have strong antitumor activity.[54]

Peptidolipids. The MurNAc moiety had, until recently, been considered to be essential for biological activity. It was all the more surprising, when it was found, that the "demuramyl" derivative of MDP-L-Ala-glycerol mycolate, i.e., L-Ala-D-isoGln-L-Ala-$OCH_2CH(OH)CH_2O$-Myc ("triglymyc"), was just as active as the MurNAc derivative in stimulating nonspecific antibacterial resistance; it is, however, entirely inactive as an adjuvant.[52] This shows that the MurNAc moiety, in this series at least, plays an essential role in stimulating humoral antibody production.

The structural requirements for biological activity in the triglymyc series have also been studied. The L-Ala-D-Glu moiety seems optimal, as in MDP itself, thus showing that there exists a specific receptor (on the macrophage?) for this structure. The replacement of the dipeptide by L-Ala-L-isoGln, β-Ala-D-isoGln, L-Ala-D-isoAsn, or L-Ala-D-Gln leads to much less active products. The importance of the length of the lipid chain has also been examined: the mycolate gives optimum activity; a "corynomycolate" (with a synthetic isomer of the C_{32} corynomycolic acid) is only weakly active.[52]

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(49) (a) T. Shiba, S. Okada, S. Kusumoto, I. Azuma, and Y. Yamamura, *Bull. Chem. Soc. Jpn.*, **51**, 3307 (1978); (b) M. Uemiya, K. Sugimura, T. Kusama, I. Saiki, M. Yamawaki, I. Azuma, and Y. Yamamura, *Infect. Immun.*, **24**, 83 (1979).
(50) Mycolic acids are bacterial α-branched β-hydroxy acids; mycobacterial mycolic acids have 60 to 90 carbon atoms (see 7), nocardomycolic acids have 40 to 60 carbon atoms, and corynomycolic acids have 30 to 36 carbon atoms.
(51) P. Lefrancier, M. Petitou, M. Level, M. Derrien, J. Choay, and E. Lederer, *Int. J. Pept. Protein Res.*, **14**, 437 (1979).
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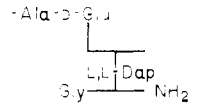
Migliore-Samour et al.[55] have recently prepared an adjuvant-active peptidolipid by N-laurylation of a cell-wall tetrapeptide (4) isolated from *Streptomyces*. A synthetic



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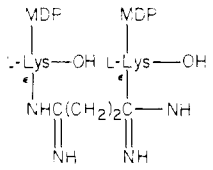
N^2-[*N*-(*N*-lauroyl-L-alanyl)-γ-D-glutamyl]-N^6-glycyl-DD,-LL-diamino-2,6-pimelamic acid stimulates delayed hypersensitivity to ovalbumin (in FIA) and protects mice against infection by *Listeria monocytogenes*. This most interesting new type of compound (a mixture of diastereoisomers) increases circulating antibodies despite the absence of the MurNAc moiety.

Even the fatty acyl chain seems not to be essential, since quite recently Mašek et al.[56] have reported that the nonapeptide L-Ala-D-isoGln-L-Lys(Ac)-D-Ala-$(Gly)_5$-OMe strongly stimulates production of delayed hypersensitivity when injected in FIA to mice with ovalbumin as antigen.

Oligomers, Polymers, and Carriers. "Small is beautiful"; thus, MDP is not antigenic.[21] It does not react with antipeptidoglycan antibodies[57] and does not sensitize to tuberculin,[21] but it is very rapidly excreted in the urine: more than 50% after 30 min, more than 90% after 2 h in mice.[58] Moreover, MDP is not active in some systems where larger peptidoglycan fragments are active. For instance, WSA (water-soluble adjuvant), a lysozyme digestion product of mycobacterial cell walls (M_r 20 000) containing an arabinogalactan linked to a peptidoglycan,[59,60] and even MPP (muramylpentapeptide = MurNAc-L-Ala-D-isoGln-*meso*-DAP-D-Ala-D-Ala) inhibit the secretion of plasminogen activator by macrophages, whereas MDP is inactive.[61]

It was thus tempting to prepare oligomers and polymers of MDP. Some cross-linked dimers and trimers, such as **5** and **6**, have been synthesized[33] and a β-D-*p*-aminophenyl



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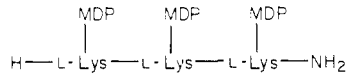

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glycoside of MDP has been cross-linked with glutaraldehyde.[62a] Table I shows that the glycoside has lost most of the activities of MDP, whereas, surprisingly, some of these were recovered and even increased after cross-linking.

Table II. Potential Risks of Therapy with Immunostimulants[a]

(1) sensitization to immunostimulant itself
(2) sensitization to common extrinsic antigens
(3) sensitization to autoantigens
(4) increased formation of blocking antibodies
(5) increased formation of suppressor cells
(6) toxicity for myelolymphoid cells
(7) neoplastic transformation of myelolymphoid cells

[a] See ref 78.

An obvious, promising possibility for increasing the size and efficiency of MDP is to couple it with an appropriate carrier[62b] or with a synthetic polypeptide such as described by Sela and colleagues.[63] Chedid et al.[64] have recently combined MDP with multi[(poly(DL-alanyl)-poly(L-lysine)] and found, that, indeed, this "macromolecularization" potentiates the antiinfectious activity of MDP 100-fold, but also its pyrogenicity. However, surprisingly the "inactive" (or even antiadjuvant or immunosuppressive) stereoisomer of MDP (MDP-D,D)[34,35] coupled to that carrier gives a molecule which is nonpyrogenic and is not an adjuvant but which increases very strongly nonspecific, antibacterial resistance.[64] Moreover, it does not sensitize nor induce an immune response to the glycopeptide moiety.

A further development in this field must be, as predicted by Sela,[63a] the combination of synthetic antigens with synthetic adjuvants, via synthetic carriers. Indeed, the immunogenicity of a synthetic MS-2 coliphage coat protein fragment linked to multi[poly(DL-alanyl)-poly(lysine)] was greatly increased by its attachment to a disaccharide tetrapeptide isolated from the peptidoglycan of *B. megaterium*.[63b] A recent patent of Ciba-Geigy covers antigen-MDP and antigen-carrier-MDP compounds.[65]

Applications of MDP. MDP has been shown to improve the efficiency of influenza vaccines in mice.[66,67]

Several groups have studied immunization of monkeys against malaria infection. Protective immunity was produced in *Aotus* monkeys[68] and in macaques[69] using MDP or nor-MDP in FIA and *Plasmodium falciparum* or *P. knowlesi* merozoites. With 6-*O*-stearoyl-MDP in liposomes a full protection of *Aotus* monkeys was reported using mature segmenters of *P. falciparum* as the antigen.[70]

Partial protection of mice against *Trypanosoma cruzi* has been obtained by implantation of MDP in an Alzet minipump.[71] Clinical applications of MDP have still not been reported.

Mechanism of Action. The mechanism of action of MDP and its derivatives has been studied in detail.[72-78]

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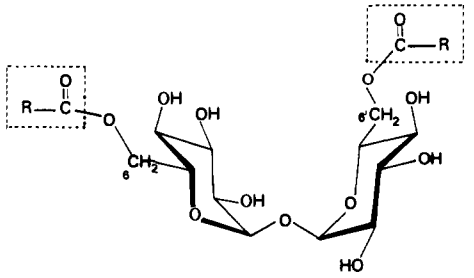

Trehalose 6,6'-diesters

	Total carbon number	Structure of $R-\overset{O}{\overset{\|}{C}}-OH$
		Mycobacterial mycolic acids, for instance:
7	C_{180} (cord factor=TDM=P_3)	$CH_3(CH_2)_{19}CH(CH_2)CH(CH_2)_{14}CH(CH_2)_{17}CH(OH)CH(C_{24}H_{49})CO_2H$ $C_{84}H_{164}O_3$
8	C_{100}	synthetic racemic $CH_3(CH_2)_{20}CH(OH)CH(C_{20}H_{41})CO_2H$ $C_{44}H_{88}O_3$
9	C_{76}	synthetic racemic $CH_3(CH_2)_{14}CH(OH)CH(C_{14}H_{29})CO_2H$ $C_{32}H_{64}O_3$

The primary action seems to be on the macrophage,[73,76] with liberation of monokines, leading to activation of B cells and T cells. In the presence of MDP, macrophages produce in vitro increased amounts of collagenase, prostaglandin, and a fibroblast proliferation factor.[73b]

Immunosuppression. It is known that most adjuvants either enhance or suppress the immune response, depending on the timing in relation to the antigenic stimulation and the dosage of both antigen and immunomodulator.[79] MDP can also be immunosuppressive[80,81] and could hopefully find useful applications against autoimmune diseases. Kishimoto et al.[82] have recently obtained a selective suppression of IgE response by administration of antigen-conjugated muramylpeptides, such as MDP-Lys(εDNP), mycolyl-MDP-Lys-(εDNP), or ovalbumin coupled to MDP-Lys or to mycolyl-MDP-Lys. The IgE suppression is explained by the induction of allergen-specific suppressor cells. This work opens a promising avenue for the treatment of allergies.

Trehalose 6,6'-Diesters

Mycobacteria, *Corynebacteria*, *Nocardiae*, and some other genera produce 6,6'-diesters of trehalose with various α-branched β-hydroxy acids, called mycolic acids.[50,83] The mycobacterial trehalose diesters are also called *cord factor*, P_3, or TDM (for trehalose dimycolate).[84]

The immunostimulant properties of mycobacterial cord factor **7** were first described by Bekierkunst et al.,[85] who stressed the role of cord factor in tumor regression, when added to deproteinized and delipidated BCG cell walls. Ribi et al.,[86] studying the antituberculosis and antitumor

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properties of mycobacterial cell walls, isolated an active lipid "P_3" which was later shown to be identical with cord factor.[84b,87]

Lower, synthetic 6,6′-trehalose diesters (such as C_{100} 8 or C_{76} 9)[94-96] have qualitatively the same biological activity (adjuvant,[88] antibacterial,[88,89] antitumor,[90,91] antiparasitic[89,93]) as mycobacterial cord factor. In some cases,[89,93] even the dipalmitate (C_{44}) was found to be active. Moreover, the synthetic analogues are nontoxic and much less granulomagenic[90] and are thus good candidates for veterinary and clinical applications.

Goren and Jiang[97] have prepared "pseudo-cord factor" analogues of TDM; these are derivatives of α-D-glucopyranuronosyl (1–1)-α-D-glucopyranuronoside (the diacid obtained by oxidation of trehalose) combined with high-molecular-weight amines or alcohols; some of these have tumor regression activity. Here again it is shown that "neither the toxicity nor the granulomagenicity of cord factor are required for expression of antitumor activity". Yarkoni et al.[98] came to the same conclusion.

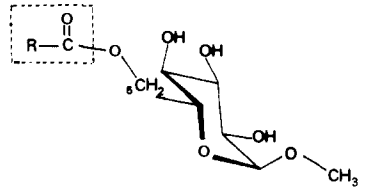
Antibacterial Activity. TDM and synthetic trehalose diesters in a bayol–water emulsion were shown to be active against infection of mice by *Salmonella typhi* and *S. typhimurium*[85d] and against *Klebsiella pneumoniae* and *Listeria monocytogenes*, in a peanut oil in water emulsion.[88] TDM was even active in saline, without oil.[99] Synthetic C_{76} 9 had the same activity as natural TDM, whereas esters with C_{22} acids, as well as trehalose dipalmitate and a dioleate of sucrose, were inactive.[88]

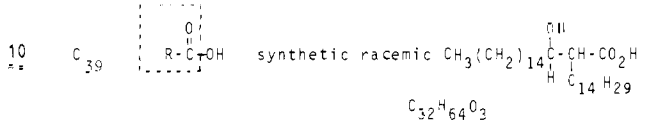
Antitumor Activity. TDM 7 and C_{76} 9 produce regression of a syngeneic murine fibrosarcoma after intralesional injection; all cured animals are also resistent to a second inoculation of the same tumor.[90] The antitumor activity depends on the oil concentration of the emulsion.

Pimm et al.[91a] have described the suppression of growth of an ascitic rat hepatoma by intraperitoneal administration of TDM in peanut oil; trehalose dibehenate was also tumor suppressive, but less so than TDM (C_{76} being inactive, C_{100} only weakly active). More recently,[91b] the use of squalene in water emulsion gave more satisfactory results, TDM being the most effective, whereas the synthetic analogues had an activity more or less proportional to the length of the acyl chains. With a severe challenge, TDM was, however, definitely more active than C_{100} (the highest synthetic analogue tested). This finding should stimulate further efforts to prepare "heavier" synthetic trehalose diesters having the full biological activity of TDM.

Antiparasitic Activity. The effect of TDM in *Babesia microti* infected mice was reported by Clark.[92] The maximum effect was obtained when mice were infected 7 weeks after receiving the TDM injection! Olds et al.[93] have studied *Schistosoma mansoni* infection in mice; TDM and even trehalose dipalmitate were protective. Natural immunity after infection protects 32% of mice, but an injection of 200 μg of trehalose dipalmitate raised this to 73%, indicating that different mechanisms of immunity are cumulative.

Mechanism. The immunostimulant mechanism of cord factor and related glycolipids has been discussed earlier.[84] Due to their amphipathic properties they interact with membranes; they are also chemotactic for phagocytic cells.[100] Considering that the balance of lipophilic to hydrophilic groups might be essential, we have compared the antitumor activity of synthetic C_{76} 9 with that of methyl-6-*O*-(2-tetradecyl-3-hydroxyoctadecanoyl) α-*O*-D-glucopyranoside (10), which is (more or less) a "half C_{76}". This C_{39} glycolipid is, however, less active than C_{76}.[101]





Thus, a disaccharide diester having appropriate acyl functions seems to be optimal. From the work of Kato and Asselineau[102] on an in vitro model (mitochondrial phosphorylation), it seems that the D-gluco configuration is the most active.

A report on the immunostimulant activity of maltose tetrapalmitate[103] has not yet been confirmed. *N*-Acylglucosamines with C_8 to C_{18} acyl chains have also been recommended as immunostimulants.[104]

Combined Immunostimulation

Bacterial endotoxins are well-known immunostimulants;[105] Ribi et al.[86c] were the first to realize the potential of combining a more or less detoxified endotoxin (ET) with

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P_3 (i.e., cord factor = TDM) to produce regression of the guinea pig line 10 hepatoma, after intralesional injection. Some synthetic analogues (for instance, 8) were also active.[86c,d] More recently they have replaced part of the activity of ET by MDP(Ser) (the serine analogue of MDP) and have obtained 100% regression with a purified ET preparation called B_4.[106] Yarkoni et al.[90] had shown that the synthetic C_{76} analogues (9) of TDM are just as active as TDM itself (with ET). Then they replaced ET by MDP and obtained 100% cures using 10% paraffin oil or, more recently, 2 to 10% squalane.[107]

At Orsay, the sequential immunostimulation of peritoneal macrophages was evaluated by (1) thymocyte mitogenic protein production in vitro and (2) cytostatic activity against a syngeneic mastocytoma P815.[108] TDM-stimulated macrophages are cytostatic in vitro against mastocytoma cells. This activity decreases during in vitro cultivation but can be maintained by MDP, MPP, or LPS.

Resident macrophages can be made cytostatic against the mastocytoma cells by sequential stimulation in vitro, first by TDM and then by MDP or MPP, thus showing a direct synergistic action of both types of immunostimulants on macrophages.[108]

Conclusions

Future research in this field will develop along several lines leading, hopefully, to applications in veterinary and human medicine: (a) In view of the rapid excretion of MDP, it will be necessary to prepare slow-release forms of MDP or its analogues, using, for instance, minicapsules, minipumps,[109] liposomes,[70,110] macromolecular carriers,[64] etc. (b) It should be possible to modulate the structure of MDP so as to produce specific molecules with high affinity to certain organs or target cells; the length of the peptide moiety, the substitutions of the glycosidic part, and, finally, the nature, position, and length of a lipid moiety can all be modified so as to improve the biological activities desired. (c) Combined immunostimulation is certainly a field with great promise; it will be necessary to find the best combinations and dosage of immunostimulants (such as trehalose diesters plus MDP) so as to produce efficient and long-lasting effects.

One important advantage of the use of these adjuvants in vaccines will be to use less antigen (these being very often expensive and difficult to prepare in large quantities). Especially the future viral vaccines will use synthetic vaccinating subunits which are much less immunogenic than the whole virus; they will need a strong adjuvant.

The nonspecific antibacterial activity of MDP (or its derivatives and combinations) will be particularly useful in cases of bacterial infections by antibiotic-resistant strains.

Experimental and clinical applications of cancer immunotherapy are now widely studied. It is hoped that, here too, the synthetic immunostimulants described in this review might be used, either in vaccines (with tumor antigens) or for stimulation of nonspecific resistance, especially for treatment of immunodepressed patients.

It is still doubtful whether the immunosuppressive properties of MDP and some of its derivatives will find useful applications (for instance, for the treatment of autoimmune diseases).

To end this review with a word of caution, let us quote Gisler et al.[78] enumerating the potential risks of therapy with immunostimulants (Table II).

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Communications to the Editor

Prevention of Ames Test Mutagenicity by Chemical Modification in a Series of Monoamine Oxidase Inhibitors

Sir:

Potential drugs sometimes surface, especially in medicinal chemistry and insecticidal research, which, while of acceptably low acute toxicity, must be abandoned when found to be carcinogenic on chronic administration to test animals. The possibility of a rational approach to removing carcinogenicity is therefore of practical, as well as of theoretical, interest. That this possibility exists was thought likely after consideration of the current understanding of the succession of events believed necessary for a great proportion of chemically induced carcinogenicity. For example, eq 1 shows the succession of events which the work of J. A. and E. C. Miller, among others,[1] shows

$$\mathrm{ArNH_2} \rightleftharpoons \mathrm{ArNHC(O)CH_3} + [\mathrm{O}]\ (\text{"microsomal enzymes"}) \rightarrow \underset{\mathbf{1}}{\mathrm{ArN(OH)C(O)CH_3}} \rightarrow \underset{\mathbf{2}}{\mathrm{ArN(OZ)C(O)CH_3}} \rightarrow \underset{\mathbf{3}}{\mathrm{Ar\overset{+}{N}C(O)CH_3}} \quad (1)$$

apparently to be required for an aryl amide, such as 2-acetaminofluorene [*N*-(2-fluorenyl)acetamide; 2-AAF], to cause cancer to experimental animals. A similar sequence is thought to apply to carcinogenic arylamines either directly or after in vivo acylation. An arylamine or aryl amide must first be oxidized to the arylhydroxyamine or arylhydroxamic acid 1. This oxidation is catalyzed by

(1) Miller, J. A. *Cancer Res.* **1970**, *30*, 559. Welsburger, J. H.; Yamamoto, R. S.; Williams, G. M.; Grantham, P. H.; Matsushima T.; Welsberger, E. K. *Ibid.* **1972**, *32*, 491.

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