Tetrahedron Vol. 45, No. 20, pp. 6331–6360, 1989
Printed in Great Britain.

0040–4020/89 $5.00+.00

TETRAHEDRON REPORT NUMBER 262

MURAMYLPEPTIDES AND LIPOPEPTIDES: STUDIES TOWARDS IMMUNOSTIMULANTS

G. BASCHANG
Pharma Research, CIBA-GEIGY AG, CH-4002 Basel

(*Received* 26 *April* 1988)

CONTENTS

1. INTRODUCTION

Knowledge of the possibility of reinforcing the bodily defences against infection extends far back into antiquity. In India and China, a form of subcutaneous administration of smallpox scabs was practised more than 2,000 years ago. The ancient Greeks knew that disease could confer immunity, as described by Thucydides in his report on the plague in Athens in 430 B.C. Hippocrates advanced the thesis that "the physis, the healing power of nature, acts in the body to generate fever, heating the noxious morbid matter, ripening it and driving it out in the crises". This formulation is all the more remarkable for the fact that in 1927 the Nobel prize for medicine was awarded to Wagner von Jauregg for his successful malarial fever therapy of tertiary syphilis. Salvarsan, otherwise a potent

therapeutic agent, cannot cross the blood–brain barrier and combat the spirochete in the brain whereas Hippocrate's physis, stimulated by malaria, can.

A large portion of this 'physis' would nowadays be equated with the immune system, including its connections to the central nervous system and to the psyche. Although the civilizations of antiquity evidently knew something about the curative power inherent in the body and its susceptibility to stimulation, it was left to modern science to identify the factors responsible. These are predominantly widely occurring components of micro-organisms, such as murein, lipopolysaccharides, lipoproteins and glycans. Substances of these classes are amongst the most active stimulants known, but their structures are complicated, and often they are very hard to isolate in a homogeneous and reproducible form. Moreover, they can also contain unrecognized structural elements which can provoke untoward effects. To surmount these difficulties, analysts and synthetic chemists will have to join forces with biologists and take up the clear challenge of identifying active structural elements and synthesizing them in pure, reproducible forms.

Abbreviations and acronyms are listed in the Appendix, p. 6360.

2. BEGINNINGS OF MODERN IMMUNOSTIMULANT THERAPY

In 1797, the English country doctor Edward Jenner succeeded, for the first time, in inoculating children against smallpox with cowpox lymph. The vaccinia virus (L. vacca, cow) gave the name to this form of therapy. Nowadays the same virus genetically combined with specific attributes of certain pathogens serves as a modern variant of a 'synthetic' vaccine.

The progress of modern medicinal chemistry in this field can be traced back to the work of the New York surgeon William Bradley Coley, who at the end of the 19th century achieved some success in the treatment of neoplasms by injecting killed bacteria (Coley's toxins). McDermott and Freund in 1942 discovered that killed tubercle bacilli, mixed with mineral oil and an emulsifying agent, yielded a potent adjuvant eliciting a complete immune response to otherwise ineffective antigens. Later on, Alexander demonstrated that mouse macrophages activated with killed tubercle bacilli (*Bacillus Calmette-Guérin*, BCG) could kill tumour cells *in vitro*. Later Benacerraf, Clarke and Old showed that mice infected with BCG acquired an enhanced resistance to bacteria and viral infections and to certain tumours.

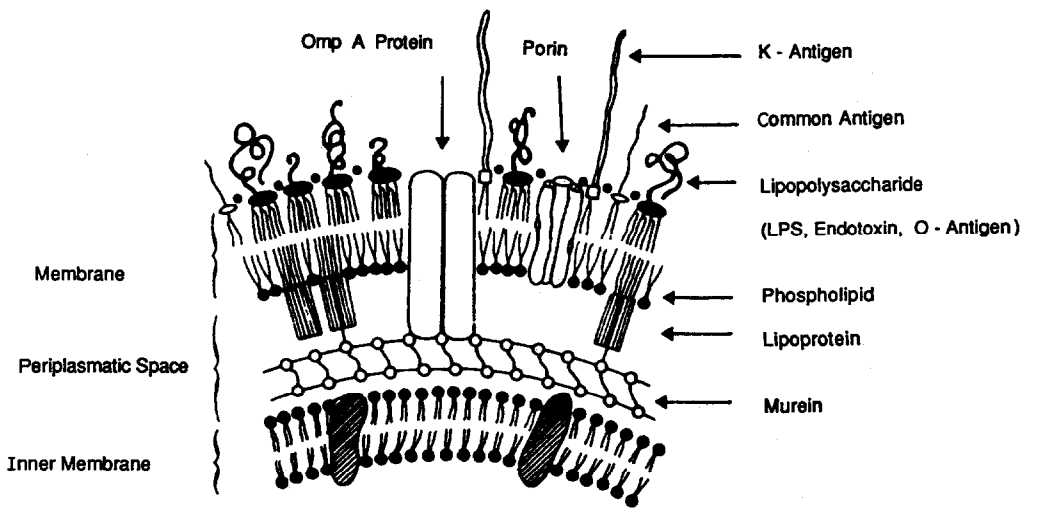


Fig. 1. Schematic representation of the cell wall of Gram-negative bacteria (after E. Rietschel, Borstel, FRG).

Muramyl peptides

These findings encouraged a systematic search for active principles in Mycobacteria. In 1974, thanks to the work of E. Lederer and S. Kotani the identification of N-acetyl-muramyl-L-alanyl-D-isoglutamine (MDP) as the smallest active unit of the cell wall, which constitutes the most active fraction of Mycobacteria was achieved.[1,2] Since 1975, many research groups have been concerned with the synthesis of this highly active glycopeptide and its derivatives. A number of reviews have been published.[3-9] A recent review on immune regulatory agents has appeared in *Tetrahedron*.[180]

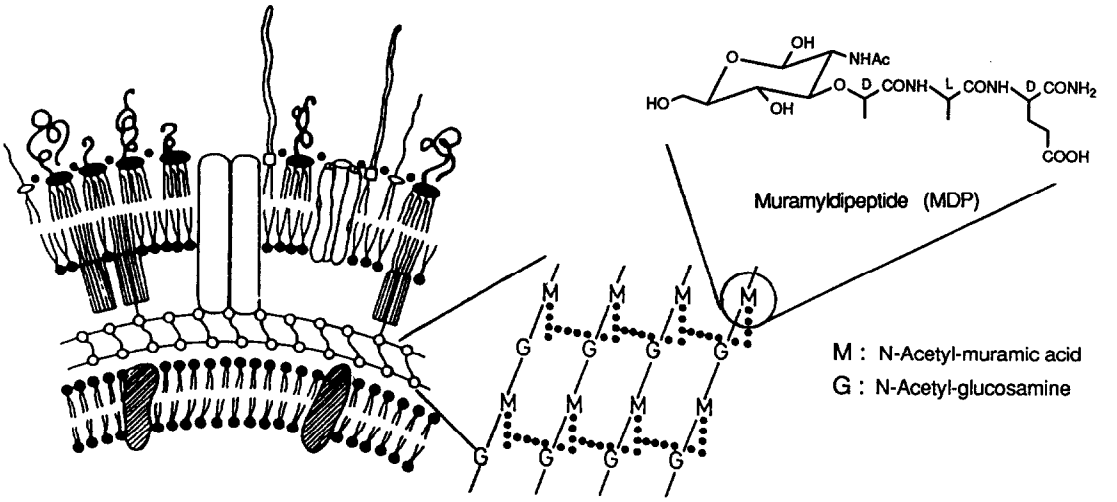


Fig. 2. Muramyldipeptide as a structural element of murein.

Lipopeptides

The muramyl peptides form a clearly circumscribed group of substances which are characterized by the general sequence N-acetyl-muramic acid-L-amino acid-α,γ-substituted D-glutamic acid. In contrast, the lipopeptides exhibit a rather confusing heterogeneity and include representatives of various origins, compositions and biological profiles. In this Report we want to distinguish three categories of lipopeptides:

1. Lipopeptides-I (LP-I) are derived from peptides of murein. They are generally associated with lipid residues in the form of prodrugs. Their amino acid composition is essential for their biological activity. D-glutamic acid is an essential element and the fatty acid residues cause a prolongation of the compound's lifetime in the body as well as its partition to lipophilic compartments. These compounds are discussed in a recent review.[9]

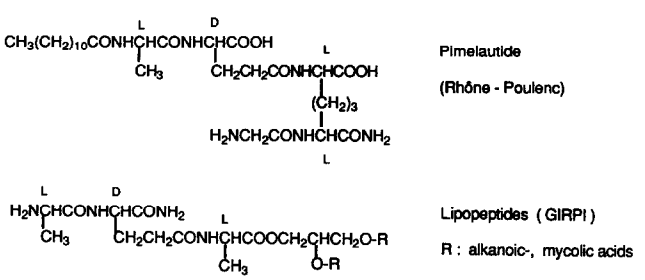


Fig. 3. Lipopeptides-I (LP-I) derived from murein.

2. Lipopeptides-II (LP-II) comprise peptides which are mostly substituted with fatty acids and possess antibiotic activities. These do not fall within the scope of this Report because they do not stimulate immune responses. Many of these peptides are derived from bacteria and for many of them a function in ion transport across membranes has been identified. They are specific for certain strains and they are not as ubiquitous as, for example, the lipophilic part of lipoprotein *E. coli* which may be common to most bacteria.[9a]

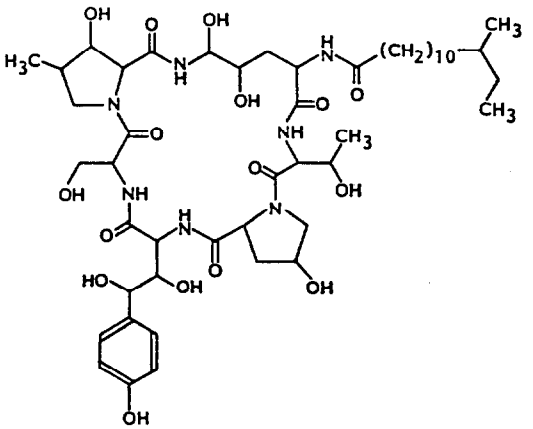
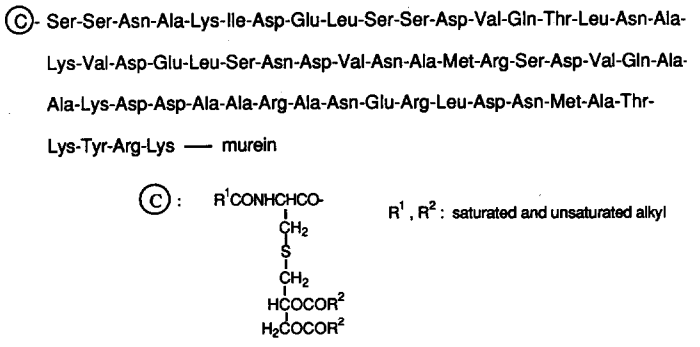


Fig. 4. Mulundocandin, a member of antifungal lipopeptides (LP-II).

3. Lipopeptides-III (LP-III), derived from lipoprotein elaborated by *E. coli*. Their biological profile is quite distinct from that of LP-I: their amino-acid composition is not of importance for their biological activity. Lipoprotein *E. coli* is one member of a widespread class of proteins which are anchored to bacterial membranes by a conserved structure based on L-cysteine.[9a] CIBA-GEIGY has been engaged in research into LP-III since 1977,[10] but the substances only became generally known as a result of the publications of Jung and Bessler.[11] *E. coli* lipoprotein is a component of the outer membrane of Gram-negative bacteria and has been largely elucidated by V. Braun and co-workers.[12] Its reported potent mitogenic action on B cells of the mouse[13] prompted CIBA-GEIGY to study its effect on antibody synthesis *in vivo*. The compound proved highly active and was considered worthy of further medicinal chemical investigation. A model of the lipoprotein was constructed.[14]



V. Braun, Biochim. Biophys. Acta 415, 335 (1975)

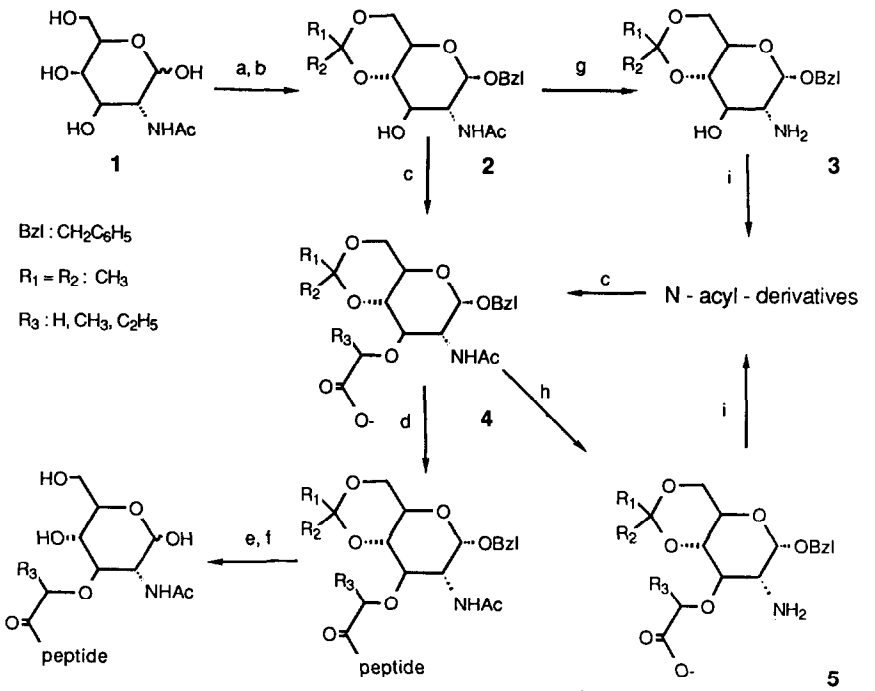
Fig. 5. Lipoprotein *E. coli*.

3. WORKING HYPOTHESES

It seems reasonable to assume that constant exposure to microbial aggression has taught the mammalian immune system to recognize specific components of micro-organisms as warning signals. This hypothesis is readily compatible with the fact that the most potent of the immunostimulants so far identified including lipid A, lipoprotein, muramyl peptides and glucans are specific for microbes and contain highly conserved structural elements. As exemplified by the muramyl peptides, the polymeric constitution does not appear to be an essential feature: one typical element of a redundant structure is sufficient. This is an important discovery because many natural immunostimulant substances are either not homogeneous or they cannot be obtained reproducibly. Moreover they are not susceptible to deliberate manipulation because of certain characteristics such as molecular weight, composition or pharmacokinetics. It is left to the chemist to prepare homogeneous and, if possible, therapeutically better products.

According to a second hypothesis advanced by H. Umezawa,[15] inhibitors of enzymes which are localized in the membranes of cells belonging to the immune system may exert either a stimulant or an inhibitory effect upon the system. Using an appropriate screening procedure, Umezawa quickly succeeded in identifying immunostimulant compounds which were capable of inhibiting enzymes as diverse as ATPase, phosphodiesterase, aminopeptidase and glycosidases. The underlying mechanisms have not yet been elucidated but this phenomenon is nevertheless in accord with the observation that traumatizing stimuli such as ultraviolet light, heat-stroke, or enzyme inhibition can lead to gene duplication, gene activation and other genetic consequences.

Finally it may be assumed that there is still a rational basis for therapy with synthetic products, even though more and more endogenous substances which act upon the immune system, for example the lymphokines (see p. 6342), are obtainable in pure form by biotechnological methods.



a: benzylalcohol, trimethylchlorosilane; b : 2.2-dimethoxy-propane, H^+; c : tosylate of L-lactic acid/ NaH d: EEDQ or DCC, HOBt or Woodwards reagent + peptide-ester; e: dilute acetic acid; f: H_2, Pd-C in acidic medium; g: KOH, EtOH, 80°; h: $N_2H_4.H_2O$, 110°; i: $(Boc)_2O$ or carbobenzoxychloride, Na_2CO_3

Fig. 6. Synthesis of N-acetyl-muramylpeptides from N-acetyl-glucosamine.

4. CHEMISTRY

4.1. *Synthesis of muramyl peptides*

A review on this subject has been published.[4] The synthesis generally follows a scheme propo by R. W. Jeanloz *et al.*,[16] which has been developed to its present status by modification of protect groups and improvement in methods of etherification.

Starting from N-acetyl-glucosamine **1** the first synthesis necessitated the separation of diastereomeric (*R,S*)-propionyl ethers. Racemization during step c which occurs with 2-(*S*)-chlc propionic acid or with 2(*S*)-halo-propionic acid esters, can be avoided by using the tosylate of L-la acid. A further improvement relates to the use of 2,2-dimethoxy-propane instead of benzaldehyd step b, to introduce a 4,6-isopropylidene protecting group (**2**). This can be removed with dilute a at room temperature and it confers a better solubility. Coupling step d with muramic acid derivati has to be done under non-racemizing conditions according to general peptide chemistry. coupling of peptides with nor-muramic acid derivatives (R_3 = H) does not need these precauti The removal of the α-glycosidic benzyl group by catalytic hydrogenation, step f, proceeds m slowly than that of the β-glycosidic benzyl group but it is easier to obtain in pure form. Alternati hydrogenation can be done under transfer conditions. A further variant has been developed Japanese authors.[25] They oxidize the α-benzyl-ether to a benzoylester with chromium trioxi pyridine complex. This ester is cleaved by mild alkali. An entry to various N-acyl-substitu muramic acid derivatives is possible via **3** or **5** which are obtained from the corresponding N-ace derivatives **2**, **4** with base. A different approach to such derivatives is shown in Fig. 8.

A short and efficient synthesis was devised by R. Gigg *et al.*[17] starting from N-benzoyl-g cosamine **6** and was extended to serve our purposes (Fig. 7).

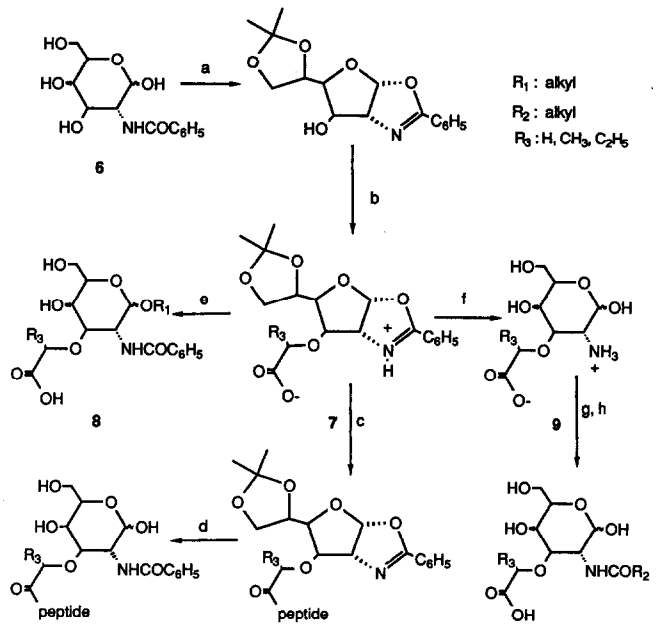


Fig. 7. Synthesis of N-benzoyl-muramylpeptides from N-benzoyl-glucosamine.

While the procedures in steps a, b, c, d, g, h are analogous to the corresponding procedures in Fig. 6, steps e and f need a comment. Intermediate **7** is suited to yield glycosides **8** under mild conditions (step e). In step f, after acid hydrolysis, which has to be done in 6 N hydrochloric acid, removal of HCl by evaporation would lead to lactam formation. This is avoided by neutralization at room temperature, evaporation and separation of the muramic acid derivative **7** from KCl by silylation and extraction into organic solvents.

A third variant, leading directly to derivatives with a free amino group, starts from D-mannose **10**,[18] Fig. 8.

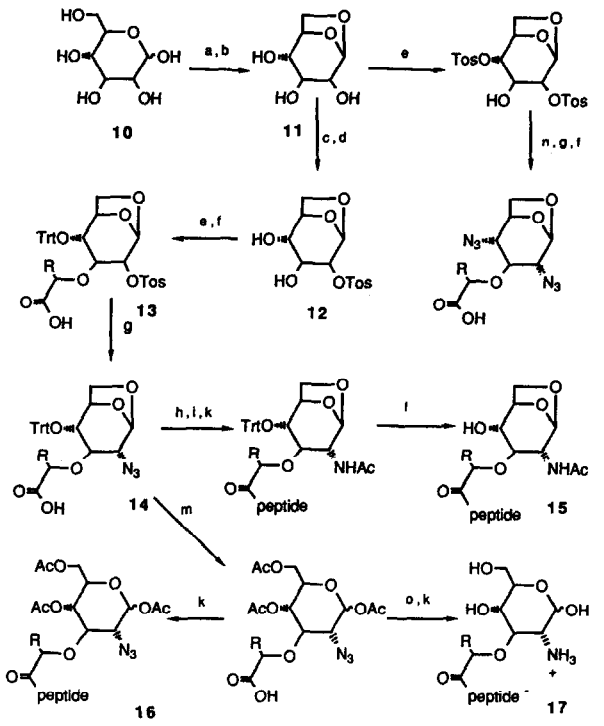


Fig. 8. Synthesis of muramylpeptides from D-mannose.

The scheme in Fig. 8 was developed to obtain an intermediate which would lead to 1,6-anhydro-muramylpeptides, glycosides of muramyl-peptides and derivatives with a free amino-group on the carbohydrate.

The 6-tosylate of mannose can be cyclized to **11** at pH 12–13 in ethanolic NaOH at $-10°C$. Reaction of **11** with Bu_2SnO and subsequent reaction with tosylchloride gives the 2-tosylate of 1,6-anhydro-mannose **12** selectively. Tritylation in position 4 leaves the less reactive hydroxyl-group in position 3 for attachment of the lactyl-ether giving **13**. Introduction of the azide group in position 2 gives **14** so the preparation of 1,6-anhydro-N-acetyl-muramylpeptides **15** is straightforward. Alternatively 2-azido-muramylpeptides **16** can be obtained, **14** also leads to glycosides with ROH/H^+

and last but not least to desacetyl-muramylpeptides **17**. As indicated in Fig. 8, (steps e, f, g, n) a 2,4-diazido-derivative of muramic acid **18** has also been obtained via 1,6-3,4-dianhydro-2-tosyl-galactopyranose.

Synthesis of labelled derivatives

For pharmacokinetic studies, various labels have been introduced into the MDP molecule. The French group synthesized lactyl-1-^{14}C-MDP,[19] while a Japanese group obtained U-^{14}C-ala-MDP.[20] The Merck group introduced tritium into muramic acid giving the 6-^{3}H-N-acetyl-muramyl dipeptide.[21] In CIBA-GEIGY, the acetyl group was labelled, yielding N-^{3}H-acetyl-muramyl dipeptide.[22]

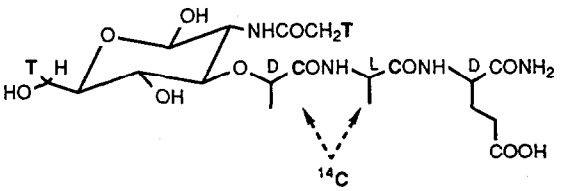
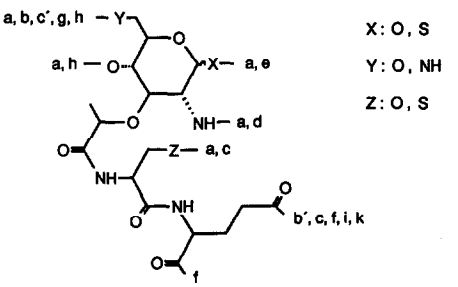


Fig. 9. Labelled muramyldipeptides.

4.2. *Prodrugs of muramyl peptides*

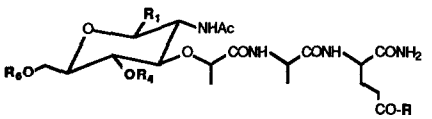
4.2.1. *Lipophilic prodrugs.* N-acetyl-muramyl dipeptide (MDP) is rapidly excreted in the urine (>90% in 2 h).[19] Lipophilic derivatives have therefore been described by several groups.[5–8,23–30,69,71–78,81,82,94,160] Only a general survey needs to be given here (Fig. 10). Special derivatives are listed in Table 1 and Fig. 11 and are mentioned in the section on 'Biology' (see pp. 6349, 6352).



a : alkanoyl, aroyl, trimethylsilyl;
b : cholesteryl-(3)-succinyl;
b': cholesteryl-(3)-succinyl-oxyethylamide;
c : dipalmitoylphosphatidyl-oxyethylamide;
c': dipalmitoylphosphatidyl-oxyethylamido-succinyl;
d : dipalmitoylphosphatidyl-oxyacetyl;
e : α,β-alkyl, -benzyl; β-amino-phenyl;
f : oxyalkyl, aminoalkyl;
g : alkylphosphoryl;
h : alkylidene (4.6);
i : ε-alkanoyl-L-lysyl;
k : dipalmitoylglyceryl-L-alanyl.

Fig. 10. Lipophilic prodrugs of muramylpeptides.

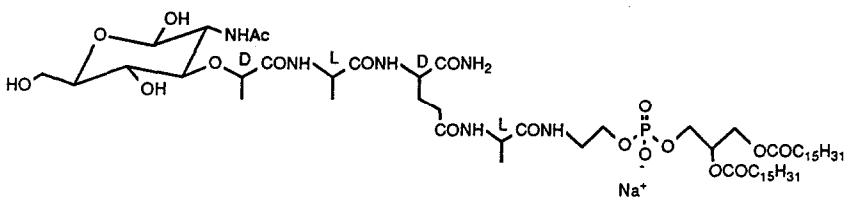
Table 1. Lipophilic prodrugs of N-acetyl-muramyldipeptide



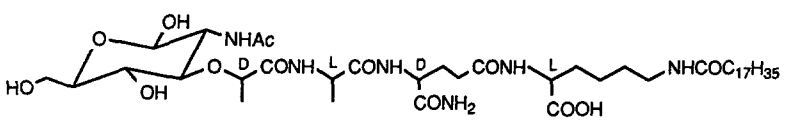
R^n		Profile*	Source
R^6: $OCOCH(C_{14}H_{29})_2$	R^1 = R: OH, R^4: H	B T	Dainippon
R^6: aminoacyl-COR'	R^1 = R: OH, R^4: H	A B	Takeda
$R^4 = R^6$: octanoyl	R^1 = R: OH	T V	Syntex
R^1: ß-S-alkanoyl	$R^4 = R^6$ = R: H	A	Gifu University
R: L-Lys-ε-$COC_{17}H_{35}$ (MDP-Lys-L18)	R^1: OH, $R^4 = R^6$: H	B T V	Daiichi
R: L-Ala-$OCH_2CHCH_2OCOC_{15}H_{31}$ $OCOC_{15}H_{31}$	R^1: OH, $R^4 = R^6$: H	B T V	GIRPI
R: L-Ala-cholesteryl-(3)	R^1: OH, $R^4 = R^6$: H	A T V	GIRPI, Ciba-Geigy
R: L-Ala-dipalmitoyl-kephalin (MTP-PE)	R^1: OH, $R^4 = R^6$: H	A B T V	Ciba-Geigy
R: L-Ala-$NHCH_2CHCH_2NH$-R" OH	R^1: OH, $R^4 = R^6$: H	T V	Ciba-Geigy

* A: adjuvant ; B: antibacterial ; T: antitumor; V: antiviral ; R": cyclooxygenase-inhibitors

MTP-PE is being developed at Ciba-Geigy and at Daiichi it is MDP-Lys (L18) (Fig. 11).



Muramyltripeptide-phosphatidylethanolamide (MTP-PE)



MDP-Lys (L18)

Fig. 11. Muramyltripeptide-phosphatidylethanolamine (MTP-PE) (Ciba-Geigy); MDP-Lys (L18) (Daiichi).

4.2.2. *Polymeric prodrugs.* As a vehicle for MDP, polyacrylamide[31] or mixed polymers from vinylpyrrolidone and L-alanine-vinyl ester[32] have been used. Nor-MDP is coupled through L-lysyl-L-lysine methyl ester to microspheres of poly-lactic acid having an average diameter of 1–8 μ.[32]

MDP and nor-MDP have also been bound to synthetic[3,34] and natural polypeptides.[35,36,36a] Synthetic vaccines with protein-(peptide)-antigens can be prepared by this technique[37,38] (Table 3).

Syntex has developed a slow-release form.[33]

4.2.3. *Liposomes.* A special type of delivery system has been produced by mixing lipophilic muramyl peptides with phospholipids which, upon agitation or ultrasonication in an aqueous medium, form multilamellar vesicles (MLV). A mixture of seven parts of lecithin to three parts of phosphatidyl serine[39,41] has proved particularly satisfactory. Both components are prepared synthetically in CIBA-GEIGY. Lipids containing mannose are also serviceable (see p. 6354).

4.3. *Lipopeptides LP-I*[9]

Lipopeptides LP-III. Before initiating a synthesis programme, we had to determine the absolute configurations of the chiral centres in the cysteine thio-ether. N-palmitoyl-(*R*)-cysteine reacted with 1-tosyl-2,3-isopropylidene-(*R*)-glycerol giving a thio-ether with the (*R,R*) configuration. (*R,S*)-glycidol and N-palmitoyl-(*R*)-cysteine gave a pair of diastereomers with the (*R,R*) and (*R,S*) configurations. Comparison of the ^{13}C-NMR spectra of these compounds with that of the N-acyl-S-(2,3-dihydroxypropyl)-cysteine obtained from the lipoprotein after hydrolysis with pronase and alkali showed the chiral carbon atom of the side-chain had the (*R*)-configuration. CD measurements demonstrated the (*R*)-configuration for the α-carbon atom with a positive Cotton effect at 226 nm.

The lipopeptides were synthesized as follows (Figs 12 and 13):

A synthesis starting from (*R*)-cysteine **19** leads to a series of N-acyl-cysteines **20**. Alkylation of the sulfhydryl group of **20** with 1,2-isopropylidene-(*R*)-glycerol-tosylate (steb b) gives N-acyl-S-[2(*R*),3-dihydroxypropyl]-(*R*)-cysteine **21**. With (*R,S*)-glycidol a mixture of N-acyl-S-[2(*R,S*),3-dihydroxypropyl]-(*R*)-cysteines, **21** and **22**, is obtained. Esterification of the side chain (step e) is done after conversion of the cysteine carboxyl-group to a benzhydrylester (step d). Mild acid hydrolysis gives **23**. Alternatively, step e is carried out after coupling of peptides lacking reactive functional groups, i.e. before step h. Step g needs special comment. The usual coupling procedure with dicyclohexylcarbodiimide and N-hydroxy-benzotriazole leads to almost complete racemization in this case. Originally this could only be detected by NMR and not until the development of a special HPLC-method was this problem solved. Use of N-hydroxy-norbornan-dicarboximide instead of N-hydroxy-benzotriazole gives a product **24** which contains less than 3% of the (*S,R*)-diastereomer.

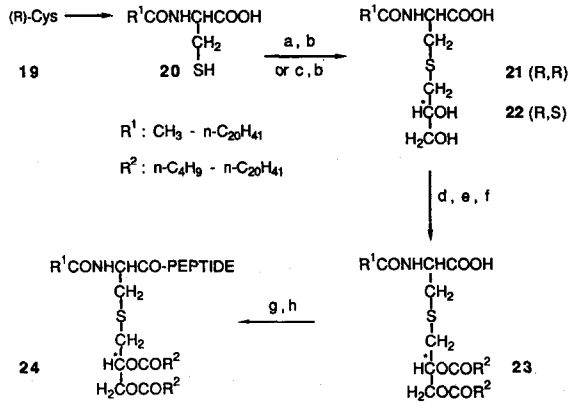
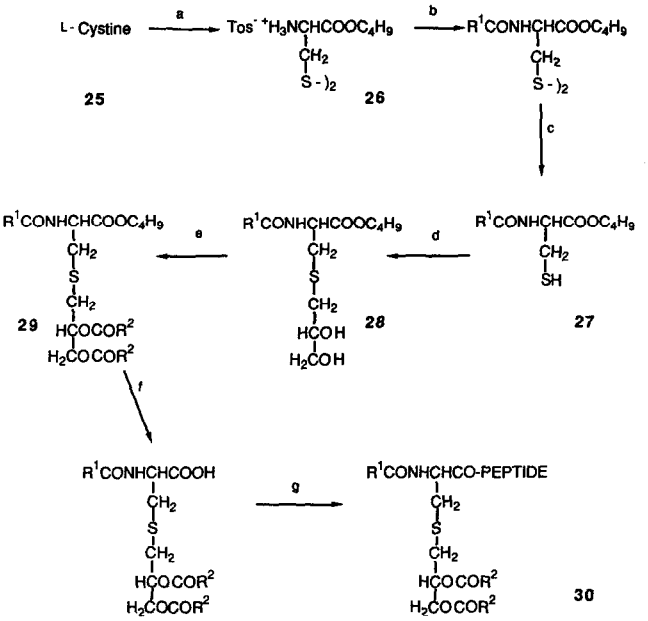


Fig. 12. Synthesis of lipopeptides-III from cysteine

A synthesis starting from (*R*)-cystine **25** gives the bis-*tert*-butylester which is isolated as the tosylate salt **26** (Fig. 13). Acylation at the amino-group (step b) and reduction with zinc and acid leads to the *tert*-butylester of N-acyl-cysteine **27**. This is alkylated at sulfur with 1-tosyl-(*R*)-glycerol. which reacts faster than 1,2-isopropylidene-glycerol-tosylate, to **28** and it is acylated to **29**. Further processing to lipopeptides **30** is straightforward, given the precautions in step g (Fig. 12).



a : $CH_3COOC_4H_9$, $HClO_4$; 50 %; *b* : R^1COCl, *N-ethyl-morpholine*, 67 %; *c* : Zn, citric acid, 96 %;
d : 1-tosyl-glycerol, DBU, DMF; 95 %; *e* : R^2COCl, pyridine, 4-dimethylamino-pyridine; 79 %;
f : CF_3COOH, 93 %; *g* : DCC, N-hydroxy-norboman-dicarboximide, peptide; 80 %.

Fig. 13. Synthesis of lipopeptides-III from cystine.

5. BIOLOGY

5.1. *Constitution of the immune system*

Tissues. The principal centres of the immune system are the thymus, spleen, bone-marrow, lymph nodes and lymphatic tissues of the mucosa such as Peyer's patches in the small intestine. In adults, these tissues amount in weight to about 6 kg. The skin possesses its own complete immune system.[40] For a recent survey on the compartmentalization of various defence systems see reference 40*a*.

Cells. A germinal centre which develops in the bone-marrow under the control of factors from stem cells (CSFs)[60] gives rise, after several differentiation stages, to various types of leucocytes. These include the B and T lymphocytes, which in the adult account for 1 kg of body-weight. B lymphocytes synthesize antibodies. T lymphocytes comprise subpopulations which fulfil helper (T_H), suppressor (T_S),[42] and killer (T_C) functions.[43,44] The killer population is additionally composed of natural killer (NK) cells and large granular lymphocytes (LGL).[45,46]

Among the granulocytes, the predominating neutrophils play an important part in ensuring a

rapid reaction against infections. The eosinophils and basophils perform special tasks such as defence against parasites. Of importance for defence against bacterial and viral infections and also against tumour metastases are the circulating monocytes and, in the tissues, the macrophages.[47] These cells can secrete more than 75 different products. They have more than 30 types of surface receptor and in the primary and secondary lysosomes contain more than 40 hydrolytic enzymes.

The functions of these cell types can be expediently assessed in a variety of test systems *in vivo* (Fig. 14). *In vitro* test systems using isolated cells, however, are of limited relevance owing to the many regulatory mechanisms which operate only in the living organism and so therefore will be left out of consideration.

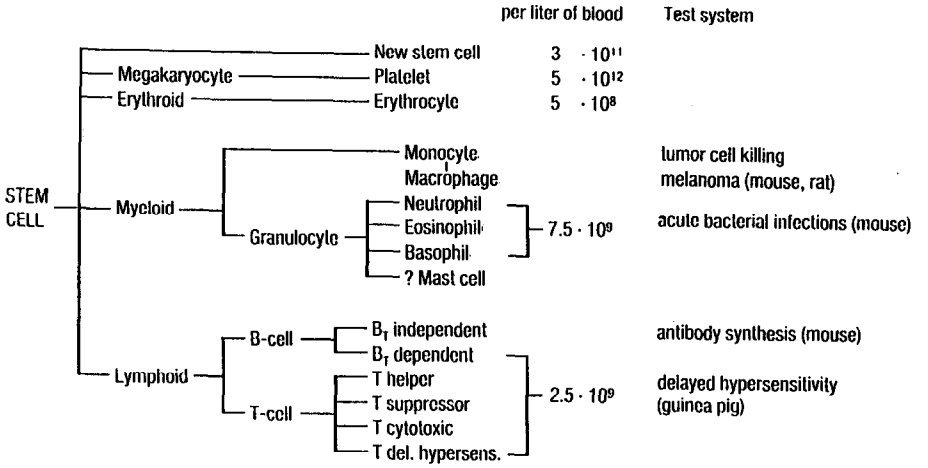


Fig. 14. Effectors of the immune system.

Factors. The various cells and cell systems communicate and are regulated by way of factors and receptors. Cell-to-cell contacts play an important role[48–54] and prominent factors include IL-1,[55] IL-2,[56] IL-3,[57] IL-4,[58] CSFs,[59,60] IFNγ[61] and suppression factors.[42] Many of these are now obtainable in pure form and are under pharmacological and clinical investigation.

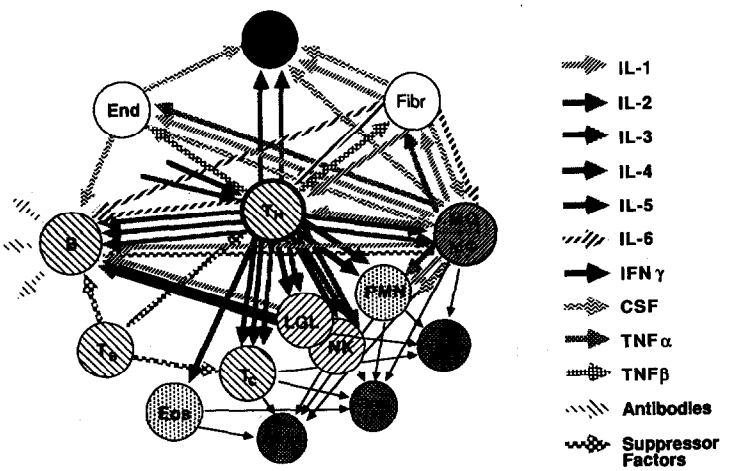


Fig. 15. Network of leucocytes and factors. B is used collectively for resting B-cells, blast cells and plasmocytes. CSF stands for G-CSF, M-CSF or GM-CSF. T_H stands for different populations of helper cells (e.g., $T_H1 \rightarrow$ IL-2+IFNγ, $T_H2 \rightarrow$ IL-4).

Besides the enormous complexity which is depicted the diagram also illustrates one line of defence against infection, composed of monocytes, macrophages, cytotoxic T cells, NK cells, neutrophils and eosinophils. The target cells are homed on by way of special cytophilic antibodies (antibody-dependent cell-mediated cytotoxicity, ADCC) (Fig. 16). Alternatively special receptors on T cells[62] mediate the contact. It is not yet known how NK cells, monocytes and macrophages distinguish their target cells from healthy cells but in the case of NK and LAK (IL-2-activated T_C cells), the ganglioside GM2 appears to play a part.[63] The modes of killing differ. Monocytes, macrophages and neutrophils mainly use cytotoxic peptides (poisons) and oxygen radicals (burns). NK cells and T_C cells use poisons and pore-forming proteins (plugs), and eosinophils predominantly use burns and plugs.[64–66] All these programmes are regulated and are evidently initiated specifically and in several phases. Bacterial components are the activators for monocytes and macrophages. The endogenous mediators (lymphokines, monokines, interleukins) are generally proteins which presumably act in concert. This can be inferred from the marked synergisms which have been demonstrated between IL-1 and TNFα, IL-2 and IFNγ, IFNγ and TNFα,β, IL-2 and IL-4, IL-1 and G-CSF, IL-3 and IL-6. These observations furnish a further argument which favours the use of signal substances specific for bacteria because they liberate a series of lymphokines.

5.2. *Pharmacology*

5.2.1. *Adjuvancy.* In immunology, an adjuvant is defined as an agent capable of intensifying humoral or cellular immune reactions, or both. The basic components of the humoral immune-defence system are specific antibodies, the titres of which are determined in specific-pathogen-free (SPF) mice. Cell-mediated immune defences function by way of a variety of components: T_C cells, which carry their own receptors, macrophages, different types of granulocytes, and NK cells, which, armed with cytophilic antibodies, become specific defence cells (ADCC). Cellular immune defences can be assessed by means of a skin reaction (delayed-type hypersensitivity) in guinea pigs.

'Adjuvant' is an imprecise term and is often also applied to materials such as liposomes, oils and $Al(OH)_3$ that contribute to the development of an immune response in a way which is different from muramylpeptides and lipopeptides. We refer to them as carriers. They may form depots of the antigen, thus hindering its elimination, or they may catalyse the uptake of antigen by phagocytes and antigen-presenting cells (APC), or both. Macromolecules or whole organisms can fulfil such functions. For example catalysis of uptake into APC, induction of interleukins and delivery of T-epitopes and B-epitopes (cf. Figs 18 and 19).

Test for antibody synthesis to heterologous serum proteins. Mice are injected intraperitoneally or subcutaneously with suboptimal doses (10 μg) of bovine serum albumin (BSA) or human γ-globulin (HGG), dissolved in 0.2 ml of phosphate-buffered saline.

Various doses of the test compounds are injected by different routes (i.p. or s.c.), either once, 30 min prior to the administration of the antigen, or daily for five consecutive days before or after administration of the antigen. The results we refer to (Table 2) were obtained after a single administration. Immune sera are collected on Days 9, 15 and 29, and antibody titres are determined by passive haemagglutination. For this purpose, sheep red blood cells are coupled with either BSA or HGG by glutaraldehyde. Haemagglutination titres are expressed as $\log_2$ of reciprocal final agglutinating dilutions. Usually results are expressed as average titres of the three serum samples.

In this test, hydrophilic muramylpeptides have to be given simultaneously or shortly after the antigen to achieve good stimulation of antibody synthesis. The dose of protein antigen does not induce antibody synthesis to any significant extent without adjuvant.

Test for delayed-type hypersensitivity. Guinea-pigs are injected on Day 0 with 1 mg BSA and different doses of test compound in incomplete Freund's adjuvant (FiA). 0.1 ml of this mixture are administered by subplantar injection into each hind foot. After three weeks the animals are challenged by intradermal injection into the back of 100 μg BSA in 0.1 ml phosphate-buffered saline. Erythema and swelling of the skin ensue, which should persist for 24–48 h. A reaction volume is

For typical effective doses see Table 8. Details of these tests are given in reference 67.

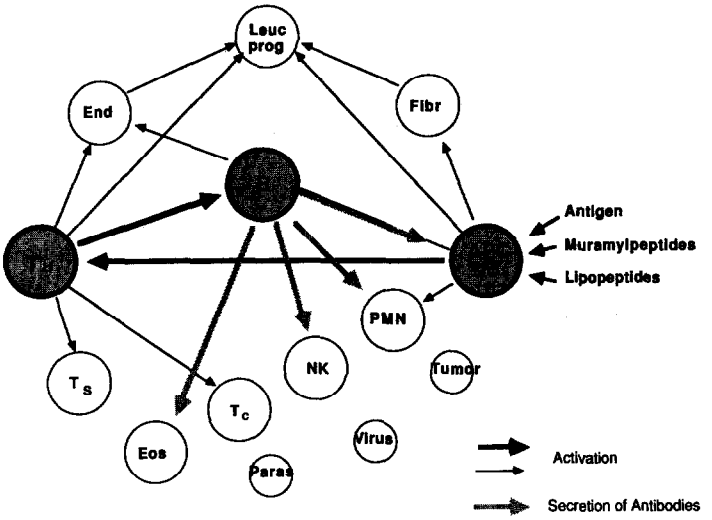
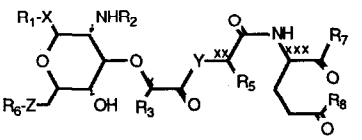


Fig. 16. Induction of antibody synthesis.

calculated from the area of the erythema and the increased thickness of the skin. BSA in FiA provokes no skin reaction.

Figure 16 schematically illustrates activations that ensue after ingestion of an antigen by antigen-presenting cells (APC), leading to the synthesis of antibodies by plasmocytes. These cells arise by differentiation of B cells after activation by T_H cells. Muramylpeptides and lipopeptides contribute by inducing IL-1, which is one proliferation signal for T_H cells (cf. p. 6348). Besides the activation of T_C cells by IL-2, the diagram also shows that cytophilic antibodies can serve as a targeting device for a variety of cells and that this leads to antibody-dependent cell-mediated cytotoxicity.

Table 2. Structure–activity relationships of muramylpeptides in adjuvant tests



Substituent		Activity		Reference
		DTH	AbS	
R1-X:	α,ß-O-benzyl	+-	+-	32
	α,ß-O-alkyl	+-	+-	32, 68
	ß-SH, ß-S-alkanoyl	+		25, 69
	α,ß-O-alkanoyl	+	+	72,91
C-1:	reduction to sorbitol	-	-	70
R2:	H	+	+	71, 93
	benzoyl	+	+-	72, 73
	alkanoyl	+	+	72, 73, 74
	tosyl	-	-	32

Table 2.—(*continued*)

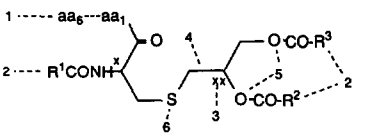
Substituent			Activity		Reference
			DTH	AbS	
R3:	CH_3, C_2H_5, C_4H_9		+	+	72, 73, 75
	$=CH_2$, $=C_3H_7$, $=CHC_6H_5$		+	+-	76
R5:	amino acid 1		+	+	3, 72, 73, 77
Y:	O		-	-	32
R6-Z:	O-alkanoyl		+	+	3, 32, 73, 78, 94
	NH_2, NH-alkanoyl		+		80
	S-alkanoyl		-		81, 82
R7:	oxyalkyl		+	+	3, 32
	aminoalkyl		-	-	3, 32
	α-amino acid		-	-	3, 32
	α-amino acylamide		+	+	3, 72, 83
R8:	oxyalkyl		+	+	3, 72
	aminoalkyl		-	-	3, 72
	amino acid		+	+	3, 72
	S-(N-acyl-cysteinyl)		+	+	32
	extension of peptide chain		+	+	3, 84, 85, 86
amino acid 2	D-Glu		+	+	3, 72, 73
	D-Gla		+	+	35
	all others		-	-	
chirality in peptide					
x:	L		-	-	3, 32
xx:	D-Ala		-	-	3
	D-Abu		+	+	32
xxx:	L		-	-	3, 32
carbohydrate derivatives					
D-galacto			+	+	71
D-manno			+	+	71
D-allo, D-gulo, D-xylo, L-ido			-	-	71
positional isomers					
N-acetyl	side-chain				
2	4		-	-	71
2	6		+-	-	71, 87
2	1-β		-	-	71, 87
2	1-β-S		-	-	32
deoxy-derivatives					
1-deoxy (2.5-anhydro)			+		88
2-deoxy			-	-	71, 92
4-deoxy			-	-	71, 92
6-deoxy			-	-	71, 92
1.6-anhydro			-		18, 89, 90

As a rule, activity in the test for antibody synthesis (AbS) is sensitive to substituents that are presumably removed slowly *in vivo*. This also holds good for multiple substitutions. In the case of unsubstituted muramylpeptides the more lipophilic the substance, the less is antibody synthesis stimulated. Stimulation of DTH has been correlated with the occurrence of IgG_2, which is cytophilic and can activate complement.[95] In comparing published data, it must be borne in mind that the intensity of the DTH reaction depends upon the antigen which is used. Azobenzenearsonate-N-acetyl-tyrosine (ABA tyrosine) is more immunogenic than bovine serum albumin (BSA). The adjuvancy of MDP in mice is genetically controlled and non-responders can be observed.[96]

Lipopeptides

LP-I. These compounds are active adjuvants.[9] They differ, however, from LP-III in being devoid of any mitogenic effect on B cells.

LP-III. Derivatives of this class are very efficient stimulants of antibody synthesis (mouse) and mitosis in splenic B cells. They do not, however, stimulate DTH against BSA in incomplete Freund's adjuvant. A summary of structure–activity relations for antibody synthesis is given in Fig. 17.



Nr.	Variation type	example	Biological effect
1	sequence	L- and D-amino acids	not much influence
2	chain length	C_2-C_{20}: $R^1 = R^2 = R^3$	C_8 - C_{20} good
3	chirality	x (R)	active
		x (S)	inactive
		xx (R), (S)	(R) slightly better than (S)
4	lipophilic substitution	alkyl, ß-acyloxyalkyl	< natural residue
5	replacement of oxygen	single and double N	< ester
6	oxidation	sulfoxide	< thioester

Fig. 17. Stimulation of antibody synthesis in mice by lipopeptides (LP-III); structure–activity relations.

Vaccines

One practical consequence of the adjuvancy of muramyl peptides is their use in vaccines. This is also a consequence of the discovery that the active principle of Freund's adjuvant (FcA) is murein, a polymeric form of MDP.

Table 3 shows examples in which it has proved to be possible to induce the formation of specific antibodies, of which some had neutralizing or protective effects, either by admixture of the muramyl peptide, or by its chemical bonding to peptide-(protein)-antigens. It is noteworthy that the simultaneous administration of several antigens leads to the formation of specific antibodies to each.[97,98] Lipopeptides (LP-III) have also been coupled directly to peptide antigens.[122]

A phenomenon of central importance in the induction of immune responses by vaccines, and generally, is the activation and proliferation of antigen-specific T-helper cells. To initiate these steps, the T_H cells must receive signals in addition to IL-1, which lipopeptides and muramylpeptides cannot

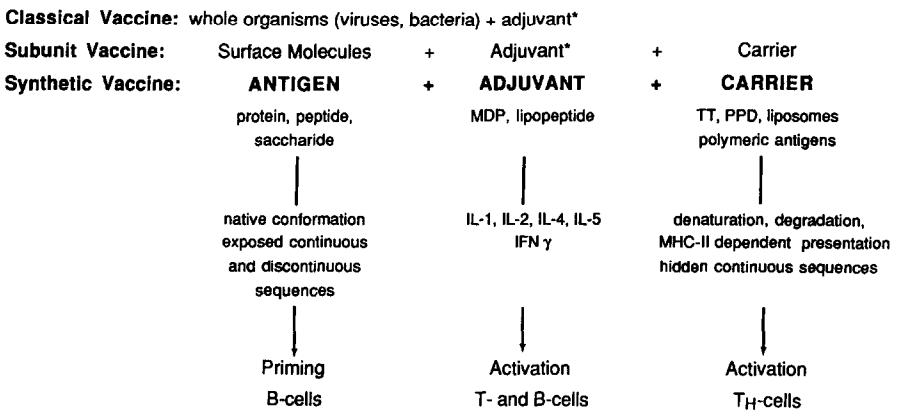


Fig. 18. Schematic representation of the typical components of vaccines and their functions.

Table 3. Vaccines constructed with MDP and MDP-derivatives

Organism	Vaccine	Effect	Reference
S. pyogenes, 24	Murabutide + M-peptide	bactericidal Abs, man	99
Cl. tetani	Murabutide + TT	specific Abs, man	100, 101
C. diphtheriae	MDP + BSA(A-L)-(toxinpeptide)$_n$	protection, guinea-pig	102
	(MDP-Lys)$_n$-toxoid	specific Abs, mouse	103
S. mutans	MDP + cell walls	IgA response, rat	104
Coliphage MS-2	PG-(A-L)-peptide	neutralization, rabbit	105
Hepatitis B	MDP + peptides + liposomes	specific Abs, mouse	106
	MDP + vaccine	specific Abs, mouse	23
	Murabutide + Hep. B-SA-TT	specific Abs, mouse	107
FMD Virus	MDP + VP1-peptide	protective Abs, mouse	98
	(MDP-Lys)$_n$-polym. peptide	protective Abs, guinea-pig	98
P. falciparum	L-18-MDP + sporozoites + liposomes	protection, monkey	108
P. knowlesi	Murabutide + (sporoz. peptide)$_n$-TT	coat disintegr. of parasites	109
	(MDP-Lys)$_n$-TT-(peptide)$_m$	specific Abs, mouse	103
S. + C. + Hep. B	Murabutide + TT-(peptide)$_n$	specific Abs, mouse, guinea-pig	97
S. + C. + Hep. B + P. knowlesi	Murabutide + condensed peptides	specific Abs, mouse	98
T blasts	(MDP)$_n$-T cells	suppression of alloreactivity in MLC, mouse	110
LH-RH	MDP-Lys-peptide	castration, mouse	111
h-Chorion-gonadotropin	n-MDP + peptide + squalene + arlacel	sterilization, rabbit, monkey	112
	(MDP)$_n$-TT-(peptide)$_m$	specific Abs, rabbit	103

Reviews: 37, 38, 113, 114, 115, 173

(A-L): synthetic poly-D,L-alanine-poly-L-lysine; PG: peptidoglycane; L-18-MDP: 6-O-stearoyl-MDP; S.: streptococc. M-peptide; C: diphtheria peptide; Hep. B: hepatitis viral peptide; TT: tetanus toxoid; MLC: mixed lymphocyte culture; Hep B-SA: hepatitis B surface antigen.

provide. According to the most recent model,[118a] proliferation results from an interaction between two sets of structures (TCR and CD 4 or CD 2) on the T_H cell with two (T-epitope–MHC-II-complex and I-a or LFA-3) on the antigen-presenting cell. Part of the original antigen, the T-epitope, that has survived degradation in APCs is presented on their surface to the T-cell receptor as a complex with MHC protein. Binding of the TCR to this complex provides one signal to T_H. The interaction of I-a on APC with CD 4 on T_H generates two further signals. One signal is delivered to T_H by CD 4. It is synergistic with the signal from TCR. The second signal is received by APC from I-a and it liberates IL-1.[117,118] In Fig. 19 the interaction of CD 2 with LFA-3 is shown because this has been analysed in more detail.[118a]

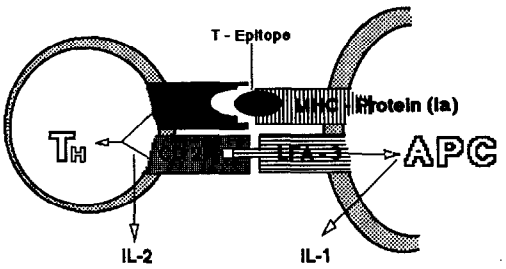


Fig. 19. Proposed molecular interactions between T-helper cells (T_H) and antigen-presenting cells (APC). CD 2: Cluster determinant 2, glycoprotein on thymocytes, NK cells, Tc cells and other mature T cells. LFA-3: Lymphocyte function-associated antigen 3, glycoprotein on epithelial cells, endothelial cells, fibroblasts and many cells of haematopoetic origin. MHC-II-protein (I-a): Carrier for presentation of antigen.

The smaller the peptide antigens, the more likely is non-responsiveness to be observed. In animal experiments, the usual device was therefore to use large, aggregating proteins as carriers, since the chances of infiltrating suitable T-cell epitopes increase proportionately with molecular weight. Another practical means of activating T_H is to use components of widespread toxins or proteins, such as tetanus toxoid (TT) or tubercle protein (PPD). The latest development consists in binding the antigen to as small as possible a synthetic T-cell determinant.[119–121] The role of the muramyl peptides and lipopeptides partly consists in liberating IL-1 to stimulate the secretion if IL-2. IL-2 in its turn leads to the expansion of the T helper-cell population, which, through the intervention of further factors (IL-4, IL-5, etc.), ensure the expansion and maturation of specific B-cell clones.

Table 4. Prophylactic effect of muramylpeptides against subcutaneous *E. coli* infections in mice

MDP-analog	Dose		Survival at day 7			
	mg	(mg/kg)	6.10^6 cells		$1.2.10^7$ cells	
			alive/total	%	alive/total	%
Controls	-	-	0-4/40	0-10	0/40	0
MDP	100	(4)	27/40	62.5	9/40	22.5
MDP-6-O-stearate	150	(6)	32/40	80	19/40	47.5
MDP-Lys-ε-stearate	179	(7.2)	37/40	87.5	20/40	50

Matsumoto et al., Infection and Immunity 39 (1983), 1029 - 1040

5.2.2. *Resistance to infections*

Muramyl peptides. Chedid *et al.*[123] first demonstrated that muramyl peptides can enhance the resistance of mice against pathogens such as *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Candida albicans*, *Escherichia coli* and *Trypanosoma cruzi*, but only when administered prophylactically.[3] Synergistic effects can be observed with antibiotics.[124] Lipophilic derivatives and polymeric prodrugs or slow-release formulations (Alzet minipump) are superior to the water-soluble muramyl peptides.

One finding worthy of note is that lipophilic muramyl peptides are both prophylactically and therapeutically active against RNA and DNA viruses.[124a,125-127]

Table 5. Prophylactic effect of MTP-PE against various experimental virus infections in mice and guinea pigs

Virus		Viral inoculum logPFU	(route)	Animal species	MED * mg/kg
Influenza	A/Victoria	3.3-4	(i.n.)	mouse	0.001
	A/Texas	0.3-1	(i.n.)	mouse	0.0001
	A/USSR	2.3-3	(i.n.)	mouse	0.1+
	B/Ann Arbor	0.3-1	(i.n.)	mouse	0.01+
	B/Hong Kong	2.3-3.3	(i.n.)	mouse	0.0001
Parainfluenza 1 (Sendai)		1-1.7	(i.n.)	mouse	0.01
Herpes simplex	1/Tup	4.8	(i.n.)	mouse	0.1+
	1/Virtue	3.3	(i.n.)	mouse	1.0
	2/Angelotti	4	(i.vag.)	guinea pig	0.05+#
	2/Alabama	3	(i.vag.)	guinea pig	0.1§
	2/MS	3.6	(i.vag.)	guinea pig	1.0+

* minimum effective dose level
i.n. administration 7 days before infection.
\+ doses lower than indicated were not tested.
\# single i. vaginal administration 7 days before infection.
§ single s.c. administration 3 days before infection.

Figure 20 illustrates another aspect of the action of muramylpeptides and lipopeptides. Both classes of compounds liberate colony-stimulating factors from macrophages and monocytes and, via IL-1 and TNF, from endothelial cells and presumably also other cells. One of the consequences is stimulation of bone-marrow (leucocyte progenitors) to increase the number of various types of leucocytes in circulation. As granulocytes and many other leucocytes are destroyed in fulfilling their defensive functions, reinforcement of the population of freshly matured defence cells is more efficient than stimulating cells that are no longer fully active.

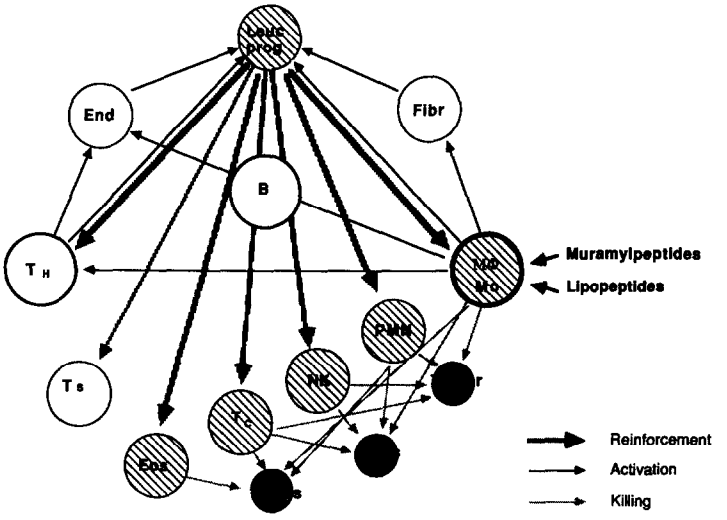


Fig. 20. Stimulation of bone-marrow (leuc. prog.) by muramylpeptides and lipopeptides.

For a survey of the role of immunomodulators in defence against microbial infections see reference 179.

Lipopeptides

LP-I. This type of lipopeptide, developed by Rhône-Poulenc and by Fujisawa, has been shown to stimulate resistance to infection and is now under clinical investigation.[9]

LP-III. Representatives of this type also display a marked anti-infective activity when administered prophylactically, or simultaneously with the induction of the infection.

Table 6. Prophylactic activity of lipopeptide CGP-31 362 against acute systemic infections in mice (60–70% survival)

Organism	Dose (mg/kg) -24h			
	i.p.	s.c.	i.n.	p.o.
E. coli 2018	1	5		
S. aureus 10 B	1	25	10	
S. pyogenes aronson	10	25		
S. pneumoniae 1126				0.5
P. aeruginosa 799	1			10

In bacterial and viral infections, the best results are obtained when the route of infection and the route of administration of the substance are the same. This is partly explained by the distinct mobilization of neutrophils; however, monocytes and macrophages are also activated, and the proliferation of T cells is stimulated.

5.2.3. *Antitumour activities*

Models. Assessments of the activity of immunostimulants against tumours naturally depend upon the experimental models used and upon their clinical relevance.[128,128a] Neither the rejection of sarcoma 180, which is normally regarded as an allogenic transplant, nor the results obtained after combined application of tumour cells and immunostimulants will be considered in this context. Similar circumstances arise upon intratumoral application. Of far greater practical interest are the experiments in which attempts are made to activate so-called unspecific defence against micrometastases systemically (i.v., p.o.). This defence system (NK cells, monocytes, macrophages, granulocytes, etc.), which normally receives powerful impetus from T cells, is generally weakened, or no longer functionally efficient after radiotherapy, chemotherapy, or long-standing neoplastic disease. An added problem is the resistance of the tumour due to induced selection as a result of the chemotherapy. It has been shown that tumour cells of different origins, like cells resistant to chemotherapy, are selectively recognized and killed by monocytes and macrophages.[129,130] The normal activation of these defensive cells is presumably mediated by factors from T cells; but bacterial components such as murein, lipopolysaccharide (LPS), lipoprotein, or fungal glucans, can replace such factors (IFNγ) and render monocytes and macrophages tumoricidal. Various components can act synergistically.[134–138] The process is also partly dependent on endogenous factors, as can be inferred from demonstrable synergies[131,132] (cf. Table 7). For T subsets in antitumour defence systems, see reference 133.

Table 7. Synergisms of muramylpeptides and lymphokines

iv-treatment of C 57 BL/6 mice alveolar Mϕs		% cytolysis	h-monocytes incubated with		% cytotoxicity for A 375 melanoma
MAF 1/2	in MLV	48	n-MDP 1	µg/ml	61
MAF 1/20	in MLV	-2	n-MDP 0.01	µg/ml	0
MDP 0.62 µg	in MLV	41	h-IFN 1000	U/ml	0
MDP 0.3 µg	in MLV	-1	h-IFN 10	U/ml	
MDP 0.3 µg + MAF 1/20		44	+ n-MDP 0.01	µg/ml	59
(131)			(132)		

Synergisms of microbial components:

MDP + LPS	cytotoxic Mϕs (134)	
MDP + TDM	resistance to:	bacterial infections (135)
		viral infections (137)
		tumour (136)
MDP + TDM + 4′-P-Lipid A	tumour regression (138)	

Abbreviations: MAF: macrophage-activating factors from T-cells; MLV: multilamellar vesicles; TDM: trehalose dimycolate; h: human

Results obtained in a melanoma model[139,140] with the lipophilic muramyl peptide MTP-PE are shown in Fig. 21.

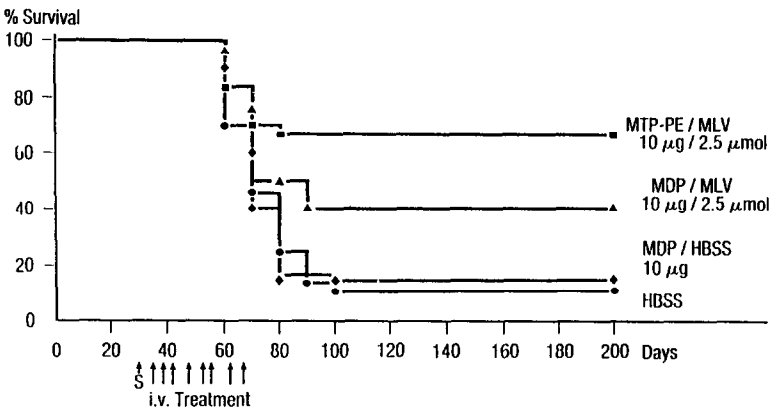


Fig. 21. Treatment of spontaneous metastases by intravenous administration of liposomes containing MDP or MTP-PE (S: surgery of the primary tumour).

It has been shown that intravenously injected liposomes render not only monocytes tumoricidal, but also macrophages in the liver and lung.[141,142] Not only does the formulation protect the substance from premature degradation and excretion, but the admixed phosphatidyl serine also catalyses its uptake into phagocytes; and, lastly, the liposomes constitute an intracellular slow-

release form, of which the rate of decomposition can be varied according to the type of phospholipid used. The approximate adjustment of the size of the liposomes also permits their temporary retention in the pulmonary capillaries. For a detailed discussion of the liposome approach see reference 142*a*.

As in these experiments with MTP-PE, the same mode of tumoricidal activation proved effective with N-acetyl-muramyl-L-alanyl-D-isoglutaminyl-L-alanyl-glycerol-dipalmitate,[143] and, strangely enough, also with N-acetyl-muramyl-D-alanyl-D-isoglutaminyl-L-alanyl-glycerol-dipalmitate.[144] It should be kept in mind that N-acetyl-muramyl-D-alanyl-D-isoglutamine is inactive in the adjuvans tests.

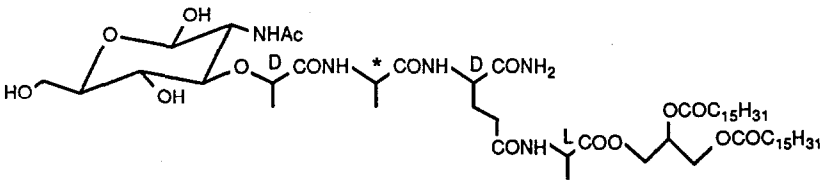


Fig. 22. N-Acetyl-muramyl-L(D)-alanyl-D-isoglutaminyl-L-alanyl-glycerol-dipalmitate.

The liposomal formulation of MTP-PE can also activate human monocytes when injected i.v.[130] Treatment of autochthonous skin tumours in the mouse has been reported.[145]

Lipopeptides (LP-III)

Compounds of the first generation with sequences corresponding to or resembling the natural sequence[10,146] proved to be potent macrophage activators (mouse and rat), but only *in vitro* and not *in vivo*. It needed a further modification of the peptide moiety with taurine, CGP 31 362, to yield compounds that were highly active *in vivo*. This also induced measurable rejection of pulmonary metastases after not only i.v., but also p.o. administration.[147]

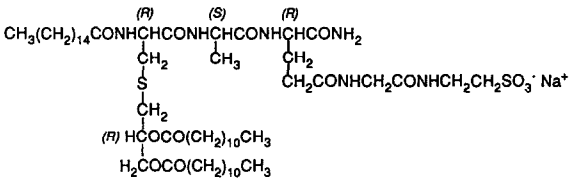


Fig. 23. CGP-31 362.

A summary of pharmacological effects is given in Table 8. It should be noticed that muramylpeptides including the lipophilic derivatives are a species different from the lipopeptides LP-III. They have a distinctly different biological profile although the overall effects so far mentioned seem to be the same. They differ in several respects:

—Substitution by fatty acids is not essential for muramylpeptides. Good prodrug forms can also be obtained with benzoyl groups or other aromatic acids, whereas for lipopeptides fatty acids are essential.

—Muramylpeptides, hydrophilic or lipophilic, do not induce secretion of TNF from macrophages by themselves. They need the cooperation of IFN-γ or LPS. LP-III function without this cooperation.

—Muramylpeptides are weak inducers of mitosis of spleen cells, LP-III are strong inducers, even stronger on a molar basis than LPS.

—Finally, in *in vitro* experiments, LP-III are more active than the most active muramylpeptides in inducing tumoricidal macrophages.

Table 8. Summary of pharmacological results

Test systems	Compounds/Judgement		Efficacy
	LPs	very active	1-10 mg/kg i.p. s.c.
Antibody synthesis (mouse)	MPS, hydrophilic	very active	1-10 mg/kg i.p. s.c.
	MPs, lipophilic	less active	
Delayed hypersensitivity, DTH (guinea pig) antigens: BSA, OVA	MPs, hydrophilic	potent in FiA	1-10 µg/kg s.c.
	LPs	inactive in FiA	
B-cell mitogenicity (mouse) in vitro incorporation of ^{3}H-thymidine into spleen cells	MPs	are weak inducers of mitosis	1-10 µg/ml
	LPs	are potent (>LPS)	0.1 µg/ml
Tumor cell killing by rat alveolar macrophages (melanoma MDBA-200)	MPs, hydrophilic	weak unless incorporated in liposomes	in vivo: 0.1-1 mg/kg
	MPs, lipophilic	very active also without liposomes	8 x i.v.
	LPs	very active without liposomes	in vivo: 1-5 mg/kg p.o.
Bacterial infections, acute and chronic (mouse)	MPs, hydrophilic	moderately active, prophylactically	10-50 mg/kg i.p. i.p.-infection
E.coli, Klebsiella pn., Candida, S.aur., P.aerug., S.aronson, S.pneum., (Toxoplasma gond. O)	MPs, lipophilic LPs	more active active prophylactically	0.5-25 mg/kg i.n. i.n.-infection
Viral infections (mouse, DNA-, RNA-viruses)	MPs, hydrophilic	inactive	
	MPs, lipophilic	very active	0.1-1 mg/kg i.n. p.o.
	LPs	less active than MPs	1-5 mg/kg i.n. p.o.

MPs: Muramylpeptides
LPs: Lipopeptides LP-III
FiA: Freund's incomplete adjuvant

5.2.4. *Sleep induction.* A factor isolated from human urine and found to induce slow-wave (deep) sleep in the rabbit and in the cat was identified as N-acetyl-muramyl-L-alanyl-D-isoglutaminyl-diaminopimelic acid.[148] The same property is also displayed by other muramyl peptides after intravenous, intraperitoneal and oral administration. The most active are the 1,6-anhydro-N-acetyl-muramyl derivatives.[149,150] MTP-PE is inactive. Lipopeptides, on the other hand, also induce slow-wave sleep in the cat but after a longer lag phase.

5.3. *Toxicity*

Muramyl peptides. Mice and rats are relatively insensitive to muramyl peptides (LD_{50} mouse: approx. 2200 mg/kg i.p.). For toxicological investigations the guinea-pig and the dog are more suitable species. The findings summarized below were made in dogs treated for 10 days with MDP (5 mg/kg) and MTP-PE (10 mg/kg) s.c. and i.v. and with MDP (5 mg/kg) administered s.c. by minipumps. For a detailed description see references 151 and 152.

There is a rapid effect on leucocytes (rise of granulocytes and monocytes) indicating a strong activation of the bone-marrow, release of pyrogenic factors as IL-I and transient increase of cortisol in plasma. Vascular effects can be observed in the eye and the arterial walls as well as involvement of mesothelial cells (epicarditis, pericarditis and synovitis) and changes in liver function (increase of acute phase proteins and increase of alkaline phosphatase).

MTP-PE in the liposomal formulation is 100 times less toxic than the free form and dogs treated over 14 days with 1 mg/kg MTP-PE daily by the intranasal route showed none of the above changes.

The role of muramyl peptides in the induction of adjuvant arthritis has been challenged. Effects of this nature could only be induced under particular conditions and in special strains of mice and rats. More recent investigations indicate that MDPs intensify an immune reaction against auto-antigens such as collagen II,[153] or the cross-reacting antigens from Mycobacteria.[154,155]

The pyrogenicity of MDP is augmented by coupling to polymeric vehicles.[156] Apyrogenic MDP derivatives, are also known including murabutide, N-acetyl-muramyl-L-alanyl-D-glutamine-α-*n*-butyl ester,[157] or N-acetyl-muramyl-L-threonyl-D-isoglutamine.[73] Liposomal MTP-PE does not cause the release of IL-1 from monocytes[158] and it is not pyrogenic up to 30 mg/kg s.c.

Fever can, however, be kept in check with antipyretics. Chedid *et al.* were surprised to find that simultaneous administration of MDP and indomethacin to mice exerted a synergistic anti-infective action.[159]

Proceeding from the hypothesis that suppression of the release of the feed-back inhibitor PGE_2 by activated macrophages could intensify or prolong the activation, we synthesized lipophilic conjugates of cyclo-oxygenase inhibitors and MDP derivatives.[160] The resultant compounds are very potent in activating macrophages to the tumoricidal state.

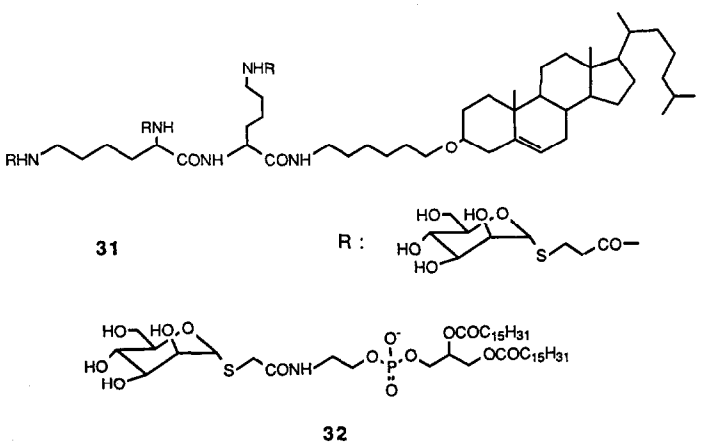
Lipopeptides

LP-I. The acute toxicity of pimelautide (Fig. 3) in the mouse is $LD_{50} = 410$ mg/kg i.v. Although the compound is pyrogenic in the rabbit, in a study in dogs, s.c. injections given three times weekly for one month were well tolerated up to a dose of 1 mg/kg.

LP-III. Apart from pyrogenicity, there are as yet no data available on the toxicity of LP-III.

5.4. *Targeting*

The targeted administration of muramyl peptides is expected to give rise to fewer side-effects. Since macrophages and monocytes are recognized as target cells, attempts have been made to find formulations ensuring that the substance is predominantly distributed among these cells. A successful formula was developed by Fidler *et al.*[41] Phosphatidyl serine introduced as a component of lecithin liposomes accelerates their uptake into monocytes and macrophages (mouse, rat and human). A conjugate such as tri-mannosyl-di-lysyl-cholesterol **31** as a component of liposomes also accelerates their uptake into macrophages.[161] By using mannose-containing phospholipids isolated from Mycobacteria, liposomes can be prepared that bind to the mannose receptors of macrophages.[162,163] Synthetic mannosyl-kephalin **32** acts in the same manner[164] (Fig. 24).



32

Fig. 24. Tri-mannosyl-di-lysyl-cholesterol, **31**; Mannosyl-kephalin, **32**.

Alternatively, soluble compounds can be used. A conjugate of bovine serum albumin, MDP and mannose is readily taken up by macrophages.[36,36a] A recent review of saccharide determinants for selective drug delivery has been published by Shen.[36b] The same effect is achieved with an antigen–antibody complex from carrier-bound MDP and a monoclonal antibody specific for MDP. In this case, uptake occurs by way of the macrophage Fc receptors.[165] A conjugate of tumour-specific monoclonal antibodies and MDP can be incorporated via the Fc receptors into macrophages at the neoplastic focus.[166]

All attempts to steer antibodies towards tumour cells are, however, confronted with the problem of antigen variation. Owing to induced selection (immune defence, chemotherapy) the phenotype of the tumour cells undergoes constant change. This is why an approach taking advantage of the natural targeting mechanism of the immune defence cells is so important.

The fact that intravenous administration of a liposomal preparation of muramylpeptides leads to tumoricidal alveolar macrophages in lung tissue, although liposomes cannot penetrate from the circulation, can be rationalized. Results from different areas of research can be combined hypothetically to explain the following chain of events:

Liposomes which are used are of a size suitable to ensure their temporary retention in the pulmonary capillaries and their uptake there into monocytes. Activated monocytes, in their turn, secrete IL-1 and TNF and thus activate the endothelium, to which they are then bound.[167,168] In response to appropriate stimuli from the tissue,[169] the cells migrate from the vessels and become activated (e.g. tumoricidal) alveolar macrophages.

5.5. *Pharmacokinetics*

The rapid elimination of water-soluble muramylpeptides after i.v. injection[19,170] and, somewhat more slowly, after administration in an oil-emulsion (incomplete Freund's adjuvant) can be considerably retarded by lipophilic substituents.[171] This has been observed with MTP-PE in the rat. Even more extreme is the repository effect that occurs after i.v. injection of MTP-PE in liposomes. The blood levels of nor-MDP in the dog after intravenous injection decline faster than the levels of MTP-PE. A similar slow decrease of serum concentrations has been reported for MDP-Lys(L18) (Fig. 11) injected subcutaneously into rats.[172]

MDP and nor-MDP are excreted in practically unchanged form, whereas MTP-PE undergoes cleavage of the N-acetyl group. N-acetyl-muramyl-dipeptide and -tripeptide also occur as metabolites.

5.6. *Biochemical mechanisms*

The mechanisms responsible for the biological effects of both lipopeptides and muramyl peptides are still unknown. Lederer's group found no demonstrable correlation between adjuvant activity, anti-infective efficacy and pyrogenicity.[3] Tentative explanations have been put forward suggesting that effects of muramylpeptides on the central nervous system could be linked with the turnover of prostaglandins and serotonin in the diencephalon.[174] Evidence purporting to show an affinity of MDP derivatives for serotonin receptors[175,176] is not very convincing because the labelled derivatives which were used, contained serotonin-like residues. A differential messenger-RNA synthesis in macrophages activated with IFNγ and MDP has been described.[177]

5.7. *Summary and conclusions*

Analysis of bacterial lead compounds has yielded extremely active, synthesizable low-molecular agents. These agents induce the release of endogenous mediators such as lymphokines from lymphocytes, monokines from monocytes and interleukins. They also act synergistically with such factors. In animal models *in vivo*, a variety of pharmacological effects can be demonstrated, and the associated cell system identified. These effects include antibody synthesis (B cells), DTH (T cells),

resistance to infection (neutrophils, monocytes, macrophages) and antitumour defence (monocytes, macrophages, neutrophils, NK cells and T_C lymphocytes).

It can be anticipated that such compounds will find application in modern vaccines constructed synthetically from a universal T epitope, a specific B epitope and an adjuvant, such as MDP. The beneficial application of these compounds in the treatment of malignant tumours, chronic infections, and perhaps also auto-immune diseases depends upon a thorough understanding of their effects in the human body. Clinical investigations of various synthetic agents are in progress. Their success will, however, be conditional upon the possibility of targeting such compounds towards the desired site of action by the development of appropriate prodrug formulations. Another decisive aspect concerns the migratory characteristics of leucocytes and their interaction with endothelial cells: these processes remain comparatively obscure. The active part played by certain types of endothelium is now being investigated in greater depth.[178] It has to be borne in mind that mammals live in symbiosis with bacteria and are to some degree adapted to bacterial metabolites and components. This is another reason for expecting that it should be possible to develop therapeutic agents capable of exploiting this principle.

Acknowledgement—The translation from the German by Mr A. H. Kirkwood, the secretarial help of Ms C. Bellin and Ms M. Herrmann and helpful suggestions by Prof. W. D. Ollis are gratefully acknowledged, as well as the contributions to our own work by many colleagues in the Departments of Biology, Chemistry, Pharmacological Chemistry and Chemical Development, which are quoted in the references.

REFERENCES

[1] F. Ellouz, A. Adam, R. Ciorbaru and E. Lederer, *Biochem. Biophys. Res. Commun.* **59**, 1317 (1974).
[2] S. Kotani, Y. Watanabe, F. Kinoshita, T. Shimono, I. Morisaki, T. Shiba, S. Kusumoto, Y. Tarumia and K. Ikenaka, *Biken J.* **18**, 105 (1975).
[3] A. Adam, J. F. Petit, P. Lefrancier and E. Lederer, *Molec. Cell. Biochem.* **41**, 27 (1981).
[4] P. Lefrancier and E. Lederer, *Fortschr. Chemie Org. Naturstoffe* **40**, 1 (1981).
[5] P. Dukor, L. Z. Tarcsay and G. Baschang, *Ann. Rep. Med. Chemistry* **14**, 146 (1979).
[6] E. Lederer, *J. Med. Chem.* **23**, 819 (1980).
[7] I. Azuma and G. Jolles, *Immunostimulants Now and Tomorrow*, Japan Scientific Societies Press, Tokyo, Springer-Verlag, Berlin, 1987.
[8] *Excerpta Medica, Intern. Congress Series* **563** (1982), Immunomodulation by Microbial Products and related Synthetic Compounds, Eds. Y. Yamamura, S. Kotani, I. Azuma, A. Koda and T. Shiba, Amsterdam.
[9] G. H. Werner, F. Floc'h, D. Migliore-Samour and P. Jolles, *Experientia* **42**, 521 (1986); [a]H. C. Wu and M. Tokunaga, *Current Topics Microbiol. Immunol.* **125**, 127 (1986).
[10] Swiss Pat. Appl. 20.6.1977, L. Z. Tarcsay, B. Kamber, J. Stanek, G. Baschang and A. Hartmann, Ciba-Geigy AG.
[11] G. Jung, C. Carrera, H. Brückner and W. G. Bessler, *Liebigs Ann. Chem.* **1983**, 1608.
[12] V. Braun, *Biochim. Biophys. Acta* **415**, 335 (1975).
[13] F. Melchers, V. Braun and C. Galanos, *J. Exptl. Med.* **142**, 473 (1975).
[14] A. D. McLachlan, *J. Mol. Biol.* **121**, 493 (1978).
[15] H. Umezawa, *Biotechnology and Bioengineering* **XXII**, Suppl. 1, 99–110 (1980).
[16] H. M. Flowers and R. W. Jeanloz, *J. Org. Chem.* **28**, 2983 (1963).
[17] R. Gigg, P. M. Carroll and C. D. Warren, *J. Chem. Soc.* **1965**, 2975.
[18] Swiss Pat. Appl. 27.3.1986, G. Baschang, Ciba-Geigy AG.
[19] M. Parant, F. Parant, L. Chedid, A. Yapo, J. F. Petit and E. Lederer, *Int. J. Immunopharmacol.* **1**, 35 (1979).
[20] S. Kusumoto, K. Ikenaka and T. Shiba, *Tetrahedron Lett.* 4055 (1979).
[21] P. L. Durette, A. Rosegay, M. A. R. Walsh and T. Y. Shen, *Tetrahedron Lett.* 291 (1979).
[22] G. Baschang, D. E. Brundish, A. Hartmann, J. Stanek and R. Wade, *J. Labelled Comp. Radiopharmaceuticals* **XX**, 691 (1983).
[23] P. L. Durette, C. P. Dorn, A. Friedmann and A. Schlabach, *J. Med. Chem.* **25**, 1028 (1982).
[24] R. K. Jain, C. M. Gupta, R. K. Saxena, R. P. Saxena, K. C. Saxena, R. Shukla, N. Anand and C. F. Costello, *Chem. Phys. Lipids* **41**, 237 (1986).
[25] A. Hasegawa, Y. Hioki, M. Kiso, H. Okumura and I. Azuma, *Carbohydr. Res.* **123**, 183 (1983).
[26] A. Hasegawa, E. Tanahashi and M. Kiso, *Carbohydr. Res.* **103**, 251 (1982).
[27] A. Hasegawa, E. Seki, Y. Fujishima, K. Kigawa, M. Kiso, H. Ishida and I. Azuma, *J. Carbohydr. Chem.* **5**, 371 (1986).
[28] I. Azuma, *Infect. Immunity* **14**, 18 (1976).
[29] Eur. Pat. Appl. EP 25495, 25.3.1981, L. Tarcsay, G. Baschang, A. Hartmann, J. Stanek, Ciba-Geigy AG; *Chem. Abstracts* **95**, 81537s (1981); EP 27258, 22.4.1981, *Chem. Abstracts* **95**, 187676b (1981); EP 34347, 26.8.1981, A. Hartmann, G. Baschang, O. Wacker and J. Stanek, Ciba-Geigy AG; *Chem. Abstracts* **97**, 24245n (1981); EP 99578, 1.2.1984, A.

Hartmann, O. Wacker, G. Baschang and L. Tarcsay, Ciba-Geigy AG; *Chem. Abstracts* **100**, 210439j (1984); EP 114787 A2, 1.8.1984, G. Baschang, A. Hartmann, O. Wacker, Ciba-Geigy AG, *Chem. Abstracts* **102**, 25037e (1984).
[30] Y. Osada, T. Otani, M. Sato, T. Une, K. Matsumoto and H. Ogawa, *Infect. Immunity* **37**, 292 (1982).
[31] A. Y. Khorlin and N. V. Bovin, *Bioorg. Khim.* **11**, 671 (1985); *Chem. Abstracts* **103**, 121257t (1985).
[32] Ciba-Geigy, unpublished.
[33] US Pat. Appl. 525190, 22.8.1983, A. C. Allison and N. E. Byars, Syntex (USA) Inc., *Chem. Abstracts* **103**, 92824t (1985).
[34] L. Chedid, M. Parant, F. Parant, F. Audibert, P. Lefrancier, J. Choay and M. Sela, *Proc. Nat. Acad. Sci.* **76**, 6557 (1979).
[35] Eur. Pat. Appl. EP 3833, 5.9.1979, G. Baschang, F. M. Dietrich, R. Gisler, A. Hartmann, J. Stanek and L. Tarcsay, Ciba-Geigy AG, *Chem. Abstracts* **92**, 116411p (1979).
[36] M. Monsigny, A. C. Roche and P. Bailly, *Biochem. Biophys. Res. Commun.* **121**, 579 (1984).
[36a] A. C. Roche, P. Midoux, C. Petit, D. Derrien, R. Mayer and M. Monsigny, in: Immunomodulators and Nonspecific Host Defence Mechanisms Against Microbial Infections, Eds. K. N. Masihi and W. Lange, *Advances in the Biosciences* **68**, 217–255 (1988); [b] T. Y. Shen, *Ann. N. Y. Acad. Sci.* **507**, 272 (1987).
[37] E. Lederer, *Drugs Exptl. Clin. Res.* **XII**, 429 (1986).
[38] M. W. Steward and C. R. Howard, *Immunology Today* **8**, 51 (1987).
[39] S. Sone and I. J. Fidler, *Cancer Immunol. Immunother.* **12**, 203 (1982).
[40] J. D. Bos and M. L. Kapsenberg, *Immunology Today* 7, 235 (1986).
[40a] C. A. Janeway Jr., *Nature* **333**, 804 (1988).
[41] A. J. Schroit and I. J. Fidler, *Cancer Res.* **42**, 161 (1982); *Biol. Cell* **47**, 87 (1983).
[42] M. E. Dorf and B. Benaceraff, *Ann. Rev. Immunol.* **2**, 127 (1984).
[43] L. L. Lanier and H. Phillips, *Immunology Today* 7, 132 (1986).
[44] R. B. Herberman, J. Hiserodt and N. Vujanovic, *Immunology Today* **8**, 178 (1987).
[45] P. Hersey and R. Bolhuis, *Immunology Today* **8**, 233 (1987).
[46] J. R. Ortaldo and R. B. Herberman, *Ann. Rev. Immunol.* **2**, 359 (1984).
[47] D. O. Adams and T. A. Hamilton, *Ann. Rev. Immunol.* **2**, 283 (1984).
[48] T. A. Springer, M. L. Dustin, T. K. Kishimoto and S. D. Martin, *Ann. Rev. Immunol.* **5**, 223 (1987).
[49] D. R. Littman, *Ann. Rev. Immunol.* **5**, 561 (1987).
[50] J. J. Woodruff and L. M. Clarke, *Ann. Rev. Immunol.* **5**, 201 (1987).
[51] R. Snyderman and M. C. Pike, *Ann. Rev. Immunol.* **2**, 257 (1984).
[52] S. K. Dower and D. L. Urdal, *Immunology Today* **8**, 46 (1987).
[53] J. B. Breitmeyer, *Nature* **329**, 760 (1987).
[54] R. J. Duquesnoy and M. Trucco, *CRC Crit. Rev. Immunol.* **8**, 103 (1988).
[55] J. J. Oppenheim, E. J. Kovacs, K. Matsushima and S. K. Durum, *Immunology Today* 7, 45 (1986).
[56] K. A. Smith, *Ann. Rev. Immunol.* **2**, 319 (1984).
[57] J. M. Garland, *Immunology Today* 7, 104 (1986).
[58] T. Kishimoto, *J. Clin. Immunol.* 7, 343 (1987).
[59] N. Hogg, *Immunology Today* 7, 65 (1986).
[60] N. A. Nicola, *Immunology Today* **8**, 134 (1987).
[61] G. Trinchieri and B. Perussia, *Immunology Today* **6**, 131 (1985).
[62] Z. Dembic, H. v. Böhmer and M. Steinmetz, *Immunology Today* 7, 308 (1986).
[63] D. S. B. Hoon, G. Sviland and R. F. Irie, *Proc. Am. Assoc. Cancer Res.* **28**, 78. Meeting, 369 (1987).
[64] P. J. Lachmann, *Nature* **321**, 560 (1986).
[65] B. B. Aggarwal, *Drugs of the Future* **12**, 891 (1987).
[66] H. S. Koren, *Immunology Today* **8**, 69 (1987).
[67] R. H. Gisler, F. M. Dietrich, G. Baschang, A. Brownbill, G. Schumann, F. G. Staber, L. Tarcsay, E. D. Wachsmuth and P. Dukor, *Drugs and Immuneresponsiveness*, in J. L. Turk and D. Parker Eds. The McMillan Press, London (1979).
[68] Y. Nagai, K. Akiyama, S. Kotani, Y. Watanabe, Y. Shimono, T. Shiba and S. Kusumoto, *Cell. Immunol.* **35**, 168 (1978).
[69] A. Hasegawa, Y. Hioki, E. Yamamoto, M. Kiso and I. Azuma, *J. Carbohydr. Chem.* **5**, 359 (1986).
[70] A. Adam, M. Devys, V. Souvannavong, P. Lefrancier, J. Choay and E. Lederer, *Biochem. Biophys. Res. Commun.* **72**, 339 (1976).
[71] I. Azuma, H. Okumura, I. Saiki, M. Kiso, A. Hasegawa, Y. Tanio and Y. Yamamura, *Infect. Immunity* **33**, 834 (1981).
[72] Swiss Pat. 614718, 14.12.1979, G. Baschang, A. Hartmann, J. Stanek and A. Sele, Ciba-Geigy AG, *Chem. Abstracts* **93**, 95665c (1979); Prior. 10.12.1975.
[73] G. H. Jones, J. G. Moffatt and J. J. Nestor, Syntex Inc. D.O.S. 2 718 010, 10.11.77, U.S. Prior. 26.4.76.
[74] H. Okumura and I. Azuma, *Agric. Biol. Chem.* (*Japan*) **47**, 847 (1983).
[75] N. E. Byars, *Infect. Immunity* **44**, 344 (1984).
[76] Swiss Pat. Appl. 27.1.1987, Prior. 27.5.1985, G. Baschang, Ciba-Geigy AG.
[77] K. I. Kamisango, I. Saiki, Y. Tanio, S. Kobayashi, T. Fukuda, I. Sekikawa, I. Azuma and Y. Yamamura, *Chem. Pharm. Bull.* (*Japan*) **29**, 1644 (1981).
[78] T. Fukuda, S. Kobayashi, H. Yukimasa, I. Imada, M. Fujino, I. Azuma and Y. Yamamura, *Chem. Pharm. Bull.* (*Japan*) **29**, 2215 (1981).
[79] T. Fukuda, S. Kobayashi, H. Yukimasa, S. Terao, M. Fujino, T. Shiba, I. Saiki, I. Azuma and Y. Yamamura, *Bull. Chem. Soc.* (*Japan*) **54**, 3530 (1981).
[80] I. Azuma, H. Okumura, I. Saiki, Y. Tanio, M. Kiso, A. Hasegawa and Y. Yamamura, *Infect. Immunity* **32**, 1305 (1981).
[81] A. Hasegawa, E. Seki, Y. Hioki, M. Kiso and I. Azuma, *Carbohydr. Res.* **129**, 271 (1984).
[82] I. Azuma, H. Okumura, I. Saiki, Y. Tanio, M. Kiso, A. Hasegawa and Y. Yamamura, *Infect. Immunity* **32**, 1305 (1981).

[83] S. Kusumoto, K. Ikenaka and T. Shiba, *Bull. Chem. Soc. (Japan)* **52**, 1665 (1979).
[84] F. Audibert, *Biochem. Biophys. Res. Commun.* **96**, 915 (1980).
[85] K. Masek, M. Zaoral, J. Jezek and V. Krchnak, *Experientia* **35**, 1397 (1979).
[86] J. Jezek, R. Straka, V. Krchnak, M. Ryba, J. Rotta, P. Mayer and M. Zaoral, *Collect. Czech. Chem. Commun.* **52**, 1609 (1987).
[87] Y. P. Abashev, T. M. Andronova, S. E. Zurabyan and A. Y. Khorlin, *Bioorg. Khim.* **7**, 980 (1981).
[88] A. Hasegawa, E. Seki, K. Kigawa, M. Kiso and I. Azuma, *Agric. Biol. Chem. (Japan)* **50**, 2133 (1986).
[89] C. Merser and P. Sinay, *Tetrahedron Lett.* 1029 (1973).
[90] H. Paulsen, P. Himpkamp and T. Peters, *Liebigs Ann. Chem.* **1986**, 664.
[91] Unpublished.
[92] A. Hasegawa, E. Tanahishi, Y. Goh, M. Kiso and I. Azuma, *Carbohydr. Res.* **92**, 75 (1981).
[93] A. Hasegawa, H. Okumura, K. Nishibori, Y. Kaneda, M. Kiso and I. Azuma, *Carbohydr. Res.* **97**, 337 (1981).
[94] T. Shiba, S. Okada, S. Kusumoto, I. Azuma and Y. Yamamura, *Bull. Chem. Soc. (Japan)* **51**, 3307 (1978).
[95] V. T. Souvannavong, A. Adam and E. Lederer, *Infect. Immunity* **19**, 966 (1978).
[96] M. J. Staruch and D. D. Wood, *J. Immunol.* **128**, 155 (1982).
[97] L. Chedid, M. Jolivet, F. Audibert, G. Przewlocki, E. H. Beachey, H. Gras-Masse and A. Tartar, *Biochem. Biophys. Res. Commun.* **117**, 908 (1983).
[98] L. Chedid, F. Audibert, M. Jolivet, C. Carelli, M. Uzan, H. Gras-Masse and A. Tartar, *Synthetic Antigens, Ann. Sclavo* **1984**, 77; Proceed. 1. Intern. Conference, Siena, 29.10.1984.
[99] M. Jolivet, F. Audibert, E. H. Beachey, A. Tartar, H. Gras-Masse and L. Chedid, *Biochem. Biophys. Res. Commun.* **117**, 359 (1983).
[100] L. Chedid, M. Jolivet, A. Morin, F. Parant, F. Audibert, F. Oberling, B. Duclos, E. H. Beachey, H. Gras-Masse and A. Tartar, *Int. J. Immunopharmacol.* 7, 398 (1985).
[101] E. Telzak, S. M. Wolff, C. A. Dinarello, T. Conlon, A. El Kholy, G. M. Bahr, J. P. Choay, A. Morin and L. Chedid, *J. Infect. Diseases* **153**, 628 (1986).
[102] F. Audibert, M. Jolivet, L. Chedid, J. E. Alouf, P. Boquet, P. Rivaille and O. Siffert, *Nature* **289**, 593 (1981).
[103] M. Jolivet, *Biological Properties of Peptidoglycan*, Eds. P. H. Seidl, K. H. Schleifer, W. de Gruyter 407–412 (1986); 2. Intern. Workshop, Munich, 20.-21.5.1985.
[104] I. Morisaki, S. M. Michalek, C. C. Harmon, M. Torii, S. Hamada and J. R. McGhee, *Infect. Immunity* **40**, 577 (1983).
[105] H. Langbeheim, R. Arnon and M. Sela, *Israel J. Med. Sci.* **13**, 1054 (1977).
[106] G. R. Dreesman, Y. Sanchez, I. Ionescu-Matiu, J. T. Sparrow, H. R. Six, D. L. Peterson, F. B. Hollinger and J. L. Melnick, *Nature* **295**, 158 (1982).
[107] F. Audibert, G. Przewlocki, P. Lefrancier, J. Choay and L. Chedid, *C.R. Acad. Sci. Paris* **295**, Serie III, 611 (1982).
[108] W. A. Siddiqui, D. W. Taylor, S. C. Kan, K. Kramer, S. M. Richmond-Crum, S. Kotani, T. Shiba and S. Kusumoto, *Science* **201**, 1237 (1978).
[109] E. R. Clough, M. Jolivet, F. Audibert, J. W. Barnwell, D. H. Schlesinger and L. Chedid, *Biochem. Biophys. Res. Commun.* **131**, 70 (1985).
[110] H. Binz, L. Z. Tarcsay, H. Wigzell and P. Dukor, *Transplant. Proceed.* **13**, 566 (1981).
[111] C. Carelli, F. Audibert, L. Chedid and J. Gaillard, *Proc. Nat. Acad. Sci.* **79**, 5392 (1982).
[112] V. C. Stevens, B. Cinader, J. E. Powell, A. C. Lee and S. W. Koh, *Am. J. Reprod. Immunol.* **1**, 315 (1981).
[113] *Ann. Inst. Pasteur/Virol* **137E**, 497 (1986).
[114] Ciba Foundation Symposium **119**, *Synthetic Peptides as Antigens*, London, 4.-6.6.1985, John Wiley (1986).
[115] R. Arnon, *Trends Biochem. Sci.* **11**, 521 (1986).
[116] R. N. Germain, *Nature* **322**, 687 (1986).
[117] P. M. Allen, *Immunology Today* **8**, 270 (1987).
[118] A. M. Livingstone and C. G. Fathman, *Ann. Rev. Immunol.* **5**, 477 (1987).
[118a] J. B. Breitmeyer, *Nature* **329**, 760 (1987).
[119] C. Leclerc, G. Przewlocki, M. P. Schütze and L. Chedid, *Eur. J. Immunol.* **17**, 269 (1987).
[120] D. Milich, A. McLachlan, G. Thornton and J. Hughes, *Nature* **329**, 547 (1987).
[121] M. J. Francis, G. Z. Hastings, A. D. Syred, B. McGinn, F. Brown and D. J. Rowlands, *Nature* **300**, 168 (1987).
[122] G. Jung, K. H. Wiesmüller, G. Becker, H. J. Bühring and W. G. Bessler, *Angew. Chemie, intern. ed.* **24**, 872 (1985).
[123] L. Chedid, M. Parant, F. Parant, P. Lefrancier, J. Choay and E. Lederer, *Proc. Nat. Acad. Sci.* **74**, 2089 (1977).
[124] F. M. Dietrich, W. Sackmann, O. Zak and P. Dukor, in: *Current Chemotherapy and Infectious Disease*, Eds. J. D. Nelson and C. Grassi, Proceed. 11. Intern. Congress of Chemotherapy, Boston 1.-5.10.1979, Vol. II, 1730–1732.
[124a] F. M. Dietrich, H. K. Hochkeppel and B. Lukas, *Int. J. Immunopharmacol.* **8**, 931 (1986).
[125] W. C. Koff, S. D. Showalter, B. Hampar and I. J. Fidler, *Science* **228**, 495 (1983).
[126] W. Brehmer, K. N. Masihi, W. Lange, E. Ribi and S. Schwartzman, *Excerpta Medica* **563**, 233 (1982).
[127] C. Leclerc, F. Audibert and L. Chedid in: *Immunochemistry of Viruses*, Eds. v. Regenmortel, M. H. V. and Neurath, A. R., Elsevier Science Publ. (1985).
[128] E. Talmagde, B. F. Lenz, M. S. Collins, K. A. Uithoven, M. A. Schneider, J. S. Adams, J. W. Pearson, W. J. Agee, R. E. Fox and R. K. Oldham, *Behring Instit. Mitt.* **74**, 219 (1984).
[128a] I. J. Fidler, M. Berendt and R. K. Oldham, *J. Biol. Response Modifiers* **1**, 15 (1982).
[129] W. E. Fogler and I. J. Fidler, *Cancer Res.* **45**, 14 (1985).
[130] I. J. Fidler, J. M. Jessup, W. E. Fogler, R. Stärkel and A. Mazumder, *Cancer Res.* **46**, 994 (1986).
[131] I. J. Fidler and A. J. Schroit, *J. Immunology* **133**, 515 (1984).
[132] S. Sone, P. Tandon, T. Utsugi, M. Ogawara, E. Shimizu, A. Nu and T. Ogura, *Int. J. Cancer* **38**, 495 (1986).

[133] T. Hamaoka and H. Fujiwara, *Immunology Today* **8**, 267 (1987).
[134] G. Barratt, J. P. Tenu and J. F. Petit, *Biological Properties of Peptidoglycan*, Eds. P. H. Seidl and K. H. Schleifer, W. de Gruyter 249–254 (1986); 2. Intern. Workshop, Munich, 20.-21.5.1985.
[135] C. A. McLaughlin, S. M. Schwartzman, B. L. Horner, G. H. Jones, J. G. Moffatt and J. J. Nestor, *Science* **208**, 415 (1980).
[136] E. Yarkoni, E. Lederer and H. J. Rapp, *Infect. Immunity* **32**, 273 (1981).
[137] N. Masihi, W. Lange, W. Brehmer and E. Ribi, *Int. J. Immunopharmacol.* **8**, 339 (1986).
[138] E. Ribi, J. L. Cantrell and K. R. Myers, *Dev. Ind. Microbiol.* **27**, 19 (1987).
[139] S. Sone and I. J. Fidler, *Cancer Immunol. Immunother.* **12**, 203 (1982).
[140] A. F. Brownbill, D. G. Braun, P. Dukor and G. Schumann, *Cancer Immunol. Immunother.* **20**, 11 (1985).
[141] Z. Xu and I. J. Fidler, *Cancer Immunol. Immunother.* **18**, 118 (1984).
[142] M. E. Key, J. E. Talmadge, W. E. Fogler, C. Bucana and I. J. Fidler, *J. Natl. Cancer Inst.* **69**, 1189 (1982).
[142a] R. Kirsh, P. J. Bugelski and G. Poste, *Ann. N.Y. Acad. Sci.* **507**, 141 (1987).
[143] N. C. Phillips, M. L. Moras, L. Chedid, P. Lefrancier and J. M. Bernard, *Cancer Res.* **45**, 128 (1985).
[144] N. C. Phillips, L. Chedid, J. M. Bernard, M. Level and P. Lefrancier, *J. Biol. Response Modif.* **6**, 678 (1987).
[145] J. E. Talmadge, B. F. Lenz, R. Klabansky, R. Simon, C. Riggs, S. Guo, R. K. Oldham and I. J. Fidler, *Cancer Res.* **46**, 1160 (1986).
[146] Swiss Pat. Appl. 25.1.1983, G. Baschang, A. Hartmann and O. Wacker, Ciba-Geigy AG.
[147] US Pat. 4 666 886A, 19.5.1987, CH Prior. 25.1.1983, *Chem. Abstracts* **108**, 38434f (1987).
[148] J. M. Krueger, J. R. Pappenheimer and M. L. Karnovsky, *J. Biol. Chem.* **257**, 1664 (1982).
[149] J. M. Krueger and M. L. Karnovsky, *Ann. N.Y. Acad. Sci.* **496**, 510 (1987).
[150] J. M. Krueger, R. S. Rosenthal, S. A. Martin, J. Walter, D. Davenne, S. Shoham, S. L. Kubillus and K. Biemann, *Brain Res.* **403**, 249 (1987).
[151] E. D. Wachsmuth, *Advances in Immunopharmacology* **3**, 482 (1986). Proceed. of 3. Intern. Conf. Immunopharmacol, Florence 6.-9.5.1985.
[152] E. D. Wachsmuth and P. Dukor, *Excerpta Medica, Intern. Congress Series* **563**, 60 (1982).
[153] K. Kakimoto, T. Tanaka and T. Koga, *Excerpta Medica* **563**, 197 (1982).
[154] W. v. Eden, J. E. R. Thole, R. v.d. Zee, A. Noordzij, J. D. A. v. Embden, E. J. Hensen and I. R. Cohen, *Nature* **331**, 171 (1988).
[155] D. B. Young, J. Ivanyi, J. H. Cox and J. R. Lamb, *Immunology Today* **8**, 215 (1987).
[156] G. Riveau, M. Parant and L. Chedid, *J. Leukocyte Biol.* **36**, 111 (1984).
[157] L. A. Chedid, M. A. Parant, F. M. Audibert, G. J. Riveau, F. J. Parant, E. Lederer, J. Choay and P. L. Lefrancier, *Infect. Immunity* **35**, 417 (1982); P. Lefrancier, M. Derrien, X. Jamet, J. Choay, E. Lederer, F. Audibert, M. Parant, F. Parant and L. Chedid, *J. Med. Chem.* **25**, 87 (1982).
[158] P. Tandon, T. Utsugi and S. Sone, *Cancer Res.* **46**, 5039 (1986).
[159] M. Parant, G. Riveau, F. Parant, C. A. Dinarello, S. M. Wolff and L. Chedid, *J. Infect. Diseases* **142**, 708 (1980).
[160] Eur. Pat. E.P. 163 286, 4.12.1985, Prior. 29.5.1984; G. Baschang, A. Hartmann and O. Wacker, Ciba-Geigy AG.
[161] M. M. Ponpipom, R. L. Bugianesi, J. C. Robbins, T. W. Döbber and T. Y. Shen, *J. Med. Chem.* **24**, 1388 (1981).
[162] G. M. Barratt, J. P. Tenu, A. Yapo and J. F. Petit, *Biochim. Biophys. Acta* **862**, 153 (1986).
[163] G. M. Barratt, D. Nolibe, A. Yapo, J. F. Petit and J. P. Tenu, *Ann. Inst. Pasteur Immunol.* **138**, 437 (1987).
[164] Eur. Pat. E.P. 233 837, 26.8.1987, Prior. 24.1.1986, B. Fechtig and G. Baschang, Ciba-Geigy AG.
[165] C. Leclerc, G. M. Bahr and L. Chedid, *Cell. Immunol.* **86**, 269 (1984).
[166] A. C. Roche, P. Bailly, P. Midoux and M. Monsigny, *Cancer Immunol. Immunother.* **18**, 155 (1984).
[167] R. S. Cotran, M. A. Gimbrone, M. P. Bevilacqua, D. L. Mendrick and J. S. Pober, *J. Exp. Med.* **164**, 661 (1986).
[168] M. P. Bevilacqua, J. S. Pober, D. L. Mendrick, R. S. Cotran and M. A. Gimbrone Jr., *Proc. Nat. Acad. Sci.* **84**, 9238 (1987).
[169] I. Saiki, L. Milas, N. Hunter and I. J. Fidler, *Cancer Res.* **46**, 4966 (1986).
[170] L. Ambler and A. M. Hudson, *Int. J. Immunopharmacol.* **6**, 119, 133 (1984).
[171] D. G. Braun, P. Dukor, B. Lukas, G. Schumann, L. Z. Tarcsay, M. Court, J. C. Schaffner, T. Skripsky, M. Fischer and P. Graepel, *Immunotoxicology*, Proceed. Intern. Seminar on the Immunol. System as a Target for Toxic Damage, Luxemburg, 6.-9.11.1984, Eds. A. Berlin, J. Dean, M. H. Draper, E. M. B. Smith and S. Spreafico, Martinus Nijhoff Publ., Dordrecht, Boston, Lancaster 219–233 (1987).
[172] H. Masayaku, K. Ono and T. Takegoshi, *Chem. Pharm. Bull. (Japan)* **33**, 5522 (1985).
[173] H. S. Warren and L. A. Chedid, *CRC Crit. Rev. Immunol.* **8**, 83 (1988).
[174] K. Masek and O. Kadlecova, *Ann. N. Y. Acad. Sci.* **496**, 517 (1987).
[175] D. H. S. Silverman, J. M. Krueger and M. L. Karnovsky, *J. Immunology* **136**, 2195 (1986).
[176] J. P. Tenu, A. C. Roche, A. Yapo, C. Kieda, M. Monsigny and J. F. Petit, *Biol. Cell* **44**, 157 (1982).
[177] M. W. Vermeulen, J. R. David and H. G. Remold, *J. Immunology* **139**, 7 (1987).
[178] V. C. Broudy, K. Kaushansky, J. M. Harlan and J. W. Adamson, *J. Immunology* **139**, 464 (1987).
[179] Immunomodulators and nonspecific host defence mechanisms against microbial infection, Eds. K. N. Masihi and W. Lange, *Advances in the Biosciences* **68**, Pergamon Press, Oxford (1988).
[180] J. P. Devlin and K. D. Hargrave, *Tetrahedron* **45**, 4327 (1989).

APPENDIX—ABBREVIATIONS AND ACRONYMS

ABA:	azobenzene arsonate
Abs:	antibody synthesis
ADCC:	antibody-dependent cell-mediated cytotoxicity
APC:	antigen-presenting cell
BCG:	bacille Calmette-Guérin
BSA:	bovine serum albumin
CD2, CD4:	cluster determinant 2, 4, surface proteins on T cells
CFU:	colony forming unit; M-: for macrophages G-: for granulocytes
CSA:	colony stimulating activity
CSF:	colony stimulating factor
DTH:	delayed-type hypersensitivity
End:	endothelial cell
Eos:	eosinophil
FcA:	Freund's complete adjuvant (with Mycobacteria)
FiA:	Freund's incomplete adjuvant (without Mycobacteria)
Fc:	fragment cristalline, stem of y-shaped immunoglobulines
Fibr.:	fibroblast
h-IFN:	human interferon γ
I-a:	protein of the MHC-complex, region I
IgG:	immunoglobulin, class G
IFN:	interferon
IL-1:	interleukin 1
i.p.:	intraperitoneal
i.v.:	intravenous
LAF-3:	lymphocyte function-associated antigen 3
LAK:	lymphokine (IL-2)-activated killer (T)-cells
Leuc. Prog.:	leucocyte progenitor cell
LGL:	large granular lymphocyte
LP-I, -II, -III:	lipopeptides type I, II, III
LPS:	lipopolysaccharide (of Gram-negative bacteria)
mAb:	monoclonal antibody
MAF:	macrophage-activating factor
MDP:	N-acetyl-muramyl-L-alanyl-D-isoglutamine
MDP-Lys(L18):	N-acetyl-muramyl-L-alanyl-D-isoglutaminyl-ε-stearoyl-L-lysine
MHC:	major histocompatibility complex
MHC-II:	protein encoded in region I of MHC, also called I-a
MLC:	mixed lymphocyte culture
MIF:	macrophage migration inhibition factor
MLV:	multilamellar vesicles
Mϕ:	macrophage
Mo:	monocyte
MTP-PE:	muramyl-tripeptide-phosphatidyl-ethanolamine
NK:	natural killer cell
nor-MDP:	N-acetyl-glucosaminyl-3-O-acetyl-L-alanyl-D-isoglutamine
Paras.:	parasite or parasite-infected cell
PG:	peptidoglycan
PGE:	prostaglandin E
PMN:	polymorphonuclear granulocyte, neutrophil
p.o.:	*per os*
PPD:	purified protein derivative (tubercle protein)
s.c.:	subcutaneous
T_C:	cytotoxic T lymphocyte
T_H:	T-helper cell
T_S:	T-suppressor cell
TCR:	T cell receptor
TNF:	tumour-necrosis factor
TT:	tetanus toxoid
Virus:	virus or virus-infected cell