Structural Aspects of Peptides with Immunomodulating Activity

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Abstract: The main function of the innate immune system from insects to mammals is to detect the presence of and act against invading microorganisms by recognizing their unique molecular signatures, most importantly, components of bacterial cell walls. A large number of peptides and derivatives, both synthetic and of natural origin, are known to influence immune responses in mammals by interacting with the conserved microbial structures, making the former attractive targets for drug development. This review focuses on structural aspects of the immunomodulating peptides and their receptors, including primary constitution, stereochemistry, conformation, binding and hydrophobic properties.

INNATE IMMUNITY

In vertebrates endowed with innate and adaptive immune systems the former is the first line of defence against invading microorganisms whereas it is the only defense in invertebrates and plants. The innate immune system, which is highly conserved from insects to mammals, recognizes pathogens using pattern recognition receptors (PRRs) that include members of the Toll-like receptor (TLR), nucleotide-binding oligomerization domain (NOD) and peptidoglycan recognition protein (PGRP) families. Microbes are carrying unique molecular signatures called pathogen-associated molecular patterns (PAMPs) which are absent from the host cells so they can serve to discriminate between self and non-self *via* binding to PRRs of the host. TLRs recognize conserved microbial structures, such as bacterial lipopolysaccharide (LPS) and viral double-stranded RNA, and activate myeloid differentiation primary-response protein 88 (MyD88) - dependent pathways to activate intracellular signalling cascades that rapidly induce the expression of a variety of overlapping and unique genes involved in the inflammatory and immune responses. NOD proteins are intracellular receptors and recognize distinct structures derived from peptidoglycan that are not ligands for TLR and do not seem to activate MyD88 (for reviews, see: [1-4]). TLRs transduce downstream signalling *via* their Toll-interleukin-1 receptor (TIR) domain. Intracellular receptors like NODs lack the TIR domain; instead, the caspase activation and recruitment domain (CARD) and PYRIN domains are involved in the signalling pathways. It was therefore proposed that TIR, CARD and PYRIN represent the three arms of innate immune detection of microorganisms in mammals [5].

Among the most important ligands for these receptor families are naturally occurring molecules that are released from microbial sources, and synthetic structures based on them. A large number of peptides and derivatives, both synthetic and of natural origin, are known to influence immune responses in mammals, making them attractive targets for drug development despite of known difficulties with rapid degradation of peptides in the organism. A lot more nonpeptidic immunomodulators of natural or synthetic origin have the potential or are being used in anti-infective therapy [6, 7]. Immunomodulating agents may either suppress or stimulate the immune response. Immunosuppressors are useful in organ transplant or autoimmune situations; stimulators or adjuvants, on the other hand, act by strengthening the host defence against pathogens. Peptide immunomodulators of both types have been discussed in an excellent review by Dutta in 2002 [8].

In the present review peptides with adjuvant activities will be discussed, with emphasis on the latest developments. A prerequisite for understanding biological function at atomic level is the knowledge of three-dimensional structures of the molecular entities involved. We will therefore focus on the structural aspects, in a broad context, of immunomodulating peptides and their receptors, including primary constitution, stereochemistry, secondary structure, conformation, binding, electrostatic and hydrophobic properties.

PEPTIDOGLYCANS

LPS, constituting the outer cell membrane of Gram-negative microorganisms, is classically regarded to be the primary agent to induce septic shock in the host organism (vide infra). The outer cell wall of Gram-positive bacteria, however, consists of a thick layer of peptidoglycan (PGN), accounting for approx. 50 % of the cell wall mass [9], intertwined with lipoteichoic acid (LTA): yet, these microorganisms are at least as efficient in inducing sepsis as the gram-negative ones [10]. LTA consists of a poly(glycerolphosphate) part connected to a gentiobiosyl diacylglycerol lipid anchor [11], its composition depending, however, on the bacterial strain [12]. LTA is similar to LPS in physicochemical properties and capable of inducing inflammatory response which is different [13] from that of LPS but of similar potency [11], or even stronger [14]. PGN is a polymer made up of alternating N-acetyl-D-glucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc: 3-*O*-(D-2-carboxy)ethyl-GlcNAc) units linked together with β-(1-4)-glycosidic bonds and crosslinked by short peptides attached to MurNAc. The amino acid at position 3 in the pending peptide chain of PGN is L-lysine in

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most Gram-positive bacteria and *meso*-diaminopimelic acid (*m*-Dap) in Gram-negative ones (Fig. **1**). The recognition of PGN by, and the mechanisms of defence response from the host's immune system have been described at length in several recent reviews (see, e.g.: [2, 9, 11, 15, 16, 17]), therefore a brief summary will suffice here.

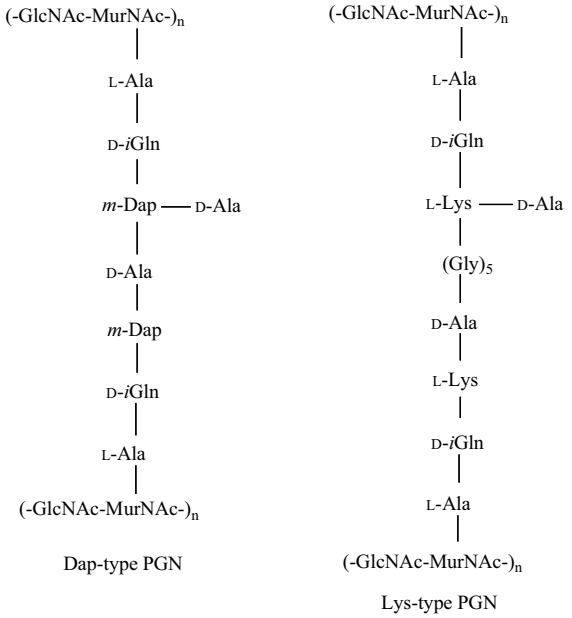


Fig. (1). Schematic structures of Dap-type and Lys-type peptidoglycan (PGN) constituents of the cell walls for Gram-negative and Gram-positive bacteria, respectively.

Insects are the best models to study innate immunity because adaptive responses may interfere with the results in mammals. In *Drosophila*, the prototype insect system, one of the most important mechanisms to fight invading bacteria is the production of anti-microbial peptides which is triggered through contact with bacterial PGN [15]. Two separate signalling pathways were identified to activate insect antimicrobial peptide genes: the *Toll* and the *Imd*. TLRs, especially TLR2, are cell surface transmembrane proteins serving as receptors for polymeric PGN (and LTA components) of Gram-positive bacteria. This function of TLRs, in insects at least, has recently been questioned suggesting that they are not PRRs to PGN but rather receptors to bind the endogenous cytokine Spätzle [15]. On the other hand, several P-GRPs on the surface of insect fat body or hemocyte cells trigger the *Imd* signalling pathway, on binding to bacterial PGN, to produce antibacterial peptides like attacin, cercopin or diptericin [15].

In mammals TLR2, which is expressed on monocytes, macrophages, dendritic cells and B-cells, *is* a receptor to polymeric PGN (but not to smaller PGN fragments) and this interaction results in the activation of NF-κB which turns on the transcription of cytokine and chemokine genes such as TNF-α and IL-8 and other mediators of inflammation. CD14, another cell-activating surface protein with multiple functions, such as LPS-binding (vide infra), is a co-receptor of TLR2. It is of note that, while LPS-signalling through the Toll pathway (using different TLRs, however) is similar to that induced by PGN, the *in vivo* effects are strikingly different: for instance, LPS is highly toxic and induces septic shock, even death; this is not the case for PGN [9, 17].

An alternative signalling pathway (in mammals) is provided by the intracellular NOD proteins: this activation is independent of TLR- and MyD88. NOD1 specifically recognizes the L-Ala-D-*i*Glu-*m*Dap sequence, characteristic for Gram-negative PGN [18], whereas the minimum PGN-fragment is MurNAc-L-Ala-D-*i*Glu (muramoyl-dipeptide: MDP) for NOD2 [17]. Human NOD1, however, was shown to specifically detect the disaccharide-tripeptide fragment GlcNAc-MurNAc-L-Ala-D-*i*Glu-*m*Dap of Gram-negative PGN [19]. MDP is a common sequence in both Gram-positive and –negative PGNs therefore, NOD2 can sense all bacteria [20]. Relatively minor differences in the primary structures of PGNs are therefore important for the recognition process leading to immune response. In other cases, the 3D spatial structure, or conformation, of epitopes are responsible for specific binding properties and, hence, biological activity, such as in the case of heterodimerization between PGRP-LCa nad PGRP-LCx induced by tracheal cytotoxin (TCT, see below, under "X-ray Structural Studies"). In order to get detected by NODs bacterial PGNs have to enter the host cells and generate NOD-activating fragments inside; this occurs, however, only with certain types of bacteria [17].

PGRPs are recently discovered [21-23] actors playing important roles in the innate immunity of animals from insects to mammals with recognition, signalling and effector functions. Insect PGRPs are involved in *Toll* and *Imd* activation mechanisms, as mentioned before, as well as in the *prophenoloxidase* cascade [21, 24] which results in the generation of polymeric melanin that promotes healing at the site of the infection. In mammals four PGRPs have been identified thus far: PGLYRP1 to -4 (formerly: PGRP-S, PGRP-L, PGRP-Iα and PGRP-Iβ, respectively; this nomenclature will be used throughout the text). The main function of mammalian PGRPs, especially that of PGRP-S which is an N-acetylmuramoyl-L-alanine amidase, seems to be digestion of bacterial PGN rather than action as PRRs, as is the case for their insect counterparts [17]. Nevertheless, the role of PGRPs in mammals is still not clear [10, 17, 25]. The bacteriolytic activity of lysozyme, a glucanohydrolase that cleaves the glycosidic bond between GlcNAc and MurNAc units of PGN, has long been known. The amidase and glycosidase activities of PGRPs both have the important function to turn off excessive immune response [15]. Bacterial LPS, on the opposite, is likely to overactivate the host's immune response: a dangerous course of events which may even lead to death of the host by septic shock (vide infra).

MUROPEPTIDES

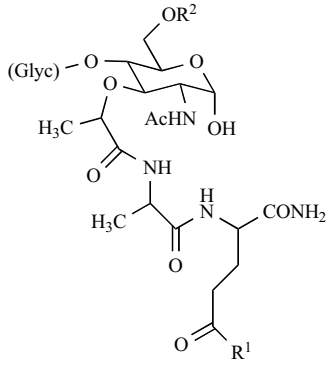
One of the main weapons the innate immune systems uses, from insects to mammals, to combat bacterial infections are antimicrobial peptides (AMPs) produced by the hosts. Some aspects of antimicrobial peptides will be discussed later in this article. In what follows we are going to briefly discuss PGN-related peptides and their activity to modulate the immune response, including induction of AMPs. The immunostimulatory activity of bacterial cell walls has long

been recognized: "Freund's adjuvant" to treat pulmonary infection consisted of a suspension in oil of killed mycobacteria [26]. Later it turned out that the cell wall peptidoglycan was responsible for this activity in several inflammatory diseases like arthritis, meningitis or septic shock. Analysis of peptidoglycan fragments led to the identification of MurNAc-L-Ala-D-*i*Gln ("muramyl dipeptide": MDP, Fig. **2**) as the minimal structure to possess biological activity [27-31]. Following this discovery hundreds of derivatives have been synthesized and tested *in vitro* and *in vivo* (for reviews, see: http://www.infectio-lille.com/diaporamas/xdlt/murabutide-02.PDF, [32-34]).

In addition to have an adjuvant effect by itself, MDP is known to synergize with LPS on the induction of synthesis of proinflammatory cytokines like TNF-α. It was also shown that MDP acts through receptors other than those responsible for transducing the effects of LPS (CD14, TLR2 or TLR4) [35]. This receptor was then identified to be the NOD2 [36, 37]. Subsequent studies showed that, in addition to MDP, GlcNAc-β(1-4)-MDP and 6-O-stearoyl-MDP were also capable of dramatically enhance LPS-induced cytokine release through monocyte activation [38].

A simple derivative of MDP, murabutide (MB; MurNAc-L-Ala-D-*i*Gln-*n*-butyl ester), shows interesting pharmacological properties and is well tolerated even by humans [39, 40]. MB was found to selectively activate CD4 lymphocytes leading to dramatic suppression of HIV replication *in vitro*. Several clinical trials indicated that the diminished innate immunity in HIV-infected subjects could be successfully boosted by administration of MB [39-42]. On the other hand, antigen-specific IgE responses were down-regulated in mice by MDP and MB [43]. MB was proposed to improve the therapeutic index interferon-alpha (IFN-α) in clinical trials. Co-administration of MB and IFN-α was well tolerated and resulted in induction of anti-inflammatory cytokines and of human HIV-1-suppressive β-chemokines [44].

Lipophilic derivatives of MDP such as B30-MDP (MDP acylated with a C30 saturated fatty acid at C-6 of MurNAc) and MDP-Lys(18) or romurtide (N^{ε}-stearoyl-MurNAc-L-Ala-D-iGln-L-Lys) display adjuvant activities on the induction of antibody response antigens or vaccines, such as hepatitis B surface antigen [45, 46], tetanus toxoid vaccine [47], or a hantavirus-inactivated vaccine [48]. MDP-Lys(L18) was found to be a potent immunoadjuvant that enhances non-



	(Glyc)	R^1	R^2
MDP	H	OH	H
GMDP	βGlcNAc	OH	H
Murabutide (MB)	H	$CH_3(CH_2)_3$-O-	H
6-O-stearoyl-MDP	H	OH	$CH_3(CH_2)_{16}$-CO-
B30-MDP	H	OH	$CH_3(CH_2)_{13}$ / $CH_3(CH_2)_{13}$ > CH-CO-
Romurtide [MDP-Lys(L18)]	H	-NH-CH-COOH | $(CH_2)_4$-NH-CO | $CH_3(CH_2)_{16}$	H
GMTP-N-DPG	βGlcNAc	CH_3 | -NH-CH-CO-NH-CH_2 | $CH_3(CH_2)_{14}COOCH$ | $CH_3(CH_2)_{14}COOCH_2$	H

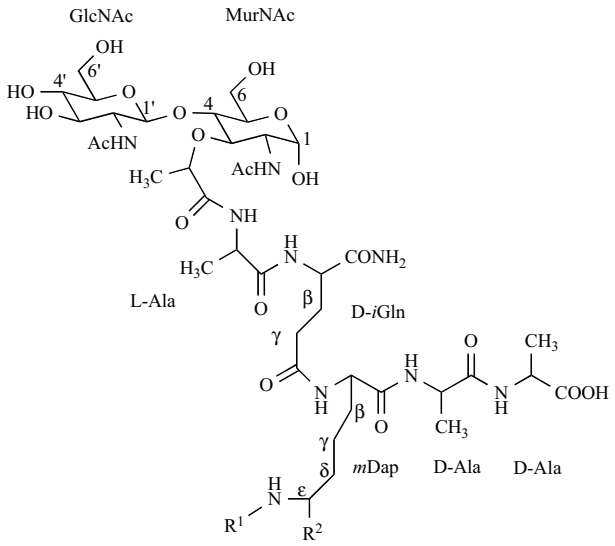
Fig. (2). Structures of MDP and its derivatives discussed in the text.

specific host resistance against mucosal Sendai and rotaviruses [49] or against a Hantaan virus strain in newborn mice [50]. Another lipophilic derivative, GlcNAc-β(1-4)-MDP-L-Ala-dipalmitoylpropylamide (GMTP-N-DPG) had adjuvant effects in mice immunized with ovalbumin (OVA) and synthetic peptide antigens [51]. It was suggested that addition of the surfactant dimethyldioctadecylammonium chloride and zinc as an L-proline complex to GlcNAc-β(1-4)-MDP has a synergistic effect on this adjuvant [52]. Production of anti-sporozoite antibodies were observed by coupling an MB-analogue, ε-amino-caproic ester of MDP, to a malaria peptide-tetanus toxoid conjugate [53, 54].

Dimers of the D-glucose analogue of MDP (GADP) showed stimulatory activities against human leukemia cells *in vitro* [55]. There are indications on the potential usefulness of muropeptides in cancer treatment. For instance, MB was found to trigger the maturation and activation of monocyte-derived immature dendritic cells conferring them greater cytostatic activity toward the tumor cell line THP-1 [56]. MDP-Lys(L18) (romurtide) is capable of enhancing host resistance and reducing tumor metastasis in murine melanoma, colon carcinoma and T lymphoma cells [57]. The same agent is clinically effective in restoration of leukocytes and platelets of cancer patients treated with chemo- or radiation therapy [33, 34]. The surface of melanoma BRO cells was shown to contain binding sites for GlcNAc-β(1-4)-MDP (GMDP). These cells were shown to react *in vitro* with GMDP by increasing the expression of melanoma-associated antigens (MAA) [58].

Peptidoglycan monomer (GlcNAc-β(1-4)-MurNAc-L-Ala-D-*i*Gln-*m*Dap-D-Ala-D-Ala: PGM, Fig. **3**) isolated from the cell wall of the Gram-negative *Brevibacterium divaricatum* is a non-toxic, non-pyrogenic immunostimulator. Detailed NMR and computational studies revealed its amphipatic character with well-separated lipophilic and hydrophilic domains [59]. It is not known whether this property plays any role in the adjuvant activity; lipophilic PGM derivatives display similar activities in immune tests (vide infra). On the other hand, lipophilic substituents attached to MDP resulted in modulation of the activity of the parent molecule in various ways as mentioned above. PGM treatment of mice challenged with ovalbumin (OVA) resulted in stimulation of IFN-γ and IL-4 production as well as both Th1 and Th2 subpopulations [60]. PGM enhanced the immunogenicity of peptides of measles virus origin [61]; co-administration with liposomes increased the adjuvant activity and induced a switch from Th1 to Th2 type of immune response [62]. It is rapidly degraded in mammals, and its metabolic products, the pentapeptide (PP) and the disaccharide (DS), are devoid of adjuvant activity [63]. Lipophilic derivatives of PGM bearing either (adamant-1-yl)-acetyl- or Boc-Tyr substituents at the ε-amino group of Dap (Ad-PGM and BocTyr-PGM, respectively, Fig. **3**) were shown by NMR and molecular modelling to assume conformations different from that of the parent PGM in solution (Fig. **4**) [64]. Their immunostimulating activities were, however, comparable to that of PGM [65].

Diastereoisomeric adamantyltripeptides, D- or L-(adamant-2-yl)-Gly-L-Ala-D-*i*Gln (AdTP1 and AdTP2), also exhibited immunostimulating activities [66]. Several dimeric



Source	R^1	R^2
Gram-positive	H	H
Gram-negative	H	COOH
Brevibacterium Divaricatum (PGM)	H	$CONH_2$
Ad-PGM	Ad	$CONH_2$
BocTyr-PGM	Boc-Tyr	$CONH_2$

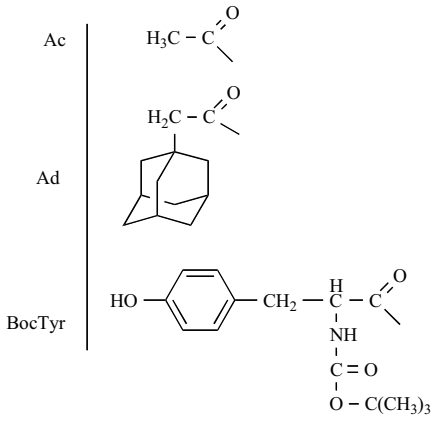


Fig. (3). Structures of peptidoglycan monomers (PGMs) and their derivatives.

PGM-type muropeptides have been synthesized with the general structure GlcNAc-β(1-4)-MurNAc(pept)-β(1-4)-GlcNAc-β(1-4)-MurNAc(pept)-β-OR, with R=H or *n*-propyl, and (pept) being di- to pentapeptide sequences of the Lys-type PGNs attached to D-Lac at position 3 of MurNAc (Fig. **5**).

Compound with R=*n*-propyl and (pep)= L-Ala-D-*i*Gln-L-Lys-D-Ala-D-Ala was found to act as a competitive inhibitor of a soluble PGRP involved in the PGN-induced melanization cascade in the insect *Tenebrio molitor* [67]. In a paper mentioned above, a series of muropeptides, MurNAc(pept) and 1,6-anhydro-MurNAc(pept), with (pept) extending from

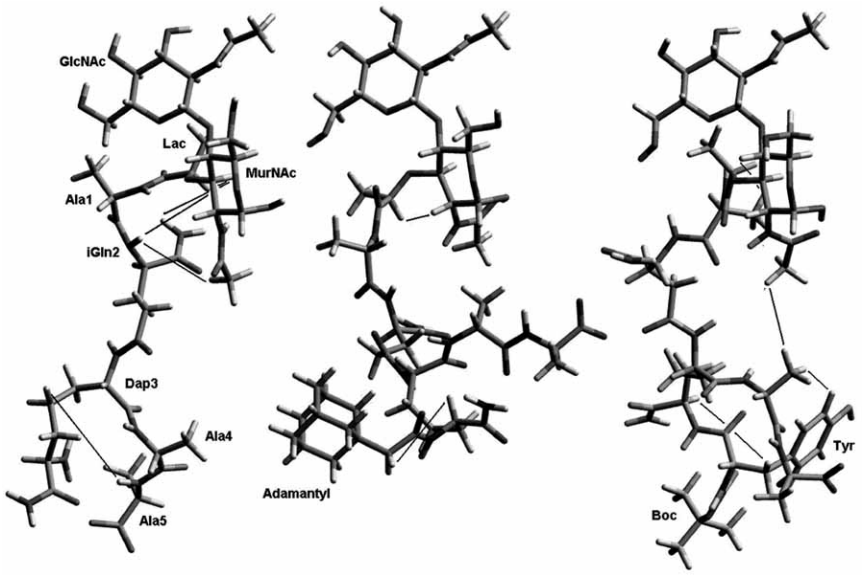


Fig. (4). Solution structures of PGM (left), Ad-PGM (centre) and BocTyr-PGM (right) as determined by NMR and molecular modelling. Thin lines indicate short (<5Å) interatomic distances as detected by NMR.

L-Ala through the tetrapeptide chain of Lys and Dap PGN-peptides, were synthesized and used to determine the structural requirements to synergize with LPS in cytokine induction [38]. Likewise, the molecular patterns allowing specific recognition by NOD1 and NOD2 were mapped using a wide array of natural or modified muramyl peptides [68]. In another study, using a series of synthetic muropeptides, two signatures of Gram-negative PGN, the presence of Dap in the peptide bridge and 1,6-anhydro-MurNAc in the glycan chain, were identified as the main structural requirements to allow discrimination of Gram-negative PGNs from Gram-positive ones [69]. MurNAc-pentapeptides (MPP), TCT, as well as a dimeric Lys-type MPP cross-linked through the peptide stem, were used to define the binding specificities of human and insect PGRPs, and it was suggested that dual strategies are used to distinguish PGNs from different bacteria: differences in the peptide stem (Lys or Dap) and detection of the crosslink between the stems. As little as a two amino acid mutation in the PGRP sequence suffices, however, to change the specificity of recognition. This observation points to an adaptive character of the innate immune system helping to counter new microbial challenges [70]. Also, the extent of recognition selectivity is variable; for instance, PGRP-IαC shows only minimal selectivity for binding Dap- or Lys-type fragments, PGRP-S, on the other hand, displays significant preference for the Dap-type [71].

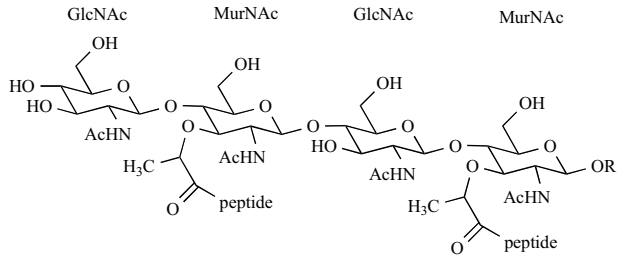


Fig. (5). Structures of dimeric PGM-type muropeptides.

Tracheal cytotoxin (TCT, GlcNAc-1,6-anhydro-MurNAc-L-Ala-D-*i*Glu-*m*Dap-D-Ala, Fig. **6**), a natural fragment of Gram-negative PGN, such as the cell wall of *Bordetella pertussis*, elicits immune responses in *Drosophila* through the *Imd* pathway [72]. In addition to immunostimulating activity [73] this muropeptide has the interesting property of being a very potent somnogenic: it significantly enhances the duration of slow wave sleep (SWS) in mammals [74]. This activity may be related to the conformation of the 1,6-anhydro-bridged MurNAc that is very different from that of the non-bridged, monocyclic glucopyranose ring. The somnogenicity is likely to be due to the indirect effect of inducing endogenous sleep factors like IL-1, TNF and NO [75]. The mammalian receptor for TCT has long been sought [69, 72] but could only recently be identified as NOD1; the detection is, however, host specific: it is poor in humans but very efficient in mice [76].

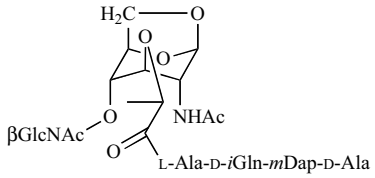


Fig. (6). Structure of tracheal cytotoxin (TCT).

X-RAY STRUCTURAL STUDIES

Considerable attention has recently been focussed on elucidating the structural basis for PGN binding by PGRPs using X-ray crystallography. The crystal structures of two *Drosophila* [77-80] and two human PGRPs [81-83] were reported and shown to possess L-shaped grooves supposed to be the PGN binding site. However, to get information on the

interactions between the proteins and their substrates requires studies of PGRP-ligand complexes. This has been done first for the C-terminal binding domain of human PGRP-Iα in complex with MurNAc-L-Ala-D-*i*Gln-L-Lys (MTP of Gram-positive bacteria), revealing an extensive network of H-bond- and van der Waals contacts of MTP with 16 residues of the protein's binding cleft. Most contacts were with the peptide part and only a few with the MurNAc. No significant conformational change of the protein was detected as a result of the binding [82]. A subsequent structure determination of the same protein complexed, however, with the complete peptide stem of PGN, i.e. MurNAc-L-Ala-D-*i*Gln-L-Lys-D-Ala-D-Ala (muramyl pentapeptide, MPP) revealed ligand-induced conformational changes in the binding cleft, and this was suggested to occur in many PGRPs [84]. Tracheal cytotoxin (TCT, vide supra) is known to induce heterodimerization between PGRP-LCa and PGRP-LCx that elicits immune responses in *Drosophila* by activating the *Imd* pathway [69]. The crystal structure of ectodomains of PGRP-LCa and PGRP-LCx bridged by TCT shows that the latter binds to LCx in the ternary LCx-TCT-LCa complex through its peptide chain and exposes the disaccharide part for interaction with LCa [85]. This is in line with results obtained by biochemical methods [86]. Both proteins undergo induced-fit conformational changes during this process. Bringing together the PGRP-LCx and –LCa receptors represents the activation step for the *Imd* pathway [85]. PGRP-LE, another protein involved in the *Imd* signalling, also binds TCT. A crystallographic study revealed that TCT induces an infinite head-to-tail dimerization in which the disaccharide moiety occupies the dimer interface [87]; this is analogous to the TCT-induced heterodimerization of PGRP-LCx and –LCa just mentioned. In both cases the Dap carboxylate group engages in a key electrostatic interaction with the guanidino sidechain of an Arg: this provides a basis for the discrimination between Gram-negative and –positive (Dap or Lys, respectively) types of PGN by PGRPs [85, 87].

ANTI-ENDOTOXIC PEPTIDES

LPS (endotoxin), the main constituent of the outer membrane of Gram-negative bacteria, is an amphiphile, consisting of the lipid A, a core oligosaccharide and an O-specific chain. The lipid A moiety (Fig. **7**) is the most highly conserved part of the structure, typically with two glucosamines, two phosphate esters and five to seven fatty acids [88]. When LPS molecules are released from the surface of bacteria, they can cause septic shock in the infected patient. The principal mechanism by which LPS is sensed is *via* an LPS-binding protein (LBP)–LPS complex and then signalling through the TLR4–MD-2 complex, a process enhanced by CD14 [89].

Neutralization and sequestration of LPS is required to block the progression of Gram-negative sepsis at early stages in addition to destroying bacteria. Polymyxin B, a small cyclic lipopeptide produced by *Bacillus polymyxa*, neutralizes LPS and has antimicrobial activity particularly against Gram-negative bacteria [90]. Due to its toxicity application of polymyxin B is limited to topical applications. Nevertheless polymyxin B is extensively used as a golden standard for benchmarking the efficiency of other compounds in neutralization of endotoxin. Development of novel endotoxin

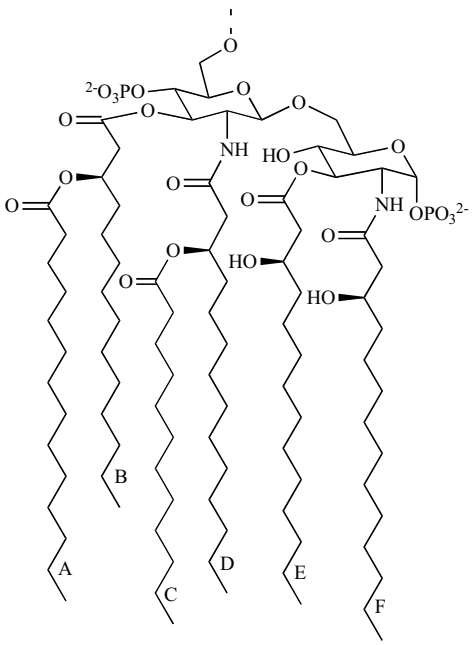


Fig. (7). Chemical structure of lipid A as found in *E. coli* strains.

neutralizing peptides without the toxicity of polymyxin B has been based on natural host defence peptides, fragments of LPS binding proteins and engineered peptides. Non-peptidic substances that have been recently described to display suppressive activity for the production of inflammatory cytokines induced by LPS-stimulated macrophages include alkyl 6-(N-substituted sulfamoyl)cyclohex-1-ene-1-carboxylate [91], furonaphthoquinones [92], coumarin – [93] and 4-aryl-3-pyridyl and 4-aryl-3-pyrimidinyl-based compounds [94], 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (PGG) from traditional Chinese herbs [95], acylated homologated spermine compounds [96], sesquiterpene phenylpropanoid derivatives [97], isosteric analogues of thalidomide [98] and bis-guanylhydrazone compounds decorated with hydrophobic functionalities [99]; they will, however, not be discussed further in this review.

The design of anti-endotoxin peptides has proven to be very difficult. Peptides in general are intrinsically flexible molecules; in addition, complexes of LPS and peptides are transient formations with unclear stoichiometry. The methods that have been applied to overcome this limitation and the large panel of peptides and proteins reported to associate with LPS have been extensively discussed in earlier reviews [100-104]. In this review we present the recent developments and state of the art in experimental and theoretical structural studies of the proteins and peptides in question, and the impact of these findings on treatment of sepsis and rational drug design.

PEPTIDES DERIVED FROM PROTEINS THAT INTERACT WITH LPS

The ability of synthetic peptides comprising the human LBP amino acid region 86-108 to interfere with the LBP-LPS interaction was reported. The analysis of selected alanine mutants of a blocking peptide corresponding to the

LBP region 86-99 suggests the importance of peptide amphipathicity for the inhibitory activity. The potency of the native peptide and a selected analogue at inhibiting LPS-induced responses was associated with their relative activity in blocking LBP-LPS interaction. Remarkably, these peptides were more active *in vivo* than *in vitro* [105]. The peptide LBP-14 (RVQGRWKVRASFFK-NH_2) derived from the same residues 86-99 of LBP was studied using NMR; in mixture with LPS the transferred NOE [106] was observed and used to determine the LPS-bound structure of LBP-14; the latter was used for docking calculations to LPS, and the derived complex used to design a peptide that displayed more than 50% increase in LPS inhibition *in vitro* [107].

An NMR and modelling study of LALF-14 (GCKPTFRR LKWKYKCG-NH_2), a synthetic cyclized fragment of the limulus anti-LPS factor (LALF) comprising residues 36-47, indicated that the β-sheet-like structure that is adopted by the fragment in the full-size protein [108] is not a prerequisite for binding of LALF-14 to LPS [109]. Cyclic cationic antimicrobial peptides of different length based on LALF were synthesized; the peptide comprising the complete LPS-binding loop of LALF was most effective in inhibiting the LPS-induced cytokine production in human mononuclear cells. The peptides were also able to displace Ca^{2+} cations from LPS monolayers [110]. Whereas a synthetic linear peptide $LALF_{28-54}$ based on the endotoxin-binding region of LALF retained activity, cyclization was associated with a decrease in the *in vitro* potency, suggesting that cyclization does not constrain the peptide in a manner that recreates the loop structure necessary for potent endotoxin antagonism [111].

A new LPS-binding domain with a strong LPS-neutralizing activity in human CD14 was identified in residues 81-100 by mapping the entire 356-amino-acid protein with synthetic 20-amino-acid peptides. The residues Leu 87, 91, and 94 were found to be essential for activity [112]. An 11-amino-acid amphipathic synthetic peptide derived from helix-1 of human lactoferrin exhibited bactericidal activity and lipopolysaccharide (LPS) binding affinity, as shown by polymyxin B displacement. Circular dichroism (CD) spectroscopy indicated that in the presence of LPS it adopted a β-strand rather than helical conformation [113]. Synthetic peptides derived from human and bovine lactoferricin were assayed for antimicrobial activity against *E. coli* and LPS mutant strains, underlining the importance of the content and relative location of tryptophan and arginine residues. Results obtained for the same assays performed with LPS mutants suggested that negative charges present in the inner core but not lipid A are the main binding site for lactoferricin; a computer model proposed that positively charged residues of the cationic peptide interact with negative charges carried by LPS and disorganise the structure of the outer membrane, thus facilitating the approach of Trp residues to the lipid A [114].

The short linear antimicrobial and endotoxin-neutralizing peptide LF11 (FQWQRNIRKVR-NH_2) based on human lactoferrin was found to bind to LPS inducing a peptide fold with a "T"-shaped arrangement consisting of a hydrophobic core and two clusters of basic residues matching the distance between the two phosphate groups of LPS [115]; this mode of binding extended the previously proposed, purely electrostatic LPS binding pattern [116], emphasizing also the importance of hydrophobic interactions in a defined geometric arrangement. In anionic micelles LF11 forms an amphipathic conformation with a smaller hydrophobic core than upon interaction with LPS, whereas in zwitterionic micelles the structure is less defined. Protection of Trp quenching in the order SDS>LPS>DPC and hydrogen exchange protection indicates the decreasing extent of insertion of the N terminus and a potential role of peptide plasticity in differentiation between bacterial and eukaryotic membranes.

Acyl analogues of peptidic fragments of human lactoferrin with weak antibacterial activity were prepared. It was found that 12 carbon units constitute the optimal acyl chain length, enhancing the antibacterial activity and binding of lipopolysaccharide by up to two orders of magnitude, even approaching the activity of polymyxin B [117]. The alkyl derivative lauryl-LF11 shows a stronger inhibition of LPS-induced cytokine induction in human mononuclear cells than LF11. Both peptides convert the mixed unilamellar/non-lamellar aggregate structure of lipid A into a multilamellar one [118].

Bactericidal/permeability-increasing protein (BPI), a 55 kDa protein causing bacterial killing and endotoxin neutralization, also has a β-turn with alternating cationic and hydrophobic residues in the putative LPS-binding domain [119]. A peptide, betapep25, was designed with 9 residues of the LPS-binding domain of BPI flanked by β-turn-inducing elements. Single alanine or norleucine replacement "walkthrough" peptides based on betapep25 were generated and tested for their ability to neutralize endotoxin [120]. Substitution of any lysine residue inhibited bactericidal activity while substitutions in hydrophobic residues from the beta-turn-inducing regions resulted in peptides that exhibited increased bactericidal activity compared to betapep25. Inhibition of LPS-neutralizing activity was seen in peptides with substituted basic or hydrophobic residues from the LPS-binding region of BPI, indicating the importance of both cationic and hydrophobic amino acid residues to bactericidal and endotoxin-neutralizing activities.

NK-2, a membrane-acting antimicrobial peptide of 27 residues derived from the cationic core region of porcine NK-lysin, a cytotoxic and antibacterial polypeptide of 9 kDa, adopts an amphipathic α-helical secondary structure, interacting specifically with membranes of negatively charged lipids. NK-2 binds to LPS with a high affinity and induces a change in the LPS aggregate structure from a cubic or unilamellar structure to a multilamellar one, leading to neutralization of the endotoxic LPS activity [121].

NATURAL ANTIMICROBIAL PEPTIDES

Both animals and plants possess a broad-spectrum of potent antimicrobial peptides, which they use to fend off a wide range of microbes, including bacteria, fungi, viruses and protozoa (for recent reviews, see: [122-126]). The structures of antimicrobial peptides and their interactions with bacterial membranes as studied by solution and solid-state NMR are the subject of a recent survey by Wang [127]. Currently there are more than 880 known antimicrobial peptides of eukaryotic origin (http://www.bbcm.univ.trieste.it/~tossi/antimic.html, http://aps.unmc.edu/AP/main.php, http://

research.i2r.astar.edu.sg/Templar/DB/ANTIMIC/). Many of the host defence peptides that have antimicrobial activity are also able to bind to and neutralize LPS; these two activities, however, do not necessarily correlate. The mode of action of many antibacterial peptides is believed to be the disruption of the plasma membrane. Studies on model phospholipid membranes have demonstrated that peptides that act preferentially on bacteria are also able to interact with and permeate efficiently anionic phospholipids, whereas peptides that lyse mammalian cells bind and permeate efficiently both acidic and zwitterionic phospholipids membranes, mimicking the plasma membranes of these cells [128].

A key feature of the recognition between cationic antibacterial peptides and endotoxin is plasticity of molecular interactions, which may have been designed for the purpose of maintaining activity against a broad range of organisms, a hallmark of primitive host defense [129]. Studies using a phage-displayed random dodecapeptide library against immobilized LPS suggested that for these short dodecapeptides to bind LPS, the potential for their structural adaptation is more important than an amphipathic structure [130].

A rational method has been proposed to dissociate the antimicrobial and hemolytic effects of cationic peptides, using site-directed substitutions of residues based on a quantitative structure-activity relationship (QSAR) analysis of designed peptide sequences and their antimicrobial, cytotoxic, and hemolytic activities [131]. The mechanism of LPS neutralization by human LL-37, magainin and others were investigated using fluorescence spectroscopy, confocal microscopy and flow cytometry, concluding that a strong binding of a peptide to LPS aggregates accompanied by aggregate dissociation prevents LPS from binding to the carrier protein LBP, or alternatively to its receptor, inhibiting cytokine secretion [132]. Heptadepsin, a novel naturally occurring cyclic heptadepsipeptide isolated from secondary metabolites of *Paenibacillus sp.*, was shown to inactivate LPS by direct interaction with LPS [133]. The antimicrobial peptide V4 was designed based on a known amphipathic cationic pattern BHPHB (B: basic; H: hydrophobic; P: polar residue, respectively) and has high binding affinity for LPS [134]. Factor C, a serine protease in the horseshoe crab ameobocytes, is sensitive to trace levels of LPS, most probably possessing an LPS binding region that exhibits exceptionally high affinity for lipid A. A peptide derived from the Sushi 3 domain of Factor C exhibits stronger binding to LPS when dimerized *via* a disulfide bridge. Circular dichroism spectrometry revealed that the S3 peptide undergoes conformational change in the presence of the disulfide bridge, transitioning from a random coil to beta-sheet structure. S3 dimers display detergent-like properties in disrupting LPS micelles [135]. A Sushi peptide from the LPS-binding domains of Factor C, able to inhibit LPS-induced septic shock in mice, was reported to interact with the lipid tail of LPS, transitioning from a random structure into an alpha-helical conformation [136]. Compounds of the cathelicidin family exhibited antimicrobial and anti-endotoxin properties [137, 138]. Fowlicidins, cathelicidins from chicken, demonstrated a strong positive cooperativity in binding lipopolysaccharide (LPS), resulting in nearly complete blockage of LPS-mediated proinflammatory gene expression [139]. Synthetic peptides derived from the beetle *Allomyrina dichotoma* defensin exhibited not only antimicrobial activities but also had a protective effect on LPS-induced mortality in mouse models [140].

These results suggest the potential use of natural antimicrobial peptides in the treatment of severe sepsis providing the problems of toxicity and fast degradation can be solved in further development of synthetic analogues.

CONCLUSIONS AND OUTLOOK

New approaches to the therapy of sepsis and antibiotic-resistant organisms are urgently needed. As the understanding of the MD-2-TLR4-LBP-CD14 pathway unfolds new targets that modify these pathways may be effective lead compounds in the treatment of septic shock. Similarly, gaining insight into the interactions of innate immune receptors with microbial PGNs and fragments will provide a basis for designing improved vaccine adjuvants and immunomodulators for the treatment of inflammatory diseases. The possibility of peptides and peptoids (peptide mimics) with increased half-life being able to protect against the severe clinical symptoms of inflammations, induced by LPS or other cell wall components, is a promising development.

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ABBREVIATIONS

BPI	=	Bactericidal/permeability-increasing protein
CARD	=	Caspase activation and recruitment domain
*m*Dap	=	*meso*-diaminopimelic acid
GlcNAc	=	N-acetyl-D-glucosamine
IFN	=	Interferon
IL	=	Interleukin
LBP	=	LPS-binding protein
LPS	=	Lipopolysaccharide
LTA	=	Lipoteichoic acid
MB	=	Murabutide
MDP	=	Muramoyl-dipeptide
MurNAc	=	N-acetylmuramic acid
MyD88	=	Myeloid differentiation primary-response protein 88
NMR	=	Nuclear magnetic resonance
NOD	=	Nucleotide-binding oligomerization domain
PAMP	=	Pathogen-associated molecular pattern
PGRP	=	Peptidoglycan recognition protein
PGM	=	Peptidoglycan monomer
PGN	=	Peptidoglycan

PRR = Pattern recognition receptor

PYRIN = Domains located at the N terminus of proteins linked to several human diseases, from cancer to inflammatory syndromes. Originally referred to the product of the familial Mediterranean fever (FMF)-associated gene

TIR = Toll-interleukin-1 receptor

TNF = Tumour necrosis factor

TLR = Toll-like receptor

REFERENCES

[1] Barton, G. M.; Medzhitov, R. *Science,* **2003,** *300*, 1524.
[2] Ulevitch, R. J. *Nat. Rev. Immunol.,* **2004,** *4*, 512.
[3] Akira, S.; Uematsu, S.; Takeuchi, O. *Cell,* **2006,** *124*, 783.
[4] O'Neill, L. A. *Curr. Opin. Immunol.,* **2006,** *18*, 3-9.
[5] Werts, C.; Girardin, S. E.; Philpott, D. J. *Cell Death Differ.,* **2006,** *13*, 798.
[6] Fasciano, S.; Li, L. W. *Curr. Med. Chem.,* **2006,** *13*, 1389.
[7] Kayser, O.; Masihi, K., N; Kiderlen, A., F. *Exp. Rev. Anti Infect. Ther.,* **2003,** *1*, 319.
[8] Dutta, R. C. *Immunol. Lett.,* **2002,** *83*, 153.
[9] Dziarski, R. *Cell. Mol. Life Sci.,* **2003,** *60*, 1793.
[10] Myhre, A. E.; Aasen, A. O.; Thiemermann, C.; Wang, J. E. *Shock,* **2006,** *25*, 227.
[11] Fournier, B.; Philpott, D. J. *Clin. Microbiol. Rev.,* **2005,** *18*, 521.
[12] Henneke, P.; Morath, S.; Uematsu, S.; Weichert, S.; Pfitzenmaier, M.; Takeuchi, O.; Muller, A.; Poyart, C.; Akira, S.; Berner, R.; Teti, G.; Geyer, A.; Hartung, T.; Trieu-Cuot, P.; Kasper, D. L.; Golenbock, D. T. *J. Immunol.,* **2005,** *174*, 6449.
[13] Strandberg, Y.; Gray, C.; Vuocolo, T.; Donaldson, L.; Broadway, M.; Tellam, R. *Cytokine,* **2005,** *31*, 72.
[14] von Aulock, S.; Schroder, N. W. J.; Traub, S.; Gueinzius, K.; Lorenz, E.; Hartung, T.; Schumann, R. R.; Hermann, C. *Infect. Immunity,* **2004,** *72*, 1828.
[15] Steiner, H. *Immuno. Rev.,* **2004,** *198*, 83.
[16] Dziarski, R. *Mol. Immunol.,* **2004,** *40*, 877.
[17] Dziarski, R.; Gupta, D. *J. Endotoxin Res.,* **2005,** *11*, 304.
[18] Girardin, S. E.; Jehanno, M.; Mengin-Lecreulx, D.; Sansonetti, P. J.; Alzari, P. M.; Philpott, D. J. *J. Biol. Chem.,* **2005,** *280*, 38648.
[19] Girardin, S. E.; Boneca, I. G.; Carneiro, L. A.; Antignac, A.; Jehanno, M.; Viala, J.; Tedin, K.; Taha, M. K.; Labigne, A.; Zahringer, U.; Coyle, A. J.; DiStefano, P. S.; Bertin, J.; Sansonetti, P. J.; Philpott, D. J. *Science,* **2003,** *300*, 1584.
[20] Viala, M.; Sansonetti, P.; Philpott, D. J. *C. R. Biologies,* **2004,** *327*, 551.
[21] Yoshida, H.; Kinoshita, K.; Ashida, M. *J. Biol. Chem.,* **1996,** *271*, 13854.
[22] Kang, D. W.; Liu, G.; Lundstrom, A.; Gelius, E.; Steiner, H. *Proc. Natl. Acad. Sci. USA,* **1998,** *95*, 10078.
[23] Liu, C.; Xu, Z. J.; Gupta, D.; Dziarski, R. *J. Biol. Chem.,* **2001,** *276*, 34686.
[24] Takehana, A.; Katsuyama, T.; Yano, T.; Oshima, Y.; Takada, H.; Aigaki, T.; Kurata, S. *Proc. Natl. Acad. Sci. USA,* **2002,** *99*, 13705.
[25] Girardin, S. E.; Philpott, D. J. *Immunity,* **2006,** *24*, 363.
[26] Freund, J. *Adv. Tubercol. Res.,* **1956,** *1*, 130.
[27] Ellouz, F.; Adam, A.; Ciobaru, R.; Lederer, E. *Biochem. Biophys. Res. Commun.,* **1974,** *59*, 1317.
[28] Kotani, S.; Watanabe, Y.; Kinoshita, F.; Shimono, T.; Morisaki, I.; Shiba, T.; Kusumoto, S.; Tarumi, Y.; Ikenaka, K. *Biken J.,* **1975,** *18*, 105.
[29] Azuma, I.; Sugimura, K.; Taniyama, T.; Yamawaki, M.; Yamamura, Y.; Kusumoto, S.; Okada, S.; Shiba, T. *Infect. Immunity,* **1976,** *14*, 18.
[30] Chedid, L.; Audibert, F.; Lefrancier, P.; Choay, J.; Lederer, E. *Proc. Natl. Acad. Sci. USA,* **1976,** *73*, 2472.
[31] Kotani, S.; Watanabe, Y.; Shimono, T.; Harada, K.; Shiba, T.; Kusumoto, S.; Yokogawa, K.; Taniguchi, M. *Biken J.,* **1976,** *19*, 9.
[32] Chedid, L.; Audibert, F.; Jolivet, M. *Dev. Biol. Stand.,* **1986,** *63*, 133.
[33] Azuma, I. *Vaccine,* **1992,** *10*, 1000.
[34] Azuma, I. *Int. J. Immunopharmacol.,* **1992,** *14*, 487.
[35] Wolfert, M. A.; Murray, T. F.; Boons, G. J.; Moore, J. N. *J. Biol. Chem.,* **2002,** *277*, 39179.
[36] Girardin, S. E.; Boneca, I. G.; Viala, J.; Chamaillard, M.; Labigne, A.; Thomas, G.; Philpott, D. J.; Sansonetti, P. J. *J. Biol. Chem.,* **2003,** *278*, 8869.
[37] Inohara, N.; Ogura, Y.; Fontalba, A.; Gutierrez, O.; Pons, F.; Crespo, J.; Fukase, K.; Inamura, S.; Kusumoto, S.; Hashimoto, M.; Foster, S. J.; Moran, A. P.; Fernandez-Luna, J. L.; Nunez, G. *J. Biol. Chem.,* **2003,** *278*, 5509.
[38] Traub, S.; Kubasch, N.; Morath, S.; Kresse, M.; Hartung, T.; Schmidt, R. R.; Hermann, C. *J. Biol. Chem.,* **2004,** *279*, 8694.
[39] Bahr, G. M.; De La Tribonniere, X.; Darcissac, E.; Ajana, F.; Bocket, L.; Sissoko, D.; Yazdanpanah, Y.; Dewulf, J.; Amiel, C.; Mouton, Y. *J. Antimicrob. Chemother.,* **2003,** *51*, 1377.
[40] Goasduff, T.; Darcissac, E. C. A.; Vidal, V.; Capron, A.; Bahr, G. M. *Clin. Exp. Immunol.,* **2002,** *128*, 474.
[41] Amiel, C.; De La Tribonniere, X.; Vidal, V.; Darcissac, E.; Mouton, Y.; Bahr, G. M. *J. Acquir. Immune Defic. Syndr.,* **2002,** *30*, 294.
[42] Vidal, V. F.; Casteran, N.; Riendeau, C. J.; Kornfeld, H.; Darcissac, E. C. A.; Capron, A.; Bahr, G. M. *Eur. J. Immunol.,* **2001,** *31*, 1962.
[43] Auci, D. L.; Carucci, J. A.; Chice, S. M.; Smith, M. C.; Dukor, P.; Durkin, H. G. *Int. Arch. Allergy Immunol.,* **1993,** *101*, 167.
[44] Darcissac, E. C. A.; Vidal, V.; Guillaume, M.; Thebault, J. J.; Bahr, G. M. *J. Interferon Cytokine Res.,* **2001,** *21*, 655.
[45] Tamura, M.; Yoo, Y. C.; Yoshimatsu, K.; Yoshida, R.; Oka, T.; Ohkuma, K.; Arikawa, J.; Azuma, I. *Vaccine,* **1995,** *13*, 77.
[46] Przewlocki, G.; Audibert, F.; Jolivet, M.; Chedid, L.; Kent, S. B. H.; Neurath, A. R. *Biochem. Biophys. Res. Commun.,* **1986,** *140*, 557.
[47] Telzak, E.; Wolff, S. M.; Dinarello, C. A.; Conlon, T.; Elkholy, A.; Bahr, G. M.; Choay, J. P.; Morin, A.; Chedid, L. *J. Infect. Diseases,* **1986,** *153*, 628.
[48] Yoo, Y. C.; Yoshimatsu, K.; Koike, Y.; Hatsuse, R.; Yamanishi, K.; Tanishita, O.; Arikawa, J.; Azuma, I. *Vaccine,* **1998,** *16*, 216.
[49] Fukushima, A.; Yoo, Y. C.; Yoshimatsu, K.; Matsuzawa, K.; Tamura, M.; Tonooka, S.; Taniguchi, K.; Urasawa, S.; Arikawa, J.; Azuma, I. *Vaccine,* **1996,** *14*, 485.
[50] Yoo, Y. C.; Yoshimatsu, K.; Hatsuse, R.; Tamura, M.; Yoshida, R.; Tonooka, S.; Arikawa, J.; Azuma, I. *Vaccine,* **1995,** *13*, 1300.
[51] Fast, D. J.; Vosika, G. J. *Vaccine,* **1997,** *15*, 1748.
[52] Grubhofer, N. *Immunol. Lett.,* **1995,** *44*, 19.
[53] Clough, E. R.; Audibert, F. M.; Barnwell, J. W.; Schlesinger, D. H.; Arnon, R.; Chedid, L. A. *Infect. Immunity,* **1985,** *48*, 839.
[54] Clough, E. R.; Jolivet, M.; Audibert, F.; Barnwell, J. W.; Schlesinger, D. H.; Chedid, L. *Biochem. Biophys. Res. Commun.,* **1985,** *131*, 70.
[55] Murata, J.; Kitamoto, T.; Ohya, Y.; Ouchi, T. *Carbohydr. Res.,* **1997,** *297*, 127.
[56] Vidal, V.; Dewulf, J.; Bahr, G. M. *Immunology,* **2001,** *103*, 479.
[57] Yoo, Y. C.; Saiki, I.; Sato, K.; Azuma, I. *Vaccine,* **1994,** *12*, 175.
[58] Petrova, E. E.; Valyakina, T. I.; Khaidukov, S. V.; Nesmeyanov, V. A. *Bioorg. Khim.,* **2001,** *27*, 218.
[59] Matter, H.; Szilágyi, L.; Forgó, P.; Marinić, Z.; Klaić, B. *J. Am. Chem. Soc.,* **1997,** *119*, 2212.
[60] Spoljar, B. H.; Cimbora, T.; Hanzl-Dujmović, I.; Dojnović, B.; Sabioncello, A.; Krstanović, M.; Tomašić, J. *Vaccine,* **2002,** *20*, 3543.
[61] Halassy, B.; Mateljak, S.; Bouche, F. B.; Putz, M. M.; Muller, C. P.; Frkanec, R.; Habjanec, L.; Tomašić, J. *Vaccine,* **2006,** *24*, 185.
[62] Habjanec, L.; Frkanec, R.; Halassy, B.; Tomašic, J. *J. Liposome Res.,* **2006,** *16*, 1.
[63] Halassy, B.; Krstanović, M.; Frkanec, R.; Tomašić, J. *Vaccine,* **2003,** *21*, 971.
[64] Fehér, K.; Pristovšek, P.; Szilágyi, L.; Ljevaković, D.; Tomašić, J. *Bioorg. Med. Chem.,* **2003,** *11*, 3133.
[65] Ljevaković, D.; Tomašić, J.; Sporec, V.; Spoljar, B. H.; Hanzl-Dujmović, I. *Bioorg. Med. Chem.,* **2000,** *8*, 2441.
[66] Tomašić, J.; Hanzl-Dujmović, I.; Spoljar, B.; Vranešić, B.; Santak, M.; Jovičić, A. *Vaccine,* **2000,** *18*, 1236.
[67] Park, J. W.; Je, B. R.; Piao, S.; Inamura, S.; Fujimoto, Y.; Fukase, K.; Kusumoto, S.; Soderhall, K.; Ha, N. C.; Lee, B. L. *J. Biol. Chem.,* **2006,** *281*, 7747.
[68] Girardin, S. E.; Travassos, L. H.; Herve, M.; Blanot, D.; Boneca, I. G.; Philpott, D. J.; Sansonetti, P. J.; Mengin-Lecreulx, D. *J. Biol. Chem.,* **2003,** *278*, 41702.
[69] Stenbak, C. R.; Ryu, J. H.; Leulier, F.; Pili-Floury, S.; Parquet, C.; Herve, M.; Chaput, C.; Boneca, I. G.; Lee, W. J.; Lemaitre, B.; Mengin-Lecreulx, D. *J. Immunol.,* **2004,** *173*, 7339.

[70] Swaminathan, C. P.; Brown, P. H.; Roychowdhury, A.; Wang, Q.; Guan, R. J.; Silverman, N.; Goldman, W. E.; Boons, G. J.; Mariuzza, R. A. *Proc. Natl. Acad. Sci. USA,* **2006,** *103*, 684.
[71] Kumar, S.; Roychowdhury, A.; Ember, B.; Wang, Q.; Guan, R. J.; Mariuzza, R. A.; Boons, G. J. *J. Biol. Chem.,* **2005,** *280*, 37005.
[72] Kaneko, T.; Goldman, W. E.; Mellroth, P.; Steiner, H.; Fukase, K.; Kusumoto, S.; Harley, W.; Fox, A.; Golenbock, D.; Silverman, N. *Immunity,* **2004,** *20*, 637.
[73] Dokter, W. H. A.; Dijkstra, A. J.; Koopmans, S. B.; Stulp, B. K.; Keck, W.; Halie, M. R.; Vellenga, E. *J. Biol. Chem.,* **1994,** *269*, 4201.
[74] Johannsen, L.; Obal, F.; Kapas, L.; Kovalzon, V.; Krueger, J. M. *Int. J. Immunopharmacol.,***1994,** *16*, 109.
[75] Chen, L. C.; Taishi, P.; Majde, J. A.; Peterfi, Z.; Obal, F.; Krueger, J. M. *Brain Behav. Immun.,* **2004,** *18*, 390.
[76] Magalhaes, J. G.; Philpott, D. J.; Nahori, M. A.; Jehanno, M.; Fritz, J.; Le Bourhis, L.; Viala, J.; Hugot, J. P.; Giovannini, M.; Bertin, J.; Lepoivre, M.; Mengin-Lecreulx, D.; Sansonetti, P. J.; Girardin, S. E. *EMBO Rep.,* **2005,** *6*, 1201.
[77] Chang, C. I.; Ihara, K.; Chelliah, Y.; Mengin-Lecreulx, D.; Wakatsuki, S.; Deisenhofer, J. *Proc. Natl. Acad. Sci. USA,* **2005,** *102*, 10279.
[78] Chang, C. I.; Pili-Floury, S.; Herve, M.; Parquet, C.; Chelliah, Y.; Lemaitre, B.; Mengin-Lecreulx, D.; Deisenhofer, J. *PLOS Biol.,* **2004,** *2*, 1293.
[79] Kim, M. S.; Byun, M. J.; Oh, B. H. *Nat. Immunol.,* **2003,** *4*, 787.
[80] Reiser, J. B.; Teyton, L.; Wilson, I. A. *J. Mol. Biol.,* **2004,** *340*, 909.
[81] Guan, R. J.; Malchiodi, E. L.; Wang, Q.; Schuck, P.; Mariuzza, R. A. *J. Biol. Chem.,* **2004,** *279*, 31873.
[82] Guan, R. J.; Roychowdhury, A.; Ember, B.; Kumar, S.; Boons, G. J.; Mariuzza, R. A. *Proc. Natl. Acad. Sci. USA,* **2004,** *101*, 17168.
[83] Guan, R. J.; Wang, Q.; Sundberg, E. J.; Mariuzza, R. A. *J. Mol. Biol.,* **2005,** *347*, 683.
[84] Guan, R. J.; Brown, P. H.; Swaminathan, C. P.; Roychowdhury, A.; Boons, G. J.; Mariuzza, R. A. *Protein Sci.,* **2006,** *15*, 1199.
[85] Chang, C. I.; Chelliah, Y.; Borek, D.; Mengin-Lecreulx, D.; Deisenhofer, J. *Science,* **2006,** *311*, 1761.
[86] Mellroth, P.; Karlsson, J.; Hakansson, J.; Schultz, N.; Goldman, W. E.; Steiner, H. *Proc. Natl. Acad. Sci. USA,* **2005,** *102*, 6455.
[87] Lim, J. H.; Kim, M. S.; Kim, H. E.; Yano, T.; Oshima, Y.; Aggarwal, K.; Goldman, W. E.; Silverman, N.; Kurata, S.; Oh, B. H. *J. Biol. Chem.,* **2006,** *281*, 8286.
[88] Raetz, C. H. R.; Whitfield, C. *Annu. Rev. Biochem.,* **2002,** *71*, 635.
[89] Cohen, J. *Nature,* **2002,** *420*, 885.
[90] Morrison, D. C.; Jacobs, D. M. *Immunochemistry,* **1976,** *13*, 813.
[91] Yamada, M.; Ichikawa, T.; Ii, M.; Sunamoto, M.; Itoh, K.; Tamura, N.; Kitazaki, T. *J. Med. Chem.,* **2005,** *48*, 7457.
[92] Kim, M. H.; Shin, H. M.; Lee, Y. R.; Chung, E. Y.; Chang, Y. S.; Min, K. R.; Kim, Y. *Arch. Pharm. Res.,* **2005,** *28*, 1170.
[93] Cheng, J. F.; Chen, M.; Wallace, D.; Tith, S.; Arrhenius, T.; Kashiwagi, H.; Ono, Y.; Ishikawa, A.; Sato, H.; Kozono, T.; Sato, H.; Nadzan, A. M. *Bioorg. Med. Chem. Lett.,* **2004,** *14*, 2411.
[94] Laughlin, S. K.; Clark, M. P.; Djung, J. F.; Golebiowski, A.; Brugel, T. A.; Sabat, M.; Bookland, R. G.; Laufersweiler, M. J.; VanRens, J. C.; Townes, J. A.; De, B.; Hsieh, L. C.; Xu, S. C.; Walter, R. L.; Mekel, M. J.; Janusz, M. J. *Bioorg Med. Chem. Lett.,* **2005,** *15*, 2399.
[95] Genfa, L.; Jiang, Z.; Hong, Z.; Yimin, Z.; Liangxi, W.; Guo, W.; Ming, H.; Donglen, J.; Lizhao, W. *Int. Immunopharmacol.,* **2005,** *5*, 1007.
[96] Miller, K. A.; Suresh Kumar, E. V.; Wood, S. J.; Cromer, J. R.; Datta, A.; David, S. A. *J. Med. Chem.,* **2005,** *48*, 2589.
[97] Motai, T.; Kitanaka, S. *J. Nat. Prod.,* **2005,** *68*, 365.
[98] Zhu, X.; Giordano, T.; Yu, Q. S.; Holloway, H. W.; Perry, T. A.; Lahiri, D. K.; Brossi, A.; Greig, N. H. *J. Med. Chem.,* **2003,** *46*, 5222.
[99] Khownium, K.; Wood, S. J.; Miller, K. A.; Balakrishna, R.; Nguyen, T. B.; Kimbrell, M. R.; Georg, G. I.; David, S. A. *Bioorg. Med. Chem. Lett.,* **2006,** *16*, 1305.
[100] Pristovšek, P.; Kidrič, J. *Mini Rev. Med. Chem.,* **2001,** *1*, 409.
[101] Pristovšek, P.; Kidrič, J. In *Drug Discovery and Design: Medical Aspects;* Matsoukas, J.; Mavromoustakos, T. Eds.; IOS Press: Amsterdam, **2002**; Vol. *55*, pp. 161.
[102] Jerala, R.; Porro, M. *Curr. Top. Med. Chem.,* **2004,** *4*, 1173.
[103] Pristovšek, P.; Kidrič, J. *Curr. Top. Med. Chem.,* **2004,** *4*, 1185.
[104] Chaby, R. *Cell. Mol. Life Sci.,* **2004,** *61*, 1697.
[105] Arana Mde, J.; Vallespi, M. G.; Chinea, G.; Vallespi, G. V.; Rodriguez-Alonso, I.; Garay, H. E.; Buurman, W. A.; Reyes, O. *J. Endotoxin Res.,* **2003,** *9*, 281.
[106] Clore, G. M.; Gronenborn, A. M. *J. Magn. Reson.,* **1982,** *48*, 402.
[107] Pristovšek, P.; Simčič, S.; Wraber, B.; Urleb, U. *J. Med. Chem.,* **2005,** *48*, 7911.
[108] Hoess, A.; Watson, S.; Silber, G. R.; Liddington, R. *EMBO J.,* **1993,** *12*, 3351.
[109] Pristovšek, P.; Fehér, K.; Szilágyi, L.; Kidrič, J. *J. Med. Chem.,* **2005,** *48*, 1666.
[110] Andra, J.; Lamata, M.; Martinez de Tejada, G.; Bartels, R.; Koch, M. H.; Brandenburg, K. *Biochem. Pharmacol.,* **2004,** *68*, 1297.
[111] Leslie, D. B.; Vietzen, P. S.; Lazaron, V.; Wasiluk, K. R.; Dunn, D. L. *Surg. Infect. (Larchmt),* **2006,** *7*, 45.
[112] Voss, S.; Welte, S.; Fotin-Mleczek, M.; Fischer, R.; Ulmer, A. J.; Jung, G.; Wiesmuller, K. H.; Brock, R. *Chembiochem,* **2006,** *7*, 275.
[113] Chapple, D. S.; Hussain, R.; Joannou, C. L.; Hancock, R. E.; Odell, E.; Evans, R. W.; Siligardi, G. *Antimicrob. Agents Chemother.,* **2004,** *48*, 2190.
[114] Farnaud, S.; Spiller, C.; Moriarty, L. C.; Patel, A.; Gant, V.; Odell, E. W.; Evans, R. W. *FEMS Microbiol. Lett.,* **2004,** *233*, 193.
[115] Japelj, B.; Pristovšek, P.; Majerle, A.; Jerala, R. *J. Biol. Chem.,* **2005,** *280*, 16955.
[116] Ferguson, A. D.; Welte, W.; Hofmann, E.; Lindner, B.; Holst, O.; Coulton, J. W.; Diederichs, K. *Structure,* **2000,** *8*, 585.
[117] Majerle, A.; Kidrič, J.; Jerala, R. *J. Antimicrob. Chemother.,* **2003,** *51*, 1159.
[118] Andra, J.; Lohner, K.; Blondelle, S. E.; Jerala, R.; Moriyon, I.; Koch, M. H.; Garidel, P.; Brandenburg, K. *Biochem. J.,* **2005,** *385*, 135.
[119] Beamer, L. J.; Carroll, S. F.; Eisenberg, D. *Science,* **1997,** *76*, 1861.
[120] Wasiluk, K. R.; Leslie, D. B.; Vietzen, P. S.; Mayo, K. H.; Dunn, D. L. *Surgery,* **2004,** *136*, 253.
[121] Andra, J.; Koch, M. H.; Bartels, R.; Brandenburg, K. *Antimicrob. Agents Chemother.,* **2004,** *48*, 1593.
[122] Brown, K. L.; Hancock, R. E. W. *Curr. Opin. Immunol.,* **2006,** *18*, 24.
[123] Sitaram, N. *Curr. Med. Chem.,* **2006,** *13*, 679.
[124] Bulet, P.; Stocklin, R. *Protein Pept. Lett.,* **2005,** *12*, 3.
[125] Imler, J.-L.; Bulet, P. In *Chem. Immunol. Allergy,* Kabelitz, D.; Schröder, J.-M. Eds.; Karger: Basel, **2005**; Vol. *86*, pp. 1-21.
[126] Zasloff, M. *Nature,* **2002,** *415*, 389.
[127] Wang, G. S. *Curr. Org. Chem.,* **2006,** *10*, 569.
[128] Papo, N.; Shai, Y. *Peptides,* **2003,** *24*, 1693.
[129] Nagpal, S.; Kaur, K. J.; Jain, D.; Salunke, D. M. *Protein Sci.,* **2002,** *11*, 2158.
[130] Zhu, Y.; Ho, B.; Ding, J. L. *Biochim. Biophys. Acta,* **2003,** *1611*, 234.
[131] Frecer, V.; Ho, B.; Ding, J. L. *Antimicrob. Agents Chemother.,* **2004,** *48*, 3349.
[132] Rosenfeld, Y.; Papo, N.; Shai, Y. *J. Biol. Chem.,* **2006,** *281*, 1636.
[133] Ohno, O.; Ikeda, Y.; Sawa, R.; Igarashi, M.; Kinoshita, N.; Suzuki, Y.; Miyake, K.; Umezawa, K. *Chem. Biol.,* **2004,** *11*, 1059.
[134] Yu, L.; Ding, J. L.; Ho, B.; Wohland, T. *Biochim. Biophys. Acta.,* **2005,** *1716*, 29.
[135] Li, P.; Wohland, T.; Ho, B.; Ding, J. L. *J. Biol. Chem.,* **2004,** *279*, 50150.
[136] Li, P.; Sun, M.; Wohland, T.; Ho, B.; Ding, J. L. *Cell. Mol. Immunol.,* **2006,** *3*, 21.
[137] Ghiselli, R.; Cirioni, O.; Giacometti, A.; Mocchegiani, F.; Orlando, F.; Bergnach, C.; Skerlavaj, B.; Silvestri, C.; Vittoria, A. D.; Zanetti, M.; Rocchi, M.; Scalise, G.; Saba, V. *Peptides,* **2006,** *27*, 2592.
[138] Cirioni, O.; Giacometti, A.; Ghiselli, R.; Bergnach, C.; Orlando, F.; Silvestri, C.; Mocchegiani, F.; Licci, A.; Skerlavaj, B.; Rocchi, M.; Saba, V.; Zanetti, M.; Scalise, G. *Antimicrob. Agents Chemother.,* **2006,** *50*, 1672.
[139] Xiao, Y.; Cai, Y.; Bommineni, Y. R.; Fernando, S. C.; Prakash, O.; Gilliland, S. E.; Zhang, G. *J. Biol. Chem.,* **2006,** *281*, 2858.
[140] Koyama, Y.; Motobu, M.; Hikosaka, K.; Yamada, M.; Nakamura, K.; Saido-Sakanaka, H.; Asaoka, A.; Yamakawa, M.; Sekikawa, K.; Kitani, H.; Shimura, K.; Nakai, Y.; Hirota, Y. *Int. Immunopharmacol.,* **2006,** *6*, 234.

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