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CUTTING EDGE

Cutting Edge: Cationic Antimicrobial Peptides Block the Binding of Lipopolysaccharide (LPS) to LPS Binding Protein[1]

Monisha G. Scott,[2]* Anita C. E. Vreugdenhil,† Wim A. Buurman,† Robert E. W. Hancock,* and Michael R. Gold*

We investigated the mechanism by which cationic antimicrobial peptides block the activation of macrophages by LPS. The initial step in LPS signaling is the transfer of LPS to CD14 by LPS binding protein (LBP). Because many cationic antimicrobial peptides bind LPS, we asked whether these peptides block the binding of LPS to LBP. Using an assay that measures the binding of LPS to immobilized LBP, we show for the first time that a variety of structurally diverse cationic antimicrobial peptides block the interaction of LPS with LBP. The relative ability of different cationic peptides to block the binding of LPS to LBP correlated with their ability to block LPS-induced TNF-α production by the RAW 264.7 macrophage cell line. ***The Journal of Immunology*****, 2000, 164: 549–553.**

Inflammation and sepsis caused by Gram-negative bacteria are due primarily to LPS that is released from the bacterial outer membrane. Treatment of Gram-negative bacterial infections would be greatly aided by substances that can effectively block LPS-induced production of inflammatory mediators. Cationic antimicrobial peptides are an evolutionarily ancient component of the innate immune system that block many of the actions of LPS (1). In mammals, these peptides are found in the blood, in secretions, and in neutrophil granules. In vitro, naturally occurring cationic peptides, as well as synthetic analogues block the ability of LPS to stimulate the production of TNF-α, IL-6, and other inflammatory mediators (2–9). Moreover, studies in mice have shown that cationic peptides can block endotoxin-induced TNF-α release and reduce the mortality associated with endotoxemia in the galactosamine-sensitized mouse model (2, 4, 6, 9). Although several cationic peptides have been shown to bind LPS (2, 7, 10), little is known about how they block the biological effects of LPS.

The mechanism by which LPS activates macrophages is now understood in some detail. LPS binding protein (LBP),[3] an acute-phase reactant that is present in the blood, binds LPS, extracts it from micelles, and transfers it to CD14, a protein that exists as a soluble form in blood and as a GPI-linked molecule on the surface of monocytes and macrophages. LPS·CD14 complexes are thought to initiate intracellular signaling reactions by binding to Toll-like receptors (TLRs) on macrophages and other cells (11). TLR4 appears to be required for LPS to initiate signaling and to induce inflammatory responses. Inbred strains of mice with loss-of-function mutations in TLR4 do not respond to LPS (12, 13). LPS·CD14 complexes activate the NF-κB transcription factor as well as the extracellular signal-regulated kinase, c-Jun N-terminal kinase, and p38 mitogen-activated protein kinases, all of which mediate the production of inflammatory cytokines (11, 14–16). By facilitating the initial step in LPS signaling, the binding of LPS to CD14, LBP greatly enhances the efficiency of LPS signaling such that 100-fold less LPS is required to trigger cytokine secretion (17).

Because cationic antimicrobial peptides that block LPS-induced macrophage activation can bind to LPS (2, 4, 5, 10, 18–20), we hypothesized that these cationic peptides act by blocking the binding of LPS to LBP. To test this, we used an ELISA-type assay that measures the ability of biotinylated LPS to bind to immobilized LBP. Using this assay, we analyzed the ability of structurally diverse cationic peptides to block the binding of LPS to LBP. We show that peptides belonging to all of the major structural groups of antimicrobial peptides can block the binding of LPS to LBP in the assay. In general, the relative ability of the different peptides to block the binding of LPS to LBP in vitro correlated with their ability to block LPS-induced production of TNF-α by RAW 264.7 cells. Thus, the ability of cationic peptides to block macrophage activation by LPS may be due in large part to their ability to block the binding of LPS to LBP and prevent the LBP-mediated transfer of LPS to CD14.

*Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada; and †Department of General Surgery, Maastricht University, Maastricht, The Netherlands

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[2] Address correspondence and reprint requests to Dr. Robert E. W. Hancock, Department of Microbiology and Immunology, University of British Columbia, 6174 University Boulevard, Vancouver, British Columbia, Canada V6T 1Z3. E-mail address: bob@cmdr.ubc.ca

[3] Abbreviations used in this paper: LBP, LPS binding protein; Bac, bactenecin; HNP-1, human neutrophil peptide-1; HBD-2, human β-defensin-2.

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Table I. *Peptides used in this study*

Peptide	Structure	Amino Acid Sequence[a]
HBD-2	β-sheet	TCLKSGAICHPVFCPRRYKQIGTCGLPGTKCCKKP
HNP-1	β-sheet	ACYCRIPACIAGERRYGTCIYQGRLWAFCC
Gramicidin S	β-structured loop	Cyclic (PF^{d}LOVPF^{d}LOV)
Polymyxin B	Cyclic lipopeptide	Cyclized isooctanoyl BTBB(BF^{d}LBBT)[c]
Indolicidin	Extended	ILPWKWPWWPWRR-NH_2
CP11CN[b]	Extended	ILKKWPWWPWRRK-NH_2
Bac 2A-NH_2[b]	Linear	RLARIVVIRVAR-NH_2
CEMA[b]	α-helical	KWKLFKKIGIGAVLKVLTTGLPALKLTK
CP29[b]	α-helical	KWKSFIKKLTTAVKKVLTTGLPALIS
CP208[b]	α-helical	KKKSFIKLLTSAKVSVLTTAKPLISS

[a] Single letter amino acid code: O, ornithine; B, diaminobutyrate; F^d, the D enantiomer of phenylalanine.
[b] Synthetic peptides.
[c] Sequence in parentheses is cyclized.

Materials and Methods

Reagents

Escherichia coli O55:B5 LPS was purchased from Sigma (St. Louis, MO) and biotinylated using biotin-LC-hydrazide (Pierce, Rockford, IL) as described previously (21). CEMA, CP29, CP208, bactenecin (Bac) 2A-NH_2, indolicidin, and CP11CN were synthesized at the Nucleic Acid/Protein Synthesis Unit at the University of British Columbia as described previously (7). Gramicidin S and human neutrophil peptide-1 (HNP-1) were purchased from Sigma. Human β-defensin-2 (HBD-2) was kindly provided by Dr. T. Ganz (University of California, Los Angeles, CA). The amino acid sequences of these peptides are shown in Table I.

Purification of recombinant LBP

Chineses hamster ovary cells expressing human recombinant LBP were a gift from Dr. P. Tobias. The recombinant LBP was purified from the culture medium as described previously (21) and its concentration determined by ELISA (22).

Measurement of LPS-LBP interactions

The anti-LBP mAb HM14 (21), which recognizes human LBP as well as LBP·LPS complexes, was diluted to 10 μg/ml in PBS and adsorbed onto 96-well Nunc MaxiSorp ELISA plates (Nunc, Rochester, NY) overnight at 4°C. Plates were blocked at room temperature for 1 h with PBS/1% BSA and washed with 0.1% Tween 20 in dH_2O. Recombinant LBP (50 ng/ml) diluted in PBS/0.1% BSA was added to the plates for 1.5 h at room temperature. After washing the plates, biotinylated LPS was added in the presence or absence of cationic peptides. Where indicated, the peptides were either preincubated with biotinylated LPS for 30 min or added to the wells at various times after the addition of biotinylated LPS. In all cases, the plates were washed 1 h after the addition of LPS. Binding of the biotinylated LPS to the immobilized LBP was detected using HRP-conjugated streptavidin diluted 1:2000 in PBS/0.1% BSA. After a 1 h incubation, 3,3′,5′5-tetramethylbenzidine (Sigma) was added. The reactions were stopped after 15 min by adding 0.1 ml of 0.18 M sulfuric acid, and the absorbance at 450 nm was determined using an ELISA reader.

TNF-α production by RAW 264.7 cells

The murine macrophage cell line RAW 264.7 was obtained from American Type Culture Collection (Manassas, VA) and grown in DMEM supplemented with 10% FCS. The cells were plated at 10^6 cells/well in 24-well plates, incubated overnight, then stimulated with LPS for 6 h. The culture supernatants were assayed for TNF-α using an ELISA (Endogen, Hornby, ON) that could detect <50 pg/ml TNF-α.

Results

Cationic antimicrobial peptides block the interaction of LPS with LBP

The mechanism by which cationic antimicrobial peptides block the activation of macrophages by LPS has not been elucidated. We hypothesized that they block the first step in the process, the interaction of LPS with LBP. To test this, we used an assay that measures the binding of biotinylated LPS to immobilized LBP. Fig. 1 shows that amounts of LPS ranging from 10 to 500 ng/ml produced a linear binding curve. Because previous studies showed that the CP29 cecropin:melittin hybrid cationic antimicrobial peptide effectively inhibited LPS-induced TNF-α production by RAW 264.7 cells (7), we asked whether this peptide could block the binding of biotinylated LPS to LBP in this assay. When 10 μg/ml (3.4 μM) CP29 was added at the same time as LPS, it substantially reduced the ability of the LPS to bind to LBP (Fig. 1). The dose-response curve for LPS binding was shifted ~10-fold, i.e., in the presence of the CP29 peptide 10 times as much LPS was required to yield the same amount of LPS bound to LBP compared with when the peptide was not present. CP29 reduced the binding of LPS to LBP almost completely when 45 ng/ml LPS was added to the well and by >80% when 450 ng/ml LPS was added to the well. Thus, the CP29 cationic peptide strongly inhibits the binding of LPS to LBP.

The dose-response and kinetic characteristics for the cationic peptide inhibition of LPS binding to LBP are shown in Fig. 2. In these experiments, we analyzed the inhibition of the LPS-LBP interaction by the CP29 peptide as well as by polymyxin B, a

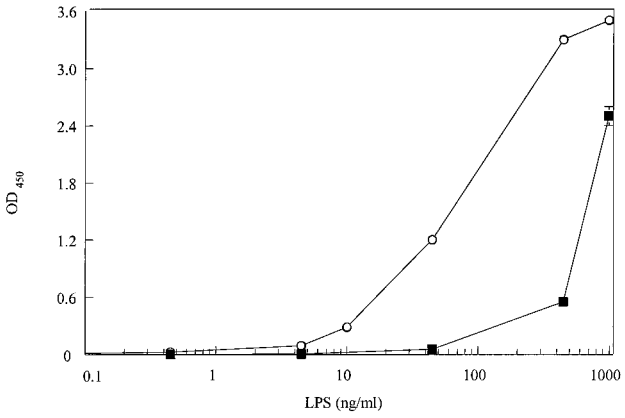

FIGURE 1. Inhibition of the binding of biotinylated LPS to immobilized LBP by CP29. Biotinylated *E. coli* O55:B5 LPS (○) or biotinylated LPS plus 10 μg/ml (3.4 μM) CP29 (■) were added to wells containing recombinant LBP immobilized using anti-LBP Abs. Binding of the biotinylated LPS to the immobilized LBP was detected using HRP-conjugated streptavidin. The values represent the mean and SE for the averages of duplicate samples from three independent experiments. Where no error bars are shown, they were smaller than the symbols.

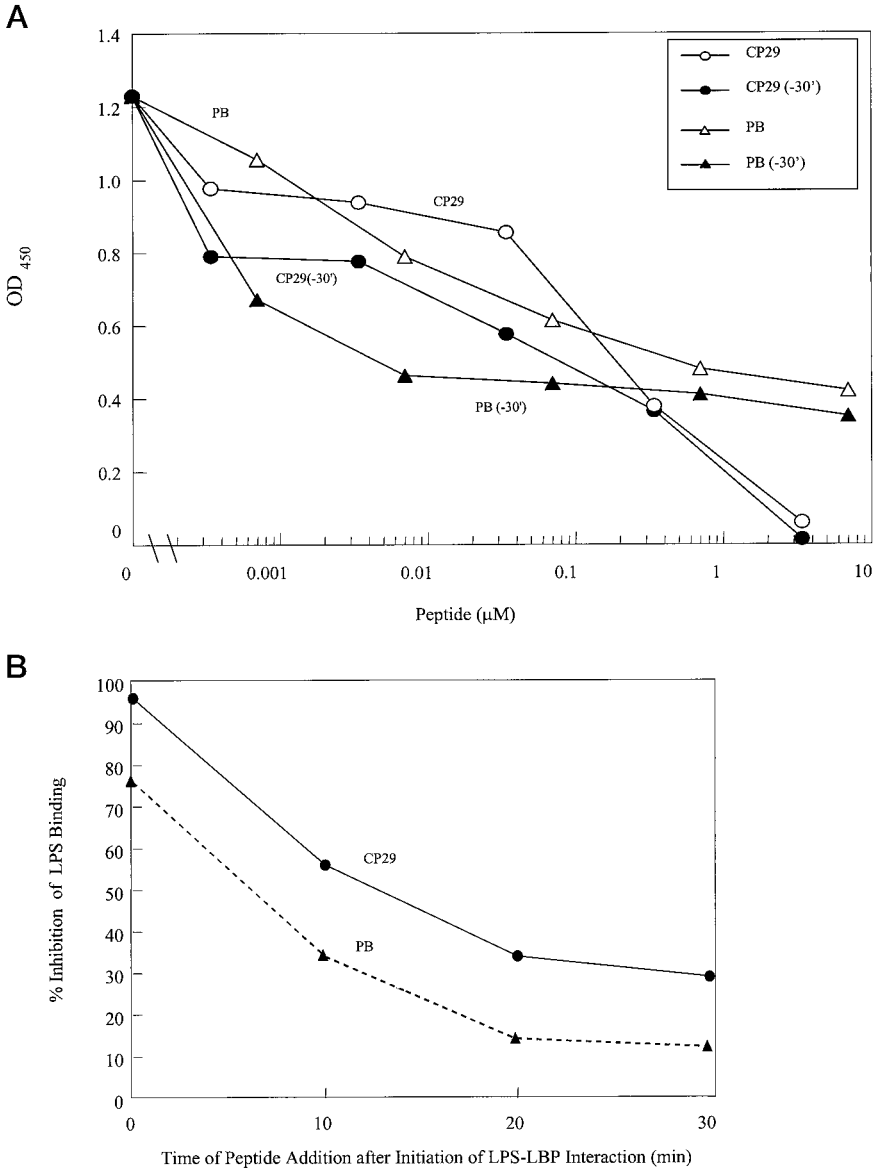

FIGURE 2. Effect of CP29 and polymyxin B on the binding of LPS to LBP. *A*, Biotinylated LPS (45 ng/ml) was mixed with the indicated amounts of CP29 (●) or polymyxin B (PB, ▲) for 30 min at 37°C before being added to the immobilized LBP. Alternatively, the LPS was added to the wells containing the immobilized LBP at the same time as either CP29 (○) or polymyxin B (PB, △), as in Fig. 1. The binding of the biotinylated LPS to the LBP was measured as in Fig. 1. Representative data from one of three similar experiments are shown. Each point represents the average and range of duplicate samples. The ranges were <10% of the average values. *B*, Biotinylated LPS (45 ng/ml) was added to wells containing the immobilized LBP. Ten micrograms per ml of CP29 (3.4 μM) or polymyxin B (7 μM) were added to the wells either at the same time (time 0) as the biotinylated LPS or at various times after the addition of the biotinylated LPS to the immobilized LBP. The data are presented as % inhibition of LPS binding. The OD_{450} for the binding of biotinylated LPS (100% value) in the absence of peptides ranged from 1.4 to 1.8. Representative data from one of three similar experiments are shown. Each point represents the average and range of duplicate samples. The ranges were generally <10% of the average values.

cationic antibiotic that causes substantial inhibition of LPS-induced TNF-α production (see Fig. 3*B*). Fig. 2*A* shows that as little as 100 ng/ml polymyxin B (70 nM) or 10 ng/ml CP29 (3.4 nM) could markedly inhibit the binding of LPS to LBP when 45 ng/ml LPS was added to the wells. The ability of polymyxin B to inhibit the LPS-LPB interaction leveled off at 60–70% inhibition when 1–10 μg/ml (0.7–7 μM) polymyxin B was added to the wells, whereas increasing the amount of CP29 from 1 μg/ml (0.34 μM) to 10 μg/ml (3.4 μM) resulted in greater inhibition, with 10 μg/ml CP29 causing nearly complete inhibition of the LPS-LBP interaction, as seen also in Fig. 1. Despite these differences, it is clear that both CP29 and polymyxin B are potent inhibitors of the LPS-LBP interaction.

We also observed that preincubating the LPS with CP29 or polymyxin B before adding it to the immobilized LBP substantially increased the ability of these peptides to block the LPS-LBP interaction (Fig. 2*A*). At least 10-fold less CP29 or polymyxin B was required to block the LPS-LBP interaction when the peptide was mixed with the LPS for 30 min before adding the LPS to the immobilized LBP-coated wells. This observation is consistent with our previous findings that CP29, polymyxin B, and other cationic peptides bind to LPS (7) and suggests that this interaction prevents LPS from binding to LBP.

While CP29 and polymyxin B can block the interaction of LPS with LBP, they cannot effectively disrupt the binding of LPS to LBP once it has occurred. Fig. 2*B* shows that when these peptides were added to the LBP-coated wells 20 min after the LPS, they were no longer able to substantially reduce the binding of LPS to LBP.

Structurally different cationic antimicrobial peptides inhibit the LPS-LBP interaction

Antimicrobial peptides have a wide variety of secondary structures (1, 3). For this study, peptides belonging to different structural groups were tested for their ability to inhibit LPS-LBP interaction. We tested 1) the human defensins HNP-1 and HBD-2, which have rigid β-sheet structures; 2) indolicidin and the indolicidin variant, CP11CN, which have an extended structure with β-turn elements (23), 3) Bac 2A-NH_2 which is a linear form of the loop peptide bactenecin (24), 4) the CP29, CEMA, and CP208 synthetic melittin:cecropin hybrids, which are amphipathic α-helical peptides (2,

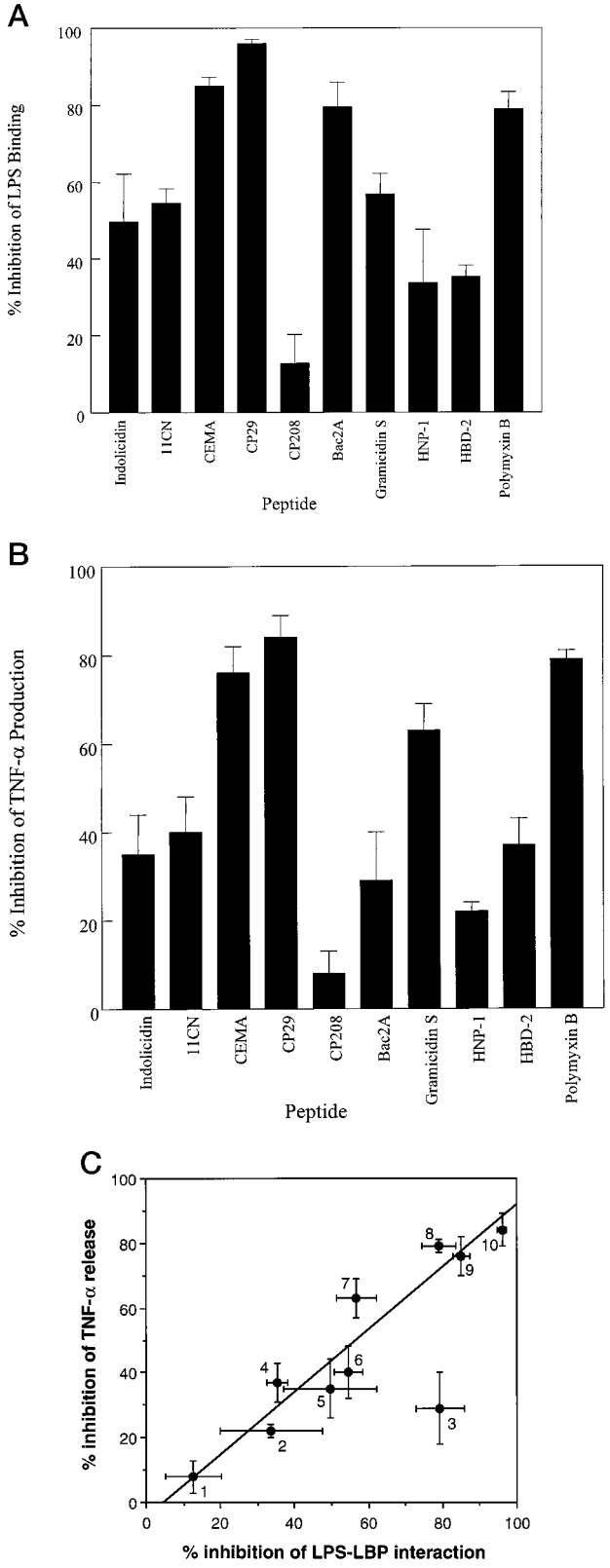

FIGURE 3. Inhibition of the LPS-LBP interaction and LPS-induced TNF-α production by structurally different cationic peptides. *A*, Biotinylated LPS (45 ng/ml) was added to wells with LBP in the presence or absence of 10 μg/ml of the indicated cationic peptides. The peptides were added to the wells at the same time as the LPS. In the absence of peptides, the binding of the biotinylated LPS to the immobilized LBP yielded an OD_{450} of 1.1–1.7. The data are expressed as % inhibition of LPS binding by the peptides. The values represent the mean and SE for three independent experiments. *B*, RAW 264.7 cells were incubated with 100 ng/ml *E. coli* O55:B5 LPS in the presence or absence of 20 μg/ml of the indicated peptides for 6 h. TNF-α was measured by ELISA. TNF-α production by unstimulated RAW 264.7 cells was always <0.3 ng/ml, while the LPS stimulation routinely resulted in 2.4–3.2 ng/ml TNF-α in the culture supernatant. The LPS-stimulated value was used as 100% and the data are represented as % inhibition of LPS-stimulated TNF-α production by the peptides. Note that the peptides did not affect the production of TNF-α by unstimulated RAW 264.7 cells. The values represent the mean and SE for three to four independent experiments. *C*, The data from *A* and *B* were graphed together as an XY scatter plot. The numbers represent the peptides as follows: 1, CP208; 2, HNP-1; 3, Bac 2A-NH_2; 4, HBD-2; 5, indolicidin; 6, CP11CN; 7, gramicidin S; 8, polymyxin B; 9, CEMA; 10, CP29. With the values for Bac 2A-NH_2 omitted, the coefficient of correlation (R^2) between the peptide-induced inhibition of the LPS-LBP interaction and the inhibition of TNF-α release was 0.921. The slope of the best fit line was 0.965, indicating that there was nearly a 1:1 correlation between the inhibition of the LPS-LBP interaction and the inhibition of TNF-α release.

7), and 5) the cyclic bacterial-derived peptide antibiotics polymyxin B and gramicidin S.

We compared the abilities of cationic peptides belonging to these different structural groups to inhibit the LPS-LBP interaction (Fig. 3*A*). At the same time, we compared their abilities to block LPS-induced TNF-α production by the RAW 264.7 macrophage cell line (Fig. 3*B*). CP29 and CEMA, synthetic α-helical peptides, were the most effective peptides at blocking both the LPS-LBP interaction and the production of TNF-α by RAW 264.7 cells. They consistently caused 85–95% inhibition of the LPS-LBP interaction while reducing LPS-induced TNF-α production by >75%. In contrast to CP29, CP208, an α-helical peptide that is related to CP29 but that lacks the tryptophan found at the N terminus of CP29, had little effect on the LPS-LBP interaction. Consistent with this observation, this peptide did not significantly reduce LPS-induced TNF-α production. We have previously shown that CP208 binds LPS poorly compared with CP29 and CEMA and that it has little antimicrobial activity (7). Thus, the ability of the α-helical cationic peptides to inhibit LPS·LBP interactions depends on structural features in addition to their positive charges.

Of the other structural groups of cationic peptides, Bac 2A-NH_2, gramicidin S, and polymyxin B all caused significant (55–80%) inhibition of the LPS-LBP interaction (Fig. 3*A*). For gramicidin S and polymyxin B, this correlated with their ability to block LPS-induced TNF-α production by 60–80% (Fig. 3*B*). In contrast, Bac 2A-NH_2 repeatedly caused only a modest (~30%) inhibition of TNF-α production even though it inhibited the LPS-LBP interaction by 75%. The human neutrophil peptide α-defensin HNP-1 and the human β-defensin HBD-2, which belong to the β-sheet class of cationic antimicrobial peptides, both exhibited only a modest ability (~40% inhibition) to block the LPS-LBP interaction, and this correlated with their modest ability (25–40% inhibition) to block LPS-induced TNF-α production. Indolicidin and the indolicidin variant CP11CN both significantly inhibited the LPS-LBP interaction (~50% inhibition), but to a lesser extent than CP29. This correlated with their lower ability to inhibit TNF-α production (~40% inhibition) as compared with CP29. Thus, with the exception of Bac 2A-NH_2, the ability of different cationic peptides to inhibit LPS-induced TNF-α production correlated well with their ability to block the binding of LPS to LBP. The strong correlation ($R^2 = 0.921$) between these two properties is illustrated in Fig. 3*C*. Moreover, the nearly 1:1 correlation between the inhibition of LPS-LBP interaction and the inhibition of TNF-α release suggests that the ability of the peptides to block the LPS-LBP interaction may be a major mechanism by which they block the ability of LPS to activate macrophages.

Discussion

In this report, we investigated the mechanism by which cationic antimicrobial peptides block the activation of macrophages by LPS. Using an assay that measures the binding of biotinylated LPS to immobilized LBP, we show for the first time that structurally diverse antimicrobial peptides can all block the interaction of LPS with LBP. Of the peptides tested, the α-helical peptide CP29 had the best ability to inhibit both the LPS-LBP interaction and LPS-induced TNF-α secretion by macrophages. It significantly inhibited the LPS-LBP interaction at concentrations as low as 10 ng/ml (3.4 nM), and 10 μg/ml (3.4 μM) CP29 caused nearly complete inhibition of the LPS-LBP interaction. The other cationic peptides tested including Bac 2A-NH_2, the indolicidins, and gramicidin S also inhibited both the LPS-LBP interaction and LPS-induced TNF-α production. Bac 2A-NH_2 was different from the other peptides in that it caused moderate inhibition of LPS-LBP interaction but had only a minor effect on LPS-induced TNF-α production. However, in general, the relative ability of different peptides to block the binding of LPS to LBP correlated strongly with their ability to block LPS-induced TNF-α production by RAW 264.7 cells. Thus, the ability of cationic peptides to block macrophage activation by LPS may be due in large part to their ability to block the binding of LPS to LBP. This would presumably block the transfer of LPS to CD14 by LBP, greatly decreasing the ability of LPS to activate macrophages.

Le Roy et al. recently showed that mAbs that block either the binding of LPS to LBP or the binding of LBP to CD14 are potent inhibitors of LPS toxicity in vivo and also block LPS-induced TNF production by RAW 264.7 cells (25). Together with our data, these results argue that inhibiting the LPS-LBP interaction is likely to be a very specific and efficient way to reduce or prevent LPS-induced inflammatory responses. The use of cationic peptides, in combination with anti-LBP mAbs, may be a very potent anti-endotoxin treatment. Our results also suggest that LPS-induced production of cationic peptides (26) may limit the magnitude of inflammatory responses by preventing further LPS signaling.

Acknowledgments

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References

1. Hancock, R. E., and R. Lehrer. 1998. Cationic peptides: a new source of antibiotics. *Trends Biotechnol. 16:82.*
2. Gough, M., R. E. Hancock, and N. M. Kelly. 1996. Antiendotoxin activity of cationic peptide antimicrobial agents. *Infect. Immun. 64:4922.*
3. Ganz, T., and R. I. Lehrer. 1998. Antimicrobial peptides of vertebrates. *Curr. Opin. Immunol. 10:41.*
4. Brackett, D. J., M. R. Lerner, M. A. Lacquement, R. He, and H. A. Pereira. 1997. A synthetic lipopolysaccharide-binding peptide based on the neutrophil- derived protein CAP37 prevents endotoxin-induced responses in conscious rats. *Infect. Immun. 65:2803.*
5. Hirata, M., Y. Shimomura, M. Yoshida, S. C. Wright, and J. W. Larrick. 1994. Endotoxin-binding synthetic peptides with endotoxin-neutralizing, antibacterial and anticoagulant activities. *Prog. Clin. Biol. Res. 388:147.*
6. Kirikae, T., M. Hirata, H. Yamasu, F. Kirikae, H. Tamura, F. Kayama, K. Nakatsuka, T. Yokochi, and M. Nakano. 1998. Protective effects of a human 18-kilodalton cationic antimicrobial protein (CAP18)-derived peptide against murine endotoxemia. *Infect. Immun. 66:1861.*
7. Scott, M. G., H. Yan, and R. E. W. Hancock. 1999. Biological properties of structurally related α-helical cationic antimicrobial peptides. *Infect. Immun. 67: 2005.*
8. van Tits, L. J., M. H. Bemelmans, S. Steinshamn, A. Waage, J. F. Leeuwenberg, and W. A. Buurman. 1994. Non-signaling functions of TNF-R75: findings in man and mouse. *Circ. Shock 44:40.*
9. VanderMeer, T. J., M. J. Menconi, J. Zhuang, H. Wang, R. Murtaugh, C. Bouza, P. Stevens, and M. P. Fink. 1995. Protective effects of a novel 32-amino acid C-terminal fragment of CAP18 in endotoxemic pigs. *Surgery 117:656.*
10. Hirata, M., J. Zhong, S. C. Wright, and J. W. Larrick. 1995. Structure and functions of endotoxin-binding peptides derived from CAP18. *Prog. Clin. Biol. Res. 392:317.*
11. Ulevitch, R. J., and P. S. Tobias. 1999. Recognition of gram-negative bacteria and endotoxin by the innate immune system. *Curr. Opin. Immunol. 11:19.*
12. Hoshino, K., O. Takeuchi, T. Kawai, H. Sanjo, T. Ogawa, Y. Takeda, K. Takeda, and S. Akira. 1999. Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. *J. Immunol. 162:3749.*
13. Poltorak, A., X. He, I. Smirnova, M. Y. Liu, C. V. Huffel, X. Du, D. Birdwell, E. Alejos, M. Silva, C. Galanos, et al. 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science 282:2085.*
14. DeFranco, A. L., M. T. Crowley, A. Finn, J. Hambleton, and S. L. Weinstein. 1998. The role of tyrosine kinases and map kinases in LPS-induced signaling. *Prog. Clin. Biol. Res. 397:119.*
15. Lee, J. C., J. T. Laydon, P. C. McDonnell, T. F. Gallagher, S. Kumar, D. Green, D. McNulty, M. J. Blumenthal, J. R. Heys, S. W. Landvatter, et al. 1994. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature 372:739.*
16. Ulevitch, R. J., and P. S. Tobias. 1995. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annu. Rev. Immunol. 13:437.*
17. Schumann, R. R., S. R. Leong, G. W. Flaggs, P. W. Gray, S. D. Wright, J. C. Mathison, P. S. Tobias, and R. J. Ulevitch. 1990. Structure and function of lipopolysaccharide binding protein. *Science 249:1429.*
18. Ooi, C. E., J. Weiss, M. E. Doerfler, and P. Elsbach. 1991. Endotoxin-neutralizing properties of the 25 kD N-terminal fragment and a newly isolated 30 kD C-terminal fragment of the 55–60 kD bactericidal/permeability-increasing protein of human neutrophils. *J. Exp. Med. 174:649.*
19. Hirata, M., Y. Shimomura, M. Yoshida, J. G. Morgan, I. Palings, D. Wilson, M. H. Yen, S. C. Wright, and J. W. Larrick. 1994. Characterization of a rabbit cationic protein (CAP18) with lipopolysaccharide-inhibitory activity. *Infect. Immun. 62:1421.*
20. Larrick, J. W., M. Hirata, R. F. Balint, J. Lee, J. Zhong, and S. C. Wright. 1995. Human CAP18: a novel antimicrobial lipopolysaccharide-binding protein. *Infect. Immun. 63:1291.*
21. Vreugdenhil, A. C., M. A. Dentener, A. M. Snoek, J. W. Greve, and W. A. Buurman. 1999. Lipopolysaccharide binding protein and serum amyloid A secretion by human intestinal epithelial cells during the acute phase response. *J. Immunol. 163:2792.*
22. Froon, A. H., M. A. Dentener, J. W. Greve, G. Ramsay, and W. A. Buurman. 1995. Lipopolysaccharide toxicity-regulating proteins in bacteremia. *J. Infect. Dis 171:1250.*
23. Ladokhin, A. S., M. E. Selsted, and S. H. White. 1999. CD spectra of indolicidin antimicrobial peptides suggest turns, not polyproline helix. *Biochemistry 38:12313.*
24. Wu, M., and R. E. Hancock. 1999. Interaction of the cyclic antimicrobial cationic peptide bactenecin with the outer and cytoplasmic membrane. *J. Biol. Chem. 274:29.*
25. Le Roy, D., F. Di Padova, R. Tees, S. Lengacher, R. Landmann, M. P. Glauser, T. Calandra, and D. Heumann. 1999. Monoclonal antibodies to murine lipopolysaccharide (LPS)-binding protein (LBP) protect mice from lethal endotoxemia by blocking either the binding of LPS to LBP or the presentation of LPS/LBP complexes to CD14. *J. Immunol. 162:7454.*
26. Russell, J. P., G. Diamond, A. P. Tarver, T. F. Scanlin, and C. L. Bevins. 1996. Coordinate induction of two antibiotic genes in tracheal epithelial cells exposed to the inflammatory mediators lipopolysaccharide and tumor necrosis factor alpha. *Infect. Immun. 64:1565.*