Lipopolysaccharide-binding protein mediates CD14-independent intercalation of lipopolysaccharide into phospholipid membranes

Andra B. Schromm[a], Klaus Brandenburg[a], Ernst Th. Rietschel[a], Hans-Dieter Flad[a], Stephen F. Carroll[b], Ulrich Seydel[a,*]

[a] *Research Center Borstel, Center for Medicine and Biosciences, Parkallee 10, D-23845 Borstel, Germany*
[b] *XOMA Corporation, 2910 Seventh Street, Berkeley, CA 94710, USA*

Received 11 November 1996

Abstract Lipopolysaccharides (LPS, endotoxin) stimulate mononuclear cells to release cytokines which initiate endotoxic effects. Interaction of LPS at low concentrations with target cells is CD14-dependent whereas at high LPS concentrations it is CD14-independent. Here, we demonstrate by resonance energy transfer (RET) technique that nonspecific, CD14-independent intercalation of LPS into membrane systems can be mediated by lipopolysaccharide-binding protein (LBP). It is proposed that in this pathway, LBP breaks down LPS aggregates, transports the smaller units to and inserts them into the phospholipid cell matrix. We furthermore show that LBP also mediates the intercalation of other negatively charged amphiphilic molecules. We propose a model explaining CD14-independent cell activation at high endotoxin concentrations.

Key words: LBP; Endotoxin; Bactericidal/permeability-increasing protein; Nonspecific intercalation; Phospholipid membrane

1. Introduction

Lipopolysaccharide (LPS, endotoxin) constitutes the major surface component of the outer membrane of Gram-negative bacteria. In mammals, LPS induces a variety of biological effects which, if left untreated, culminate in manifestations of septic shock (fever, tachypnoe, tachycardia, hypotension, and multi-organ failure). The initiation of these effects is due to an activation of monocytes/macrophages, leading to the secretion of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-8 [1,2]. Several types of interactions between endotoxin and host cells have been observed. Thus, the binding of LPS to a specific cellular receptor molecule, the phosphatidylinositol-anchored protein mCD14, has been described as a critical event in the activation of cells [3–6]. In the case of CD14-negative endothelial and epithelial cells a soluble form of CD14 (sCD14) mediates LPS binding [7]. The binding of LPS to CD14 is facilitated by LPS-binding protein (LBP) [8,9]. According to present knowledge, LBP associates with LPS and the LPS/LBP complex is then thought to interact with CD14 [3,10–12].

*Corresponding author. Fax: (49) 4537-188-632

Abbreviations: BPI, bactericidal/permeability-increasing protein; BSA, bovine serum albumim; CAC, critical aggregate concentration; LBP, lipopolysaccharide-binding protein; LPS, lipopolysaccharide, endotoxin; NBD-PE, *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)-phosphatidylethanolamine; PE, phosphatidylethanolamine; $PL_{M\Phi}$, mixture resembling the phospholipid matrix of a macrophage membrane; RET, resonance energy transfer; Rh-PE, *N*-(rhodamine B sulfonyl)-phosphatidylethanolamine

In addition to these receptor-mediated processes, several observations indicate that a CD14-independent direct activation of cells may be operative, particularly at higher endotoxin concentrations (0.1–10 μg/ml). Such concentrations have been measured in human meningococcal sepsis [13]. In in vitro experiments it has been shown that CD14-negative cells are reactive to high doses of LPS and the inhibition of CD14-dependent cell activation by anti-CD14 antibodies may be overcome by higher endotoxin doses [4,14,15]. The molecular mechanisms of CD14-independent cell activation have not yet been elucidated. Also, at saturating LPS concentrations, the molar ratio of LPS bound to CD14 is considerably higher than one [16], suggesting the existence of other cell activation mechanisms. Thus, a CD14-independent activation mechanism which is based on nonspecific hydrophobic intercalation of endotoxin molecules into the phospholipid matrix of the host cell membrane would be in accordance with these observations. It has been postulated that the activation of host cells is based on the association of LPS with the target cell, resulting in an interdigitation of the lipid A acyl residues into the cell membrane [17]. Subsequent experiments have provided supportive evidence for a receptor-independent intercalation of LPS [18–21]. However, we recently showed that in the absence of transport proteins nonspecific intercalation of endotoxin aggregates into PL membranes driven only by hydrophobic interactions does not take place in the time scale of cell activation [22].

Endotoxins are amphiphilic molecules and, therefore, tend to form multimeric clusters in an aqueous environment above a critical aggregate concentration (CAC). The absolute values of CAC for endotoxin of various chemical structures will likely depend on the acylation pattern and the length of the sugar moiety. Therefore, they should be lowest for free lipid A and deep rough mutant LPS but have not yet been determined. From a comparison with the published value for dipalmitoylphosphatidylcholine [23], the CAC of LPS should be in the order of 10^{-10} M or below. Under normal experimental in vitro conditions, endotoxins should therefore form aggregates. There is, however, evidence that endotoxin monomers are biologically more active than aggregates [24]. There is also evidence that LBP has a critical function in the activation of cells at low endotoxin concentrations by breaking down the size of LPS aggregates, by binding endotoxin in a 1:1 stoichiometry and by presenting endotoxin monomers to the host cell recognition structures as well as to other endotoxin binding proteins such as soluble CD14 and high-density lipoproteins [10,12]. In this way, LBP would act as a disaggregating protein and would guide LPS to several targets.

We have investigated the role of LBP and other proteins

PII S0014-5793(96)01338-5

which are known to bind LPS such as bovine serum albumin (BSA), lysozyme, the complement component C1q, and the bactericidal/permeability-increasing protein (BPI) in the interaction of endotoxin aggregates with the lipid matrix of target cell membranes. Membranes were constructed as protein-free phospholipid liposomes resembling the composition of the macrophage membrane ($PL_{M\Phi}$). Resonance energy transfer (RET) was applied as a sensitive technique to detect the intercalation of endotoxin molecules into the liposomal membrane.

2. Materials and methods

2.1. Preparation of lipopolysaccharides and phospholipids

Rough muant LPS was extracted according to the phenol–chloroform–petroleum ether method [25] from *Salmonella enterica* serovar Minnesota strains R595 (Re), R5 (Rc), and R60 (Ra). LPS preparations were used in their natural salt form. Free lipid A was obtained by acetate buffer treatment (1 M, 100°C, 1.5 h) of Re LPS and subsequent dialysis and conversion to the triethylammonium salt form. For preparation of dephosphorylated LPS (P^- LPS), Re LPS isolated from *Escherichia coli* strain F515 was treated with hydrofluoric acid (48%, 4°C, 72 h). After purification the phosphate content was determined to be lower than 5%. For intercalation experiments, free lipid A and LPS were prepared as 1 mM aqueous dispersions in phosphate-buffered saline (PBS) at pH 7.2, mixed thoroughly, and sonicated with a Branson sonifier for 1 min (1 ml solution). Subsequently, the preparations were cooled for 30 min at 4°C, heated for 30 min at 56°C, and recooled to 4°C. Preparations were stored at 4°C overnight prior to experiments.

Bovine brain L-α-phosphatidyl-L-serine, egg L-α-phosphatidylcholine, and sphingomyelin from bovine brain were obtained from Sigma (Deisenhofen, Germany). L-α-phosphatidylethanolamine (PE) from *E. coli* was from Avanti Polar Lipids, Inc. (Birmingham, AL). For preparation of liposomes from a single phospholipid or from a phospholipid mixture resembling the composition of the cell membrane of macrophages ($PL_{M\Phi}$) — phosphatidylcholine, phosphatidylserine, PE, and sphingomyelin in a molar ratio of 1:0.4:0.7:0.5 [26] — the lipids were solubilized in chloroform, the solvent was evaporated under a stream of nitrogen, lipids resuspended in the appropriate volume of PBS and further treated as described for LPS.

2.2. Proteins

Recombinant human LBP (456 amino acid holoprotein $rLBP_{50}$) in 10 mM HEPES, pH 7.5, and recombinant human BPI (199 amino acid N-terminal fragment $rBPI_{23}$) in 20 mM sodium citrate, pH 5.0, have been described [27,28]. BSA, chicken egg white lysozyme, and human complement component C1q were purchased from Sigma and dissolved in PBS.

2.3. Fluorescence measurements

For the RET assay, phospholipid liposomes were double-labeled with the fluorescent dyes *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)-PE (NBD-PE) and N-(rhodamine B sulfonyl)-PE (Rh-PE), which were purchased from Molecular Probes (Eugene, OR). NBD-PE and Rh-PE were dissolved in chloroform and added to the lipids in the chloroform phase to a final molar ratio [PL]/[NBD-PE]/[Rh-PE] = 100:1:1. The emission wavelength of one dye, the donor (NBD-PE), is in the range of the excitation wavelength of the second dye, the acceptor (Rh-PE). The energy transfer between these two dyes is sensitive to spatial separation. Intercalation of unlabeled molecules into the double-labeled liposomes leads to probe dilution and with that to a decrease in the efficiency of RET: the emission intensity of the donor increases and that of the acceptor decreases (for the sake of clearity, in the figures only the donor emission intensity is shown). A preparation of 900 µl of the double-labeled PL liposomes (0.1 mM) at 37°C was excited at 470 nm (excitation wavelength of NBD-PE), and the fluorescence emissions of NBD-PE (531 nm) and Rh-PE (593 nm) were adjusted to yield identical intensities and recorded for 50 s under continuous stirring to determine the base line. After 50 s, unlabeled LPS or PL preparation (100 µl, 1 mM) was added, respectively. After 100 s, the appropriate amount of protein was added, and the emission signals were usually recorded for another 300 s. These relatively high concentration were chosen to obtain an optimal signal-to-noise ratio. In control experiments we verified that also at 2–3 orders of magnitude lower concentrations of each of the two lipids detectable changes in the fluorescence intensities were obtained, however, the signals were relatively noisy. We, therefore, decided to perform the experiments at the higher concentrations.

3. Results

Upon addition of proteins to an equimolar mixture of $PL_{M\Phi}$-liposomes and aggregates prepared from deep rough mutant LPS of *Salmonella enterica* serovar Minnesota strain R595 (Re LPS), the donor emission intensity rises for LBP and BPI, indicating incorporation of LPS into the liposomal membrane (Fig. 1). In contrast, BSA, lysozyme, or C1q did not mediate incorporation but rather induced a slight decrease in emission intensity which is due to the dilution of the labeled liposomes by the aqueous protein solution. The kinetics of the increase in emission intensity exhibits a biphasic behavior with a very rapid first phase of a few seconds followed by a rather slow phase continuing even beyond the observation period.

To further characterize the transport activity of LBP, we performed experiments on the concentration dependence and substrate specificity. The effect of LBP was concentration-dependent and detectable above a threshold concentration of 10 µg/ml (data not shown), which is in the range of the physiological concentration in normal human serum [8]. The effect is also dependent on the molar ratio of the LPS/$PL_{M\Phi}$ mixture, being more pronounced for higher molar ratios (Fig. 2). From these experiments the transport stoichiometry between LBP and LPS could be assessed. Calibration measurements with liposomes made from constant amounts of $PL_{M\Phi}$ and different amounts of LPS, which were labeled with the dyes in a constant molar ratio PL/NBD-PE/Rh-PE (100:1:1 M), showed that the lower detection limit for intercalated endotoxin is around 1 mole% (corresponding to an increase in donor emission intensity of 8%) and that the intensity increases with the amount of intercalated endotoxin (data not shown). This calibration allowed the conclusion that for a mixture of $PL_{M\Phi}$ liposomes and LPS aggregates in a 1:1 molar ratio the addition of 50 µg LBP leads to an intercalation of

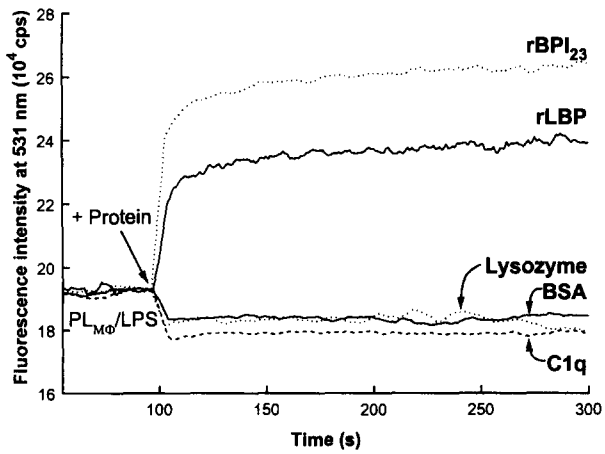

Fig. 1. Time kinetics of changes in donor emission intensity in RET experiments upon addition of various proteins (50 µg/ml) to 1 ml of equimolar mixtures (0.1 mM) of double-labeled liposomes composed of a phospholipid mixture ($PL_{M\Phi}$) resembling the lipid matrix of mononuclear cells and aggregates prepared from Re LPS of *Salmonella enterica* serovar Minnesota strain R595.

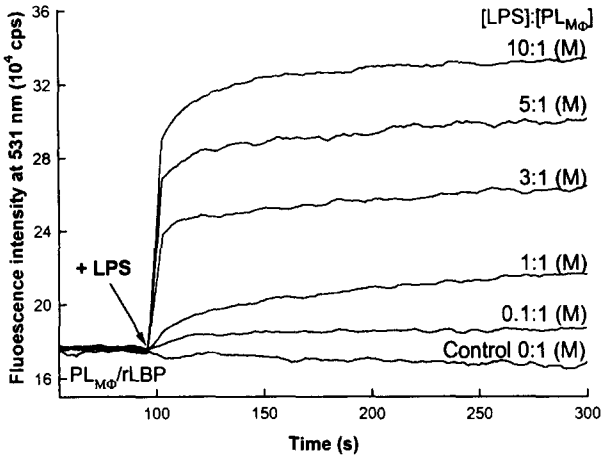

Fig. 2. Time kinetics of changes in donor emission intensity in RET experiments upon addition of Re LPS suspensions (100 μl) with increasing concentrations to mixtures of 0.9 ml of 0.1 mM double-labeled liposomes from $PL_{M\Phi}$ and $rLBP_{50}$ (50 μl, 1 mg/ml). The final molar ratio of LPS aggregates and $PL_{M\Phi}$ liposomes ranged from 0.1:1 to 10:1.

about 10 mole% LPS. From the concentration of LBP (50 μg in 1 ml) and the amount of LPS molecules transported, the molar ratio between LBP and transported LPS molecules was determined to be 1:10. For lipid mixtures with a molar ratio of [LPS]/[$PL_{M\Phi}$] higher than 1:1, the amount of LPS transported by LBP was even higher. Thus, one LBP molecule transported several molecules of LPS to the lipid surface, in a manner similar as has been described for LBP-mediated transport processes with high-density lipoprotein or soluble CD14 as targets [10,12]. Surprisingly, the transport activitiy of LBP is not restricted to endotoxin, but was also observed with dephosphorylated Re LPS carrying two negative charges on the 2-keto-3-deoxyoctonate units and negatively charged phospholipids like phosphatidylserine, -glycerol, and -inositol, but not zwitterionic (neutral) phospholipids like phosphatidylcholine and -ethanolamine. For the sake of clarity, only some of the data are shown exemplarily in Fig. 3A. LPS and phospholipid intercalation was strongly dependent on the composition of the phospholipid liposomes and was restricted to negatively charged liposomes as targets (Fig. 3B). Thus, LBP requires negative charges both for the donor lipid (phospholipid, LPS) and on the acceptor side (membrane). For different LPS chemotypes, the transport activity of LBP varied, being highest for free lipid A and Re LPS and decreasing with the length of the core oligosaccharide over Rc to Ra mutant LPS (Fig. 4). This phenomenon may be related to the disaggregating capacity of LBP, which is less effective for those endotoxins having a higher CAC and consequently an a-priori higher monomer concentration (higher water solubility). Note that, in our system, soluble CD14 (a generous gift of Dr. Chr. Schütt, University of Greifswald, Germany) had no effect on the transport of endotoxin in the presence or absence of LBP (data not shown).

To further elucidate the role of BPI in comparison to that of LBP, we have performed experiments in which identical mass amounts of $rLBP_{50}$ (the 456 amino acid holoprotein) and $rBPI_{23}$ (the 199 amino acid N-terminal BPI fragment) were added in separate experiments to liposomes of different phospholipid composition in the absence of LPS (Fig. 5). Apparently, both proteins intercalate into negatively charged (but not into uncharged) liposomal membranes. However, this effect is much less pronounced for LBP (Fig. 5B) and might be explained by a lower hydrophobicity of LBP. Fig. 5A shows that BPI, in contrast to LBP, leads to intensity fluctuations starting about 200 s after BPI addition which is most likely be due to BPI-induced aggregation and precipitation of phospatidylserine liposomes (a similar effect has been observed at high molar excess of Ca^{2+}, data not shown).

4. Discussion

Our data show that LBP acts as an effective carrier of LPS and other negatively charged amphiphiles such as phosphatidylserine and dephosphorylated Re LPS, and that one LBP molecule transports more than one LPS molecule. It has been reported that the stoichiometry of binding of endotoxin to LBP is 1:1 [12]. From these facts it is concluded that LBP breaks down the lipid aggregates to smaller units, transports these to, and intercalates them into phospholipid target membranes. This intercalation takes place within a physiologically relevant time scale.

The observed transport capability of BPI for LPS may be unexpected in view of the fact that BPI reduces rather than increases LPS bioactivity. This apparent contrast may be explained by intercalation of BPI/LPS assemblies in which LPS

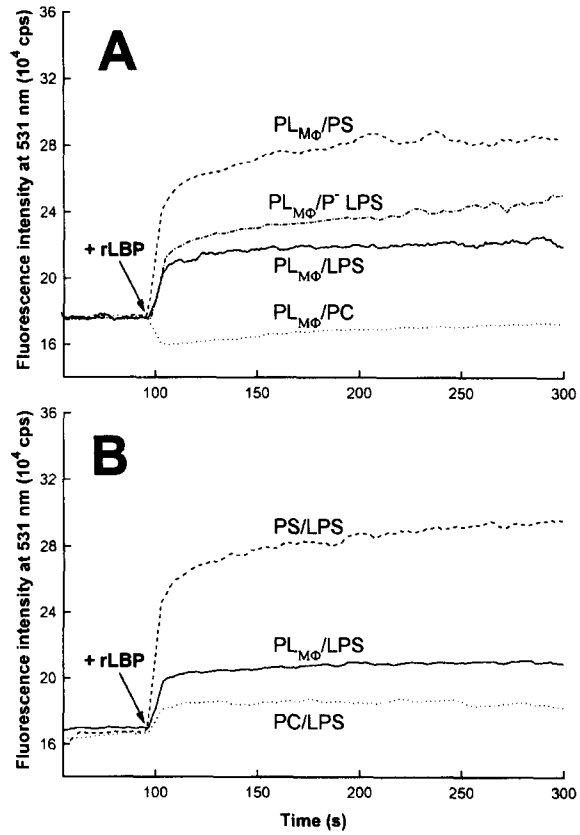

Fig. 3. Time kinetics of changes in donor emission intensity in RET experiments upon addition of 50 μg/ml of $rLBP_{50}$ to 1 ml of equimolar mixtures (0.1 mM) of (A) $PL_{M\Phi}$ liposomes and aggregates prepared from Re LPS, dephosphorylated Re LPS (P^- LPS), phosphatidylserine (PS), and phosphatidylcholine (PC), respectively, and of (B) aggregates prepared from Re LPS and double-labeled liposomes composed of the negatively charged PS, the zwitterionic PC, and $PL_{M\Phi}$, respectively.

activity is neutralized, thus not contributing to cell activation. The obvious membrane activity of BPI, i.e., its intercalation into negatively charged phospholipid membranes, may be surprising, but would be in accordance with the observation of surface bound BPI on mononuclear cells [29].

The LBP-driven intercalation of endotoxin does not require CD14 or any other membrane-associated protein. In living cells it may lead either to LPS neutralization or cell activation. CD14-independent activation has been described [4,14,15] and the data presented may provide the physico-chemical basis for this phenomenon. Thus, it is reasonable to assume that one prerequisite for the activation of host cells is the insertion of endotoxin molecules into their lipid matrix. This can be achieved in different ways, (1) via direct intercalation by hydrophobic interaction of a-priori existing endotoxin monomers which are present in sufficient numbers at least for those chemotypes with longer sugar moieties, (2) via the intercalation of monomers or smaller endotoxin aggregates which are produced by the disaggregating properties of LBP, or (3) via the mediating role of mCD14. The mere intercalation of endotoxin molecules into the lipid matrix will not be sufficient for activation. We, therefore, propose a model postulating the existence of a transmembrane signal transducing protein which is triggered by binding of endotoxin molecules (Fig. 6). The triggering signal requires, however, a particular conformation of the lipid A moiety of the endotoxin molecule. Thus, only those endotoxins will activate which possess a lipid A part leading, in the isolated form, to nonlamellar aggregate structures. This implies that the conformation of the lipid A monomer is conical with the cross-section of the hydrocarbon moiety being larger than that of the polar backbone. In contrast, a lipid A with a cylindrical conformation – leading to a lamellar aggregate structure – would not activate the signal transducer [30]. We furthermore postulate that for binding to the signalling protein, the existence of a sufficient number of hydroxy fatty acids in the endotoxin molecules is necessary allowing the formation of hydrogen bonds. This conformational concept would readily explain the antagonistic action of biologically not active endotoxins. In these cases, the binding sites of the transmembrane protein would be occupied by the inactive molecules, thus inhibiting the binding of the active structures. A candidate for the proposed transmembrane signal transducer could be an ion channel [31]. The result of the endotoxin interaction with the channel protein could lead to mismatches in intracellular ion concentrations provoking the activation of the succeeding signalling cascade.

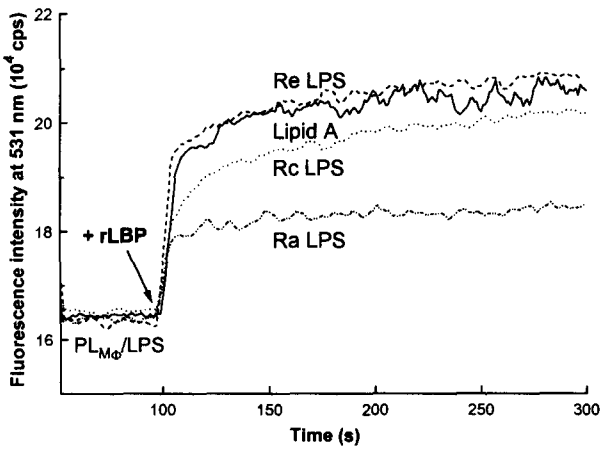

Fig. 4. Time kinetics of changes in donor emission intensity in RET experiments upon addition of $rLBP_{50}$ (50 μg/ml) to 1 ml of equimolar mixtures (0.1 mM) of double-labeled liposomes composed of $PL_{M\Phi}$ and aggregates prepared from free lipid A, Re LPS, Rc LPS (strain R5), and Ra LPS (strain R60).

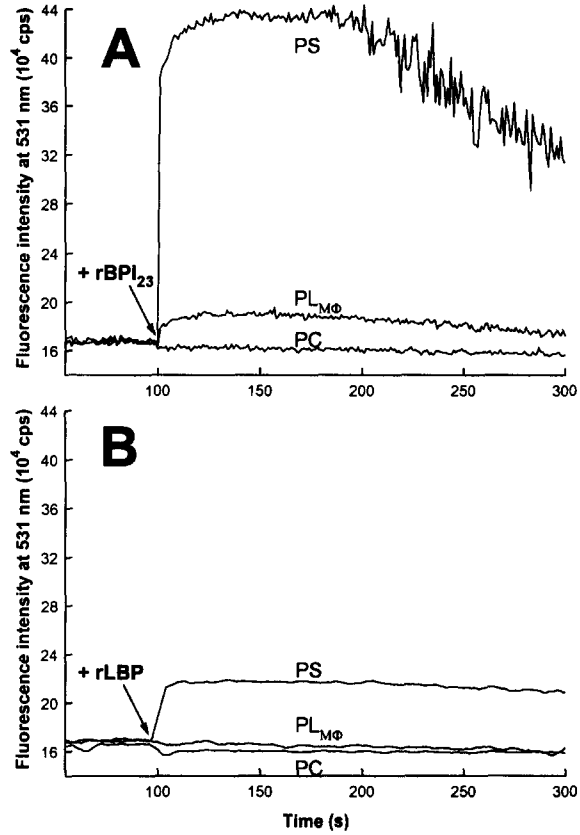

Fig. 5. Time kinetics of changes in donor emission intensity in RET experiments upon addition of 50 μg/ml of $rBPI_{23}$ (A) and $rLBP_{50}$ (B) to 1 ml of 0.1 mM of double-labeled liposomes composed of the negatively charged phosphatidylserine (PS), the zwitterionic phosphatidylcholine (PC), and $PL_{M\Phi}$.

In our model, the abovementioned types of endotoxin intercalation should express different efficiencies. Thus, the direct intercalation of monomers and the LBP-mediated process will lead to an intercalation somewhere in the lipid matrix, whereas the mCD14-mediated process will bind the endotoxin directly to the signalling protein assuming that mCD14 is located in the direct vicinity of the signal transducer. This assumption is backed by the observation that mCD14-mediated activation can be blocked by anti-CD14 antibodies. At high endotoxin concentration the blockade by anti-CD14 antibodies can be overcome [14], and obviously in that case the CD14-independent activation pathway is initiated. One further point deserves attention. LBP, initially defined as lipopolysaccharide-binding protein, turns out not to be LPS-specific but rather to interact with and transport other negatively charged lipids. Thus, LBP seems to be a lipid transfer protein in a more general sense. This aspect will be subject to further investigations.

Acknowledgements: This work was financially supported by the Deutsche Forschungsgemeinschaft (SFB 367, projects B2 and B8), the Federal Minister of Education, Research and Technology BMBF (FKZ 01 KI 9471), and the Fonds der Chemischen Industrie

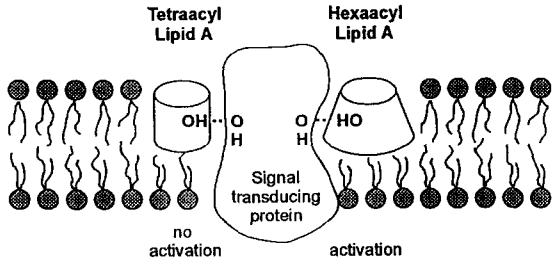

Fig. 6. Proposed model of cell activation by endotoxin. The endotoxin molecules bind with their lipid A moiety to a transmembrane signal transducing molecule, probably an ion channel. Binding is facilitated via hydrogen bonding. This requires the existence of hydroxy fatty acids in the lipid A part. A further prerequisite for activation is a particular conformation of lipid A.

(LthR). We gratefully acknowledge the technical assistance of Mrs. Christine Hamann.

References

[1] Morrison, D.C. and Ryan, J.L. (1992) Bacterial Endotoxic Lipopolysaccharides. Vol. 1. Molecular Biochemistry and Cellular-biology, CRC Press, Boca Raton, FL.
[2] Rietschel, E.T., Brade, H., Holst, O., Brade, L., Müller-Loennies, S., Mamat, U., Zähringer, U., Beckmann, F., Seydel, U., Brandenburg, K., Ulmer, A.J., Mattern, T., Heine, H., Schletter, J., Loppnow, H., Schönbeck, U., Flad, H.-D., Hauschildt, S., Schade, U.F., Di Padova, F., Kusumoto, S. and Schumann, R.R. (1996) in Curr. Top. Microbiol. Immunol. Vol. 216, Pathology of Septic Shock (Rietschel, E.T. and Wagner, H. eds.) pp. 39–81, Springer, Berlin.
[3] Wright, S.D., Ramos, R.A., Tobias, P.S., Ulevitch, R.J. and Mathison, J.C. (1990) Science 249, 1431–1433.
[4] Lee, J.D., Kato, K., Tobias, P.S., Kirkland, T.N. and Ulevitch, R.J. (1992) J. Exp. Med. 175, 1697–1705.
[5] Golenbock, D.T., Liu, Y., Millham, F.H., Freeman, M.W. and Zoeller, R.A. (1993) J. Biol. Chem. 268, 22055–22059.
[6] Ferrero, E., Jiao, D., Tsuberi, B.Z., Tesio, L., Wei Rong, G., Haziot, A. and Goyert, S.M. (1993) Proc. Natl. Acad. Sci. USA 90, 2380–2384.
[7] Pugin, J., Schürer-Maly, C.C., Leturcq, D., Moriarty, A., Ulevitch, R.J. and Tobias, P.S. (1993) Proc. Natl. Acad. Sci. USA 90, 2744–2748.
[8] Schumann, R.R., Leong, S.R., Flaggs, G.W., Gray, P.W., Wright, S.D., Mathison, J.C., Tobias, P.S. and Ulevitch, R.J. (1990) Science 249, 1429–1431.
[9] Gallay, P., Heumann, D., Le Roy, D., Barras, C. and Glauser, M.P. (1993) Proc. Natl. Acad. Sci. USA 90, 9935–9938.
[10] Hailman, E., Lichenstein, H.S., Wurfel, M.M., Miller, D.S., Johnson, D.A., Kelley, M., Busse, L.A., Zukowski, M.M. and Wright, S.D. (1994) J. Exp. Med. 179, 269–277.
[11] Gegner, J.A., Ulevitch, R.J. and Tobias, P.S. (1995) J. Biol. Chem. 270, 5320–5326.
[12] Yu, B. and Wright, S.D. (1996) J. Biol. Chem. 271, 4100–4105.
[13] Brandtzaeg, P., Mollnes, T.E. and Kierulf, P. (1989) J. Infect. Dis. 160, 58.
[14] Lynn, W.A., Liu, Y. and Golenbock, D.T. (1993) Infect. Immun. 61, 4452–4461.
[15] Ingalls, R.R. and Golenbock, D.T. (1995) J. Exp. Med. 181, 1473–1479.
[16] Kirkland, T.N., Finley, F., Leturcq, D., Moriarty, A., Lee, J.-D., Ulevitch, R.J. and Tobias, P.S. (1993) J. Biol. Chem. 268, 24818–24823.
[17] Kabir, S., Rosenstreich, D.L. and Mergenhagen, S.E. (1978) in Bacteria Toxins and Cell Membranes (Jeljaszewic, J. and Wadstrøm, T. eds.), pp. 59–87, Academic Press, New York.
[18] Larsen, N.E., Enelow, R.I., Simons, E.R. and Sullivan, R. (1985) Biochim. Biophys. Acta 815, 1–8.
[19] Portoles, M.T., Pagani, R., Diaz-Laviada, I. and Municio, A.M. (1987) Cell. Biochem. Funct. 5, 55–61.
[20] Jacobs, D.M., Yeh, H. and Price, R.M. (1990) Adv. Exp. Med. Biol. 256, 233–245.
[21] Jackson, S.K., James, P.E., Rowlands, C.C. and Mile, B. (1992) Biochim. Biophys. Acta 1135, 165–170.
[22] Schromm, A.B., Brandenburg, K., Rietschel, E.T. and Seydel, U. (1995) J. Endotox. Res. 2, 313–323.
[23] Smith, R. and Tanford, C. (1972) J. Mol. Biol. 67, 75–83.
[24] Takayama, K., Mitchell, D.H., Din, Z.Z., Mukerjee, P., Li, C. and Coleman, D.L. (1994) J. Biol. Chem. 269, 2241–2244.
[25] Galanos, C., Lüderitz, O. and Westphal, O. (1969) Eur. J. Biochem. 9, 245–249.
[26] Kröner, E.E., Peskar, B.A., Fischer, H. and Ferber, E. (1981) J. Biol. Chem. 256, 3690–3697.
[27] Theofan, G., Horwitz, A.H., Williams, R.E., Liu, P.S., Chan, I., Birr, C., Carroll, S.F., Meszaros, K., Parent, J.B. and Kasler, H. (1994) J. Immunol. 152, 3623–3629.
[28] Gazzano-Santoro, H., Parent, J.B., Grinna, L., Horwitz, A., Parsons, T., Theofan, G., Elsbach, P., Weiss, J. and Conlon, P.J. (1992) Infect. Immun. 60, 4754–4761.
[29] Dentener, M.A., Francot, G.J.M. and Buurman, W.A. (1996) J. Infect. Dis. 173, 252–255.
[30] Brandenburg, K., Mayer, H., Koch, M.H.J., Weckesser, J., Rietschel, E.T. and Seydel, U. (1993) Eur. J. Biochem. 218, 555–563.
[31] Maruyama, N., Yasunori, K., Yamauchi, K., Aizawa, T., Ohrui, T., Nara, M., Oshiro, T., Ohno, L., Tanura, G., Shimura, S., Saschi, H., Tahishima, T. and Shirato, K. (1994) Am. J. Respir. Cell Mol. Biol. 10, 514–520.