Biochimica et Biophysica Acta, 774 (1984) 261–268
Elsevier

BBA 72131

INTERACTION OF RADIOLABELED ENDOTOXIN MOLECULES WITH HUMAN MONOCYTE MEMBRANES

NANCY E. LARSEN and RICHARD SULLIVAN *

Section of Hematology, Department of Medicine, and Evans Memorial Department of Clinical Research, University Hospital, Boston University School of Medicine, 75 East Newton Street, Boston, MA 02118 (U.S.A.)

(Received November 7th, 1983)
(Revised manuscript received March 8th, 1984)

Key words: Endotoxin; Monocyte; Plasma membrane; Membrane-toxin interaction; Lipid A; (S. typhi)

Radiolabeled and biologically active endotoxin molecules were prepared, and their binding to monocyte plasma membranes was studied. The binding of ^{3}H-endotoxin and ^{51}Cr-lipid A to isolated membranes was found to be less specific and of lower apparent affinity than that observed using whole cells. Plasma membranes isolated from intact, viable ^{51}Cr-lipid A-pretreated monocytes were found to contain a significant portion of the cell-associated ^{51}Cr-lipid A following Percoll density gradient fractionation of post-nuclear homogenates. When monocytes were pretreated with ^{3}H-endotoxin under the same experimental conditions, all of the label was recovered in the extracellular medium, and subcellular fractionation revealed no fractions which contained tritium. Taken together, our results suggest that specific and high affinity interactions between monocyte membranes and endotoxin molecules are likely to depend on plasma membrane structures which are assembled in intact monocytes but which are disrupted when plasma membranes are isolated from these cells.

Introduction

Bacterial lipopolysaccharides, or endotoxins, comprise a class of membrane-active molecules derived from the cell wall of Gram-negative bacteria [1–3] which exert a variety of effects in a number of types of mammalian cells [4–6]. Interaction of these complex macromolecules with surface structures of susceptible cells is the first step in, and a prerequisite for, initiation of many of the pathophysiologic responses elicited by endotoxins [2]. The ability of endotoxins to associate with cell surfaces and to produce most of their biologic effects is apparently conferred by the lipid moiety, lipid A [7]. The polysaccharide components of endotoxins, when separated from lipid A, do not possess endotoxic bioactivity but may be involved in the initial attachment of the molecules to the cell surface [8]. It has been suggested that plasma membranes of cells sensitive to endotoxins regulate their response to stimuli initiated by these macromolecules either by a receptor-mediated process (involving specific endotoxin-binding structures [8–10] which are integral membrane components) or through a more general type of membrane interaction (which is dependent, for the most part, on the physical and structural state of the cell surface and the plasma membrane [11]). The molecular events which take place at the level of the cell membrane and which give rise to endotoxin-induced stimulation of the cell have not been well defined.

A primary biologic target for the action of bacterial endotoxins is known to be the mono-

* To whom correspondence should be addressed.

nuclear phagocyte, an immunologic effector cell which exhibits a rapid change in numerous cell activities [12–16] upon exposure to endotoxins or to lipid A. We [15] and others [17–20] have observed that although monocytes and macrophages initially respond to endotoxins by synthesizing and/or secreting a variety of proteins, such as granulocyte-monocyte colony-stimulating factors [15,17–20], β-glucuronidase [21], or endogenous pyrogen [22], these cells rapidly become highly refractory or 'tolerant', to further stimulation by endotoxins [15,21,22]. In order to gain insight into the nature of the resistance to endotoxins which human monocytes appear to acquire, our laboratory has recently begun to investigate the characteristics of the binding of endotoxins to the surface of these cells before and after induction of endotoxin resistance in vitro. The present report describes specifically the nature of the interaction of radiolabeled preparations of *Salmonella typhi* endotoxin and lipid A with plasma membranes isolated from monocytes obtained from human peripheral blood.

Methods

Preparation of human monocytes. Monocytes were isolated by adherence separation from normal human peripheral blood [23] following centrifugation on sodium metrizoate-ficoll gradients [24]. Viability assessed by the trypan blue exclusion method consistently exceeded 91%, and the percentage of monocytes, calculated from 45 experiments in our laboratory and determined by alpha-naphthylbutyrate staining [25], was 91.2 ± 1.0 (mean ± S.E.).

Incubation of monocytes with ^{3}H-endotoxin and ^{51}Cr-lipid A; density gradient fractionation of monocytes. Monocytes were incubated in McCoy's 5A medium at a concentration of $(0.1–1) \cdot 10^{6}$/ml for 16 h at 37°C in a humid incubator containing 5% CO_2 in the presence or absence of either ^{51}Cr-lipid A (2–5 μg/ml) or ^{3}H-endotoxin (1 μg/ml). Free ligand was removed by multiple centrifugations after which the cell pellets were resuspended in 1–2 ml 0.012 M phosphate-buffered 0.15 M saline (pH 7.4), and radioactivity was then measured. Monocytes were also subjected to fractionation by centrifugation in the presence of preformed self-generated gradients of Percoll [26]. Centrifuge tubes containing 10 ml of iso-osmotic Percoll solution (density = 1.075 g/ml) were prepared by mixing 5.3 ml Percoll with 1 ml 0.12 M phosphate-buffered 1.5 M saline (pH 7.4) and 3.7 ml distilled water. A gradient was formed by centrifugation at 4°C for 30 min at $15000 \times g$. 1.0 ml of the cell suspension was then layered on top of the preformed gradient, and centrifugation was carried out at $2000 \times g$ for 30 min. Fractions (1.0 ml) were removed and assayed for radioactivity and cell number.

Isolation of plasma membranes. Monocytes were suspended in 15–20 ml cold buffer containing 25 mM Tris-HCl (pH 7.4)/5 mM KCl/2 mM $MgSO_4$/10 mM Na_2ATP (lysis buffer) and equilibrated in a cell disruption chamber at 450 lb/inch^2 at 4°C. After 20 min the cells were lysed by release from the cavitation chamber and collected into a tube containing Na_2EDTA at a final concentration of 1.0 mM. The cell homogenate was centrifuged at $500 \times g$ for 10 min at 4°C to remove nuclei and undisrupted cells. 1.0 ml of each supernate was then mixed with 1.22 ml Percoll, 0.24 ml of 10-fold concentrated lysis buffer, and 0.96 ml distilled water to give a starting density of 1.05 g/ml [27]. Centrifugation was performed at $26000 \times g$ for 30 min at 4°C. 0.5–1.0-ml fractions were removed by pipette, pooled, diluted two times with lysis buffer, and subjected to centrifugation at $100000 \times g$ for 90 min to separate the Percoll beads from membrane material.

Radioactivity, density, enzyme and protein assays. Radioactivity was measured by β- or γ-spectrometry. The density of Percoll-containing fractions was determined using density marker beads (Pharmacia Fine Chemicals, Raritan, NJ). Alkaline phosphatase activity was used as a marker for plasma membranes and was quantitated using *p*-nitrophenylphosphate as the substrate in 0.1 M glycine buffer, pH 10.4 [28]. Lysozyme, which was used to identify the presence of granules, was assayed according to a previously described procedure [29] using *Micrococcus leisodykticus* as the substrate. Protein was measured in diluted fractions by the method of Lowry et al. [30] or by the Coomassie brilliant blue method of Bradford [31].

Cell surface labeling. Monocytes were labeled with tritium by the galactose oxidase method [32].

$(20–50) \cdot 10^6$ monocytes were incubated in 2 ml of 0.012 M phosphate-buffered 0.15 M saline (pH 7.4) with 12.5 International Units of neuraminidase and 5 units of galactose oxidase for 30 min at 37°C. The cells were washed twice by centrifugation, resuspended in 2.0 ml of 0.012 M phosphate-buffered 0.15 M saline (pH 7.4) and reduced with 0.5 mCi of tritiated sodium borohydride (^{3}H-$NaBH_4$, 20 Ci/mmol, ICN Radiochemicals) for 15 min at room temperature. The cells were washed, lysed by nitrogen cavitation, and fractionated on Percoll gradients as described. Distribution of radioactivity was measured in each 0.5 ml fraction.

Preparation of ^{3}H-endotoxin. Lyophilized *S. typhi* lipopolysaccharide B (Lot No. 680016, Difco Laboratories, Detroit, MI) was used as starting material. Details regarding our subsequent purification of this preparation of endotoxin have been previously reported [33]. This material was labeled with tritium using tritiated sodium borohydride (^{3}H-$NaBH_4$, 20 Ci/mmol, ICN Radiochemicals, Irvine, CA) using a modification of the method of Tack et al. [34] which we have previously described [33]. Tritiated *S. typhi* endotoxin (^{3}H-endotoxin) prepared in this manner was then subjected to gel filtration using a Sepharose 6B column under conditions which we have previously outlined [33] in order to obtain from the void volume a single peak of ^{3}H-endotoxin which had a minimal molecular weight of approx. 10^6 daltons. Endotoxin purified in this manner had a specific activity of 10 Ci per mmol (approx. 20000 cpm/μg) as determined by β-spectrometry and retained potent biologic activity as assessed by its ability to coagulate *Limulus* amebocyte lysate, to elevate the plasma concentration of granulocyte-monocyte colony-stimulating factor within 4 h after intravenous injection into CF_1 mice [35], and to stimulate isolated human monocytes to secrete granulocyte-monocyte colony-stimulating factor when added to liquid suspension cultures of these cells at concentrations of 0.5 to 1.0 μg/ml [15]. The percent of protein in each sample was determined by the method of Lowry et al. [30] or estimated by amino acid analysis which was kindly carried out for us in the laboratory of Dr. Carl Franzblau in the Department of Biochemistry [33]. Although the starting preparation of *S. typhi* lipopolysaccharide B contained 12.4% protein by weight, the final preparation of ^{3}H-endotoxin isolated by gel filtration contained negligible contamination by protein. In order to investigate this specific question, we isolated the carbohydrate moiety of the ^{3}H-endotoxin by mild acid hydrolysis [36] and demonstrated that this fraction contained > 99% of the radiolabel [33]. We further showed that the carbohydrate moiety of the ^{3}H-endotoxin, which contained virtually all of the radiolabel, contained only trace amounts of the amino acids asparagine, serine, glutamine, and glycine [33].

Preparation of ^{51}Cr-lipid A. Lipid A was extracted from *S. typhi* lipopolysaccharide B by mild acid hydrolysis in 1% acetic acid as described [36] and stored at −20°C. Lack of contamination by polysaccharide was demonstrated by assaying each sample for 2-keto-3-deoxyoctonate using a colorimetric assay [37]. ^{51}Chromium was incorporated into lipid A according to a procedure described by Braude et al. [38]. $^{51}CrCl_3$ (1 mCi, 304.7 mCi/mg, 10 mg/ml; ICN Radiochemicals, Irvine, CA) was added to lipid A (1 mg/ml in 0.012 M phosphate-buffered 0.15 M saline (pH 7.4)) and stirred for 24 h at 37°C. Excess label was removed by dialysis and by repeated centrifugations in the same buffer. Specific activity was based on the quantity of starting material and the organic phosphorus content [39] and ranged between 2000 and 4000 cpm/μg (1.8–7.2 mCi/mmol).

Membrane binding assays. Plasma membranes were prepared from human monocytes as described and were suspended in 0.012 M phosphate-buffered 0.15 M saline (pH 7.4). In the binding assay, 100 μl of membrane material (10–30 μg membrane protein) was incubated with ^{3}H-endotoxin or ^{51}Cr-lipid A at 37°C in the presence or absence of excess endotoxin (0.2 mg/ml) in a final volume of 0.25 ml of 0.012 M phosphate-buffered 0.15 M saline (pH 7.4) for periods of time varying from 15 to 240 min. The reaction was stopped either by the addition of 2.0 ml iced 0.15 M NaCl or 0.5 ml boiling 10% trichloroacetic acid. Samples were collected on Whatman GF/B filters under vacuum and were washed with an additional 8 ml of cold saline or 5% trichloroacetic acid. Nonspecific binding was defined as the radioactivity that remained bound in the presence of excess (0.5

mg/ml) unlabeled endotoxin, and background radioactivity was defined as the amount that remained associated with the filter in the absence of cells. These values were subtracted from all data points in order to produce a uniform estimate of specific binding.

Binding of ^{3}H-endotoxin and ^{51}Cr-lipid A to membranes was also studied by Percoll gradient fractionation following incubation of membrane material with each ligand. Percoll-free preparations of monocyte membranes (50–100 mg/ml) in 0.012 M phosphate-buffered 0.15 M saline (pH 7.4) or lysis buffer were incubated with ^{3}H-endotoxin or ^{51}Cr-lipid A (2 μg/ml) at 37°C for up to 16 h. Following the incubation period, 1.5 ml samples were mixed with 9 ml of isotonic Percoll solution and centrifuged at 27000 × g for 30 min at 4°C. 0.50–0.75-ml fractions were removed and analyzed as described above.

Results

Separation of monocyte plasma membranes by density gradient centrifugation

Two major bands were distinctly visible after centrifugation of post-nuclear homogenates on continuous Percoll gradients: a buoyant band (Band I), which had a density of 1.017–1.03 g/ml and which contained primarily plasma membrane fragments, and a denser band (Band II), which had a density of 1.058–1.064 g/ml and which consisted predominantly of intact granules (Fig. 1A). Band I contained alkaline phosphatase activity but no lysozyme, and 90% of the ^{3}H-labeled surface glycoproteins were included in this fraction. Band II was strongly positive for lysozyme but contained no alkaline phosphatase activity, and was essentially free of ^{3}H-labeled surface glycoproteins.

Density gradient centrifugation of monocytes after incubation with ^{3}H-endotoxin and ^{51}Cr-lipid A

In multiple experiments, we observed that monocytes which had been preincubated with ^{51}Cr-lipid A for 24 h retained a significant proportion of the total label 12.0 ± 2.1% (mean ± S.D.) which remained associated with the cells during centrifugation on pre-formed Percoll gradients. To investigate the geographic distribution of the retained radiolabel, post-nuclear supernates from cells treated with ^{51}Cr-lipid A were prepared and fractionated by density gradient centrifugation. Using this approach, the retained radiolabel was found to be distributed approximately evenly between the membrane and granule fractions (Fig. 1B). In contrast, monocytes which had been incubated with ^{3}H-endotoxin in an analogous manner were essentially free of measurable radiolabel after washing the cells, and after density gradient centrifugation of the post-nuclear homogenates, no radioactive fractions were found (Fig. 1B).

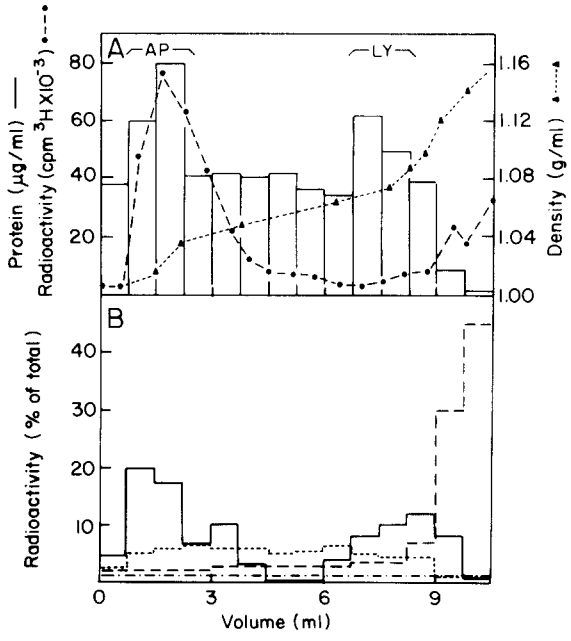

Fig. 1. (A) The distribution of subcellular fractions of disrupted monocytes on a continuous Percoll gradient. Post-nuclear homogenates from untreated and ^{3}H-labeled monocytes were isolated, disrupted by nitrogen cavitation, and fractionated as described. Each fraction was then analyzed for density (▲), radioactivity (●), protein (bars), alkaline phosphatase activity (AP), and lysozyme activity (LY).
(B) The distribution of radioactivity in membrane and granule fractions from monocytes after incubation of the cells with ^{3}H-endotoxin or ^{51}Cr-lipid A. In these experiments, monocytes were incubated with the appropriate radioligands as described, disrupted by nitrogen cavitation, and subjected to fractionation on continuous Percoll gradients. Shown are the distributions of radioactivity in subcellular fractions from cells incubated with ^{3}H-endotoxin (·–·–·) or ^{51}Cr-lipid A (———). As controls, ^{3}H-endotoxin (- - - - -) or ^{51}Cr-lipid A (— — —) were fractionated in cell-free suspensions on identical Percoll gradients.

Binding of ^{3}H-endotoxin and ^{51}Cr-lipid A to monocyte membranes

^{3}H-Endotoxin was incubated with increasing concentrations of plasma membrane in the presence or absence of excess unlabeled endotoxin, and binding was measured after precipitation of the sample with 5% trichloroacetic acid (Fig. 2). Specific binding of ^{3}H-endotoxin was maximal (80.0 ± 5.0% (mean ± S.D.)) when 20–40 μg/ml of membrane protein was present. At higher concentrations (⩾ 40 μg/ml) of membrane protein, the increase in non-specific binding of ^{3}H-endotoxin was considerable and resulted in an apparent decrease in specific binding. In subsequent membrane binding studies, < 40 μg/ml samples of membrane protein were used. We were not able to detect specific binding of ^{3}H-endotoxin to membranes when mixtures of ^{3}H-endotoxin and membrane preparations were subjected to rapid filtration on glass fiber filters following incubation periods of 0–240 min at 37°C (Fig. 2). For that reason, membrane binding of ^{3}H-endotoxin was measured after precipitating the samples with 5% trichloroacetic acid. Using this technique, we found that the association between ^{3}H-endotoxin and monocyte membranes at 37°C was complete within 2 min and remained constant for as long as 4 h from the start of the incubation period (data not shown).

In additional experiments, membranes were exposed to increasing concentrations of ^{3}H-endotoxin in the presence and absence of unlabeled endotoxin (Fig. 3). Specific ^{3}H-endotoxin binding to membranes was determined and was found not to be saturable even at concentrations as high as 8 μg/ml of ^{3}H-endotoxin.

In similar experiments, ^{51}Cr-lipid A (2.4–3.2 μg/ml) was incubated with increasing concentrations of monocyte membrane, and total, non-specific, and specific binding were measured.

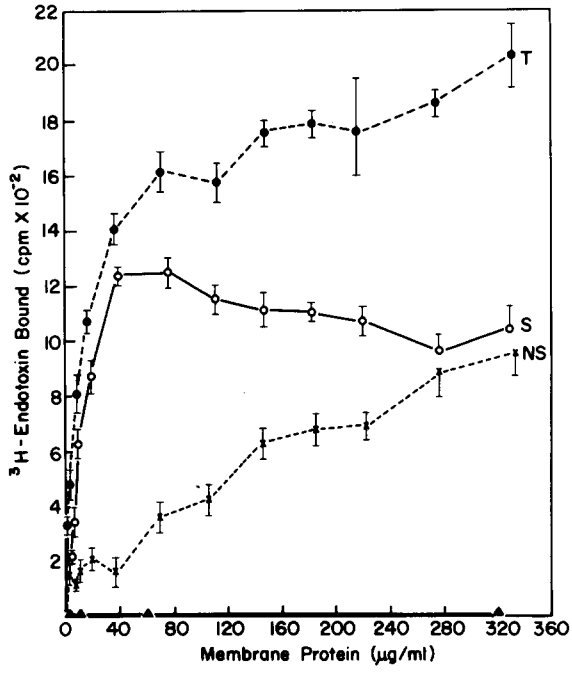

Fig. 2. Effect of increasing membrane concentrations on the binding of ^{3}H-endotoxin to monocyte membranes. ^{3}H-Endotoxin (0.4–0.6 μg/ml) was incubated at 37°C for 20 min with increasing concentrations of membrane (5–320 μg/ml) in the absence (●) or presence (x) of unlabeled endotoxin (5 mg/ml). Total (T), specific (S), and non-specific (NS) binding are shown. Binding was measured by precipitation with 5% trichloroacetic acid (○, ●, x) or by rapid filtration (▲) of each sample. Each data point shown represents mean ± S.D. of duplicate experiments.

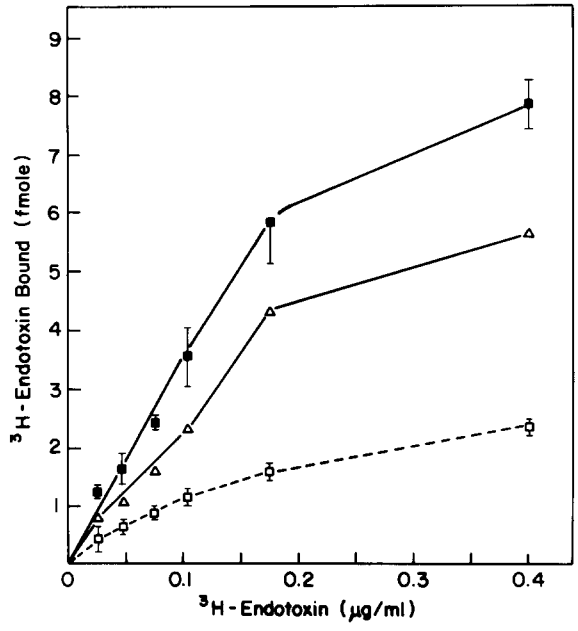

Fig. 3. Binding of ^{3}H-endotoxin to monocyte membranes as a function of the concentration of ^{3}H-endotoxin. Membranes (24 μg/ml) were incubated with serial concentrations of ^{3}H-endotoxin, and total (■), non-specific (□), and specific (△) binding were measured. Each point represents the mean ± S.E. of three samples. Results shown are from a representative experiment repeated four times.

Specific binding of ^{51}Cr-lipid A to membranes was maximal (67.0 ± 19.0% (mean ± S.D.)) at low concentrations of membrane protein; however, determinations of specific binding varied widely. At membrane concentrations greater than 10 μg/ml, nonspecific binding generally exceeded 60% of the total amount of ^{51}Cr-lipid A bound. Under these limiting conditions, the probability of an accurate determination of specific binding was very low and precluded accurate estimation of binding parameters. Binding of ^{51}Cr-lipid A to membranes was not observed when mixtures of ^{51}Cr-lipid A and membranes were subjected to rapid filtration on glass fiber filters following the incubation period. The radioactivity associated with the filters was the same in the presence and absence of membrane material.

Relatively high concentrations of unlabeled endotoxin were required to inhibit the binding of ^{3}H-endotoxin and ^{51}Cr-lipid A to isolated monocyte membranes effectively. A 1.0 μM (1.0 mg/ml) concentration of endotoxin was required to produce 50% inhibition of ^{3}H-endotoxin binding, and

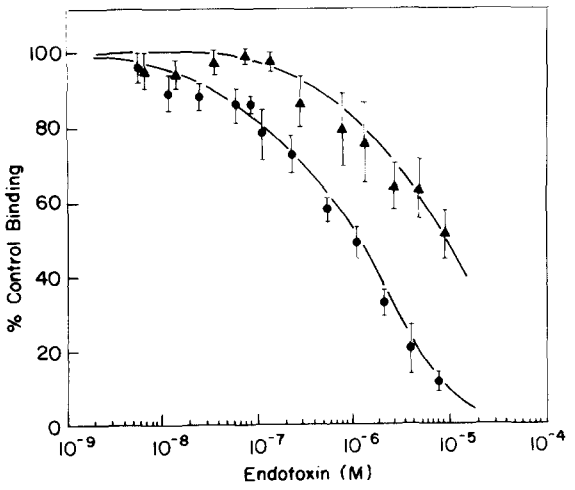

Fig. 4. Inhibition of binding of ^{3}H-endotoxin and ^{51}Cr-lipid A to monocyte membranes by unlabeled endotoxin. Membranes (10–50 μg/ml) were incubated in 0.25 ml 0.012 M phosphate-buffered 0.15 M saline with ^{3}H-endotoxin (0.4–0.6 μg/ml) or ^{51}Cr-lipid A (2–3 μg/ml) in the presence of increasing concentrations of unlabeled endotoxin ranging from 1 μg/ml to 20 μg/ml for 20 min at 37°C. Binding was measured as described. Results are expressed as percent of control binding which was defined in each experiment as the amount of ^{3}H-endotoxin (●) or ^{51}Cr-lipid A (▲) which bound in the absence of unlabeled endotoxin. Each point represents the mean ± S.E. of data from a minimum of three experiments.

a concentration of 10 μM (10 mg/ml) was needed to inhibit ^{51}Cr-lipid A binding to a similar degree (Fig. 4). Unlabeled lipid A had no inhibitory effect on the binding of either ^{3}H-endotoxin or ^{51}Cr-lipid A.

Discussion

In the present report, we describe the interaction of the radiolabeled endotoxin molecules, ^{3}H-endotoxin and ^{51}Cr-lipid A, with plasma membranes from human peripheral monocytes. Our data indicate that both of these radioligands bind to isolated monocyte membranes with lower affinity and with apparently less specificity than we have previously observed to be the case in similar studies using intact, viable monocytes [33]. Taken together, our results suggest that specific, high affinity, and presumably physiologically effective interactions between bacterial endotoxins and monocytes are likely to require complex plasma membrane structures which are part of the intact cell, and cannot be accounted for strictly on the basis of classical ligand-receptor binding.

It has long been recognized that when monocytes and macrophages are exposed to bacterial endotoxins, an extraordinary array of physiologic processes are triggered within these cells [40], which includes the production of a number of glycoproteins [15,17–25], among which are the specific granulopoietic growth factors commonly referred to as granulocyte-monocyte colony-stimulating factors [15,17–19]. We have previously observed that when human monocytes are activated by endotoxins, they initially synthesize and secrete large quantities of granulocyte-monocyte colony-stimulating factors but rapidly become refractory, or 'tolerant', to the stimulatory effects of endotoxins, as assessed by the production of these particular glycoproteins [15]. In an earlier study, in order to investigate the subcellular mechanisms involved in the acquisition of the endotoxin-refractory state which we have observed to develop rapidly in these cells, we examined the binding of radiolabeled endotoxin to intact monocytes that were maintained in liquid suspension cultures [33]. We found that the binding of ^{3}H-endotoxin to viable monocytes consisted of a rapid ($t_{1/2} < 5$ min), reversible, temperature-independent phase of

surface adsorption followed by a slower ($t_{1/2} > 20$ min), temperature-dependent period of cellular uptake which could not be reversed by the addition of competing concentrations of endotoxin. We further noted that pretreatment of the cells with native endotoxin resulted in a decrease in the capacity of the monocytes to bind ^{3}H-endotoxin which appeared to be due to a decrease in the number of binding sites on the cell surface available for the attachment of endotoxin molecules and not to a decrease in the avidity of binding. To pursue these initial observations which were made using viable, intact monocytes, the experiments summarized in this report were undertaken.

In our present study, we found that the properties of the binding of ^{3}H-endotoxin and ^{51}Cr-lipid A to isolated monocyte membranes differed considerably from the kinetics of the binding of these radioligands to the surface of the intact cells. Results from competition binding experiments indicated that the affinity of both radioligands for isolated membranes appeared to be considerably lower than for intact monocytes, since large quantities of unlabeled endotoxin were required to inhibit 50% of the binding of ^{3}H-endotoxin and ^{51}Cr-lipid A (1.0 mg/ml and 10.0 mg/ml, respectively). These concentrations of unlabeled endotoxin were 100-fold greater than those required to produce a similar degree of inhibition of binding to whole cells [33]. In these experiments, the binding of ^{3}H-endotoxin to isolated membranes appeared to be of low affinity and low specificity. In addition, distinct complexes between ^{3}H-endotoxin and membrane, or ^{51}Cr-lipid A and membrane, were not detected after density gradient centrifugation or rapid filtration of membrane suspensions which had been incubated with each of these radioligands, a finding which suggested that isolated membrane structures did not interact specifically with these molecules and further strengthened this conclusion.

It appears, then, that endotoxin molecules bind avidly to plasma membranes of intact monocytes but not to isolated membrane fractions from these cells. In addition, our data indicate that lipid A may remain firmly attached to, or embedded in, membrane and intracellular structures derived from whole monocytes preincubated with ^{51}Cr-lipid A. Although ^{3}H-endotoxin apparently does not remain associated with monocytes even after prolonged incubation periods, this finding may indicate either than endotoxin is effectively prevented from insinuating itself into the plasma membrane because of the hydrophilic properties imparted to the molecule by its carbohydrate moiety, or, since the tritium label in this preparation is confined to the polysaccharide portion of ^{3}H-endotoxin, cell-mediated hydrolysis of the molecule with subsequent release of ^{3}H-labeled carbohydrate may make it impossible to detect the presence of residual lipid A associated with the membrane.

In summary, our data may be taken to indicate that recognition and attachment of endotoxin molecules to the monocyte membrane do not depend solely on the presence of specific cell surface receptors. Instead, specificity and affinity of endotoxin for cell membranes may be attributed to the particular physical and structural state of the membrane which isolation procedures may alter. Precise spatial arrangements and structural interrelationships of phospholipids and proteins as well as the surface charge distribution, the surface area, and the resting membrane potential of the cell are factors which may dictate membrane interactions with these macromolecules by directly affecting hydrophobic and ionic associations between endotoxin molecules and the cell membrane which are necessary for effective binding.

Acknowledgements

We are indebted to Dr. Carl Franzblau and George Crombi in the Department of Biochemistry, Boston University School of Medicine, for graciously performing the amino acid analyses, Laura McCarroll for excellent technical assistance, and Christina Gray-Miller for typing this manuscript. This work was supported by grants No. 5-S07 RR0 5487-16, AM 27455, and 5T-32-HL07501-03, from the National Institutes of Health; Institutional Research Grant IN - 97G from the American Cancer Society, Inc.; the Medical Foundation of Boston, Inc.; and the Leukemia Research Foundation, Inc.

References

1 Shands, J.W., Jr. (1973) J. Infect. Dis. 128 (Supl.), 197–201
2 Springer, G.F. and Adye, J.C. (1975) Infect. Immunol. 12, 978–986
3 Utili, R., DiDonato, A., Draetta, G., Paolisso, G., Abernathy, C.O. and Illiano, G. (1982) Experientia 38, 831–833
4 Des Prez, R.M., Horowitz, H.I. and Hook, E.W. (1961) J. Exp. Med. 114, 857–874
5 Buckingham, R.B. and Castor, C.W. (1972) J. Clin. Invest. 51, 1186–1194
6 Skidmore, B.J., Chiller, J.M., Morrison, D.C. and Weigle, W.O. (1975) J. Immunol. 114, 770–775
7 Hämmerling, U. and Westphal, O. (1967) Eur. J. Biochem. 1, 46–50
8 Coutinho, A., Forni, L. and Watanabe, T. (1978) Eur. J. Immunol. 8, 63–67
9 Watson, J. and Riblet, R. (1975) J. Immunol. 114, 1462–1468
10 Haeffner-Cavaillon, N., Chaby, R., Cavaillon, J.-M. and Szabó, L. (1982) J. Immunol. 128, 1950–1954
11 Benedetto, D.A., Shands, J.W., Jr. and Shah, D.O. (1973) Biochim. Biophys. Acta 298, 145–157
12 Edelson, P.J., Zwiebel, R. and Cohn, Z.A. (1975) J. Exp. Med. 142, 1150–1164
13 Mørland, B. and Kaplan, G. (1977) Exp. Cell Res. 108, 279–288
14 Ruco, L.P., Meltzer, M.S. and Rosenstreich, D.L. (1978) J. Immunol. 121, 543–548
15 Sullivan, R., Gans, P.J. and McCarroll, L.A. (1983) J. Immunol. 130, 800–807
16 Ganguly, N.K., Lloyd, B., Price, C.P., Kingham, J.G.C., Lloyd, R.S. and Triger, D.R. (1978) Lancet 1, 1073–1075
17 Moore, M.A.S., Williams, N. and Metcalf, D. (1973) J. Natl. Cancer Inst. 50, 591–602
18 Ruscetti, F.W. and Chervenick, P.A. (1974) J. Lab. Clin. Med. 83, 64–72
19 Cline, M.J., Rothman, B. and Golde, D.W. (1974) J. Cell Physiol. 84, 193–196
20 Eaves, A.C. and Bruce, W.R. (1974) Cell Tissue Kinet. 7, 19–30
21 Snyder, S.L., Eklund, S.K. and Walker, R.I. (1979) Can. J. Microbiol. 25, 1245–1251
22 Dinarello, C.A., Bodel, P.T. and Atkins, E. (1968) Trans. Assoc. Am. Phys. 81, 334–344
23 Shaw, G.M., Levy, P.C. and LoBuglio, A.F. (1978) Blood 52, 696–705
24 Bøyum, A. (1968) Scand. J. Clin. Lab. Invest. 21 (Supp 97), 77–89
25 Koski, I.R., Poplack, D.G. and Blaese, R.M. (1976) in In Vitro Methods in Cell-Mediated and Tumor Immunity (Bloom, B.R. and David, J.R., eds.), pp. 359–362, Academic Press, New York
26 Brandslund, I., Rasmussen, J.M., Fisker, D. and Svehag, S.-E. (1982) J. Immunol. Methods 48, 199–211
27 Perret, B., Chap, H.J. and Douste-Blazy, L. (1979) Biochim. Biophys. Acta 556, 434–446
28 Kimball, H.R., Ford, G.H. and Wolff, S.M. (1975) J. Lab. Clin. Med. 86, 616–630
29 Shugar, D. (1952) Biochim. Biophys. Acta 8, 302–309
30 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
31 Bradford, M.M. (1976) Anal. Biochem. 72, 248–254
32 Gahmberg, C.G. and Andersson, L.C. (1977) J. Biol. Chem. 252, 5888–5894
33 Larsen, N.E. and Sullivan, R. (1984) Proc. Natl. Acad. Sci. USA 81, 3491–3495
34 Tack, B.F., Dean, J., Eilat, D., Lorenz, P.E. and Schechter, A.N. (1980) J. Biol. Chem. 255, 8842–8847
35 Sullivan, R., Zuckerman, K.S. and Quesenberry, P.J. (1980) Br. J. Haematol. 44, 365–374
36 Galanos, C., Rietschel, E.T., Lüderitz, O. and Westphal, O. (1971) Eur. J. Biochem. 19, 143–152
37 Dröge, W., Lehmann, V., Lüderitz, O. and Westphal, O. (1970) Eur. J. Biochem. 14, 175–184
38 Braude, A.I., Carey, F.J., Sutherland, D. and Zalesky, M. (1955) J. Clin. Invest. 34, 850–857
39 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468
40 Elin, R.J. and Wolff, S.M. (1976) Annu. Rev. Med. 27, 127–141