

Interaction of Bifidobacterial Lipoteichoic Acid with Human Intestinal Epithelial Cells

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Binding of the lipoteichoic acids of *Bifidobacterium bifidum* to human colonic epithelial cells appeared to be specific, reversible, and cell concentration and time dependent. A single population of approximately 8.3×10^8 binding sites per cell was detected, with a dissociation constant of 125 μM . Ester-linked fatty acids are essential for binding.

Lipoteichoic acids (LTA) of gram-positive bacteria have been shown to possess a high binding affinity to mammalian cell membranes (2-4, 8, 9, 14, 26). Binding occurs spontaneously via the lipid part of the LTA (4, 9, 17). Studies concerning the binding of LTA are of special interest, because LTA appears to play an important role in the adherence of several gram-positive bacteria to epithelial and other mammalian cells (1, 5, 7, 9). LTA may also serve as a carrier for other antigens and bind them to target tissues, in which they may provoke immunocytotoxic reactions (9, 11).

Bifidobacterium bifidum subsp. *pennsylvanicum* is a gram-positive, nonmotile, asporogenous, anaerobic organism normally present in the human intestine (13). Previously we described the structures of the rather unique teichoic acid (27) and LTA (19a) of this bacterium. The present investigations, concerning the binding of the bifidobacterial LTA to human colonic epithelial cells (colonocytes), were performed to obtain more information about the biological activities and functions of the LTA.

B. bifidum subsp. *pennsylvanicum* was grown in a chemically defined medium (16) for 16 h at 37°C under an N₂-CO₂ (90%:10%) atmosphere. For labeling, [¹⁴C]oleic acid (250 $\mu\text{Ci/liter}$) was included in the medium, and the Tween 80 concentration was lowered from 0.5 to 0.1 g/liter. Unlabeled LTA and [¹⁴C]oleic acid-labeled LTA ([¹⁴C]LTA) were extracted from cytoplasmic membranes with aqueous phenol and purified as described before (19a). The purified [¹⁴C]LTA had a specific activity of about 190 dpm/ μg . For preparation of free colonocytes, biopsies from colon resection surgery were incubated in appropriate buffers with the chelation-elution method described by Pinkus (20). The suspension of isolated colonocytes was washed three times in 0.02 M phosphate-0.15 M NaCl (PBS) (pH 7.4), resuspended in PBS, and counted in a hemacytometer. Cell viability was estimated by trypan-blue exclusion. Although the viability of the isolated colonocytes varied between 40 and 80%, we could not detect any significant differences in their ability to bind LTA.

Binding assays were performed in 1.5-ml Eppendorf tubes (Brinkmann Instruments, Inc., Westbury, N.Y.). Tubes containing 150 μl of PBS with various amounts of [¹⁴C]LTA and colonocytes were incubated at 37°C with shaking. After a specified time, the assay was terminated by adding 1.5 ml of PBS to each tube. The cell-[¹⁴C]LTA complexes were collected by centrifugation, washed three times with 1.0 ml

of PBS, resuspended in 0.5 ml of 0.1 M NaOH, and heated for 5 min at 96°C. Bound [¹⁴C]LTA was determined by liquid scintillation counting in an Opti-Fluor (Packard Instrument, Brussels, Belgium).

The binding of LTA to colonocytes was cell concentration (Fig. 1) and time dependent (Fig. 2). Binding reached a maximum within 60 min and was comparable with the binding of streptococcal LTA to buccal epithelial cells (26). Binding of bifidobacterial LTA appeared to be reversible, since 45% of the bound [¹⁴C]LTA was displaced by a 50-fold excess of unlabeled LTA within 10 min. [¹⁴C]LTA remained bound in PBS controls. Displacement of 80% of labeled LTA has only been described for leukocytes (9). Other authors have found 40 to 55% displacement (2, 4, 8, 26). The occurrence of the added unlabeled LTA in micelles may be responsible for the inability to displace all of the bound LTA (8). Internalization is not expected. Control incubation with a 50-fold excess of unlabeled LTA included in the binding assay revealed aspecific binding values which were always less than 10% of the experimental values.

To study the influence of deacylation of LTA on the binding, we labeled the polar part of the LTA with [U-¹⁴C]glucose. Glycerol could not be used for labeling since it is not incorporated by *B. bifidum*. Extraction and purification of LTA was as described above. Experiments with [¹⁴C]glucose-labeled LTA gave identical results as those with [¹⁴C]oleic acid-labeled LTA, but the low specific activity of the former made it less useful for all experiments. Deacylation of the [U-¹⁴C]glucose-labeled LTA, by treatment with 0.1 M NaOH in ethanol for 30 min at 37°C, abolished the binding of the LTA. This is consistent with the requirement of ester-linked fatty acids for binding (1, 2, 8). To determine the number of LTA binding sites per colonocyte and the affinity constant, we incubated 4×10^5 colonocytes with various amounts of [¹⁴C]LTA (50 to 1,000 μg) for 1 h at 37°C. Bound [¹⁴C]LTA was calculated, and the data were analyzed by the method of Scatchard (22) (Fig. 3). The Scatchard plot revealed a maximal binding (at saturation) of 5.49 μg of LTA per 4×10^5 colonocytes. Assuming a molecular weight of 10,000 for the bifidobacterial LTA (19a), we calculated that colonocytes possess a single population of about 8.3×10^8 binding sites per cell. The dissociation constant was 125 μM . Since bifidobacterial LTA also binds to human erythrocytes, for comparison we determined the population of binding sites on these cells. Human erythrocytes appeared to possess a single population of about 2.1×10^6 binding sites per cell, with a dissociation constant of 44

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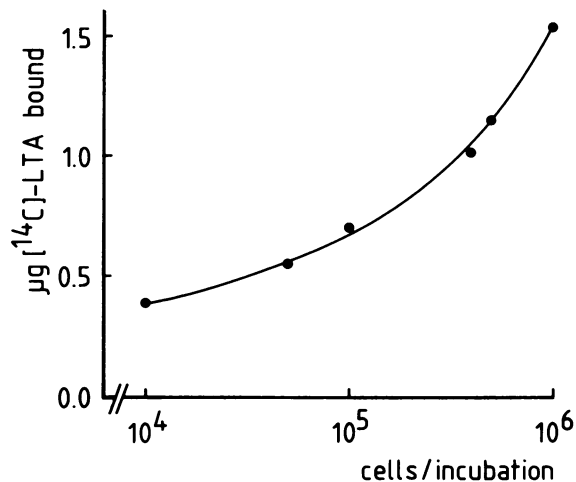


FIG. 1. Relationship between the number of colonocytes and binding of LTA. The indicated number of cells were incubated with 50 µg of [¹⁴C]LTA for 1 h at 37°C, and bound [¹⁴C]LTA was determined. Values are means of two separate experiments and are corrected for nonspecific binding.

µM. The values for colonocytes as well as for erythrocytes are comparable with those described for other LTA (8, 26).

We also tested the effect of some sugars and albumin on the binding of [¹⁴C]LTA to colonocytes. None of the sugars tested (fucose, glucose, galactose, mannose, arabinose, and xylose) inhibited the binding at concentrations up to 25 mg/ml, indicating that sugar moieties are not involved in the interaction between the epithelial cell membrane and the LTA. Albumin (5 mg/ml) markedly reduced the binding to 34 ± 3% that of the control, most probably by masking of the lipid part of the LTA. Simpson et al. (25) showed that streptococcal LTA binds readily to the fatty acid binding sites of serum albumin.

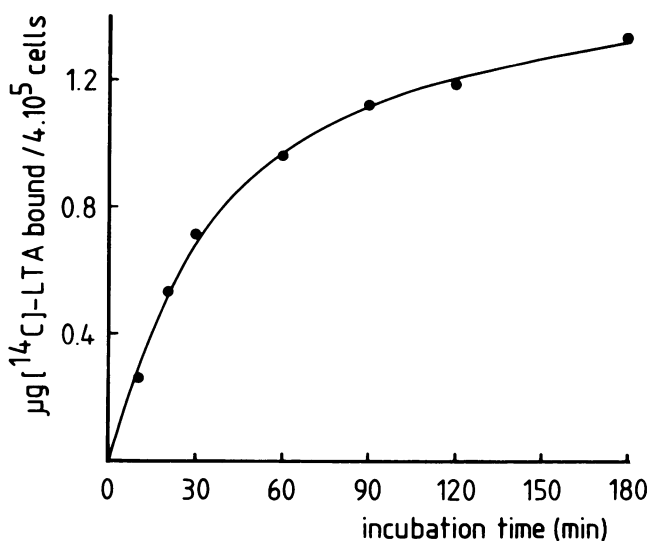


FIG. 2. Time-dependent binding of LTA to human colonocytes. 50 µg of [¹⁴C]LTA was incubated with 4 × 10⁵ colonocytes at 37°C. At the indicated time intervals, bound [¹⁴C]LTA was determined. Values are means of two separate experiments and corrected for nonspecific binding.

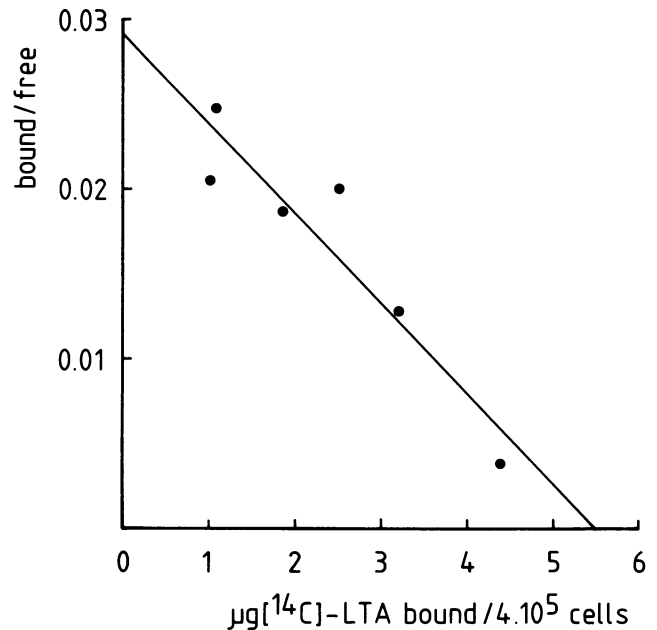


FIG. 3. Scatchard plot of the binding of LTA to human colonocytes. The ratio of bound to free [¹⁴C]LTA is plotted as a function of bound [¹⁴C]LTA. The intercept of the abscissa (5.49 µg) corresponds to the maximal binding capacity of human colonocytes.

The present study revealed that bifidobacterial LTA binds in a specific and reversible way to colonocytes. Binding characteristics were comparable with those described for oral epithelial cells (26). The LTA binds via the ester-linked fatty acids of the lipid part of the molecule, since deacylated LTA did not show binding activity and albumin effectively inhibited the binding. Because of the specificity of the binding and the importance of the lipid part, a protein or glycoprotein with fatty acid-binding sites appears to be the most probable receptor in the membrane of the colonocytes. The binding of closely related fatty acids to this putative receptor will be about equal since the rather high percentage of oleic acid in the bifidobacterial LTA compared to streptococcal LTA (40 versus 24%) (21) did not change the binding characteristics. Glatz and Veerkamp (12) reported that palmitic and oleic acid bound to the fatty acid-binding sites of albumin with the same affinity. Recently, evidence was obtained for the presence of fibronectin as a membrane receptor for streptococci on epithelial cells (6, 23, 24). Fibronectin is capable of binding LTA via its fatty acid-binding sites (10) as described for albumin (25). The importance of the lipid part of the bifidobacterial LTA in its binding to colonocytes correlates well with the role of LTA as the main component responsible for surface hydrophobicity of whole bacteria (15, 19). If the model depicted by Ofek et al. (18), concerning the interaction of LTA with cell wall proteins and its orientation, can be applied to other bacteria, hydrophobic interaction mediated by LTA may play an important role in the adherence of bifidobacteria to intestinal epithelial cells.

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