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Attachment of *Helicobacter pylori* to Monohexosylceramide with Various Ceramides

Maan Abul-Milh
College of Dentistry, Al-Muthanna University, Al-Muthanna, Iraq
E.mail address: mamilh@hotmail.com
Phone: (00964)7809099948

Abstract

Binding of Helicobacter pylori to glycolipids separated on thin-layer chromatogram and incorporated into liposomes has been carried out. Wild type clinical isolates recognized well galactosylceramide with non-hydroxylated fatty acid of ceramide [Galβ1Cer (non-OH)] and weaker bound galactosylceramide with hydroxylated fatty acid[Galβ1Cer(OH)]. Rough and culture collection strainsdemonstrated contrarily different binding profile to hydroxylated glucosylceramide [Glcβ1Cer (OH)] and hydroxylated galactosylceramide [Galβ1Cer (OH)]in comparison with the wild type strains. All tested bacterial strainsfailed to recognize glucosylceramide with non-hydroxylated fatty acid[Glcβ1Cer (non-OH)], separated on TLC palte. Glucosylceramide [Glcβ1Cer (OH)] was bound likely well as non-hydroxylated galactosylceramide [Galβ1Cer (non-OH)]by clinical isolates, whereasbinding to these sugars was weak and variable when using rough and culture collection strains. [Galβ1Cer (OH)] has been recognized by wild type strains in the liposome assay, whereas[Glcβ1Cer (non-OH)] was detected weakly and variably by both types of strains using liposome aggregation assay. Lactosylceramide (lacer),with hydroxylated fatty acid of the ceramide [Galβ1-4Glcβ1Cer (OH)] was detected by bacteria using TLC plate. Both clinical isolates andculture collection strainsstrongly recognized lacCer with hydroxylated fatty acid of ceramide on thin-layer chromatogram. Lipids from non-acid fraction of the same preparation were recognized by H. pylori characterized bypositive inding to gangliotriaosylceramide, GgO3(GalNAcβ1-4Galβ1-4Glcβ1Cer) and gangliotetraosylceramide, GgO4 (Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1Cer).PE was the only receptor active phospholipid detected byH. pylori. No binding was shown to globoseries glycolipids-containing Galα1-4Gal.

Introduction

Attachment of microbes to cell surface receptors on the target tissue is considered an essential step in the initiation, establishment, and maintenance of infection. *H. pylori* colonizes the stomach of a majority of the global human population and is implicated in several diseases of the gastrointestinal tract including chronic gastritis, duodenal and gastric ulcers, and gastric adenocarcinoma (3, 14, 25). Clinical symptoms appear in about 10–20% of the infected individuals resulting in worldwide medical problems.

In recent years, a largenumber of studies have aimed at the identification of potentialmicrobial host receptors, the majority of which appear to beglycoconjugates (6, 15, 24). Glycoconjugates exhibit a characteristic and specific pattern of expression, which is dependent on theanimal species, age, individual, and cell type (29). In1994 this pathogen was classified by the World Health Organization (WHO) as a class I carcinogen (13). Like many other microbes, *H. pylori* bacteria recognizes carbohydrates, probably mediating essential attachment to host cells (16, 17, 24). The mechanism for infection is not yet fully understood, but it probably includes binding to glycoconjugates on the host cell surface. Several binding specificities are expressed by the bacterium, including interactions with some neutral, sulfated, and sialylated carbohydrates (16). However, *H. pylori*is unusually complex in its binding to carbohydrates as shown by interaction with sialylated oligosaccharides (7, 19, 27), gangliotetraosylceramide (19), Lewis b antigen (4), and lactosylceramide (1), lactotetraosylceramide (31), sulfatide (28), and heparan sulfate (5). Thus, the recognition of a specific carbohydrate receptor on the host cell surface determines, at least in part the host, the infected tissue, age and specificities correlated to microbial infections.

Adherence of the gastric pathogen *H. pylori* to human gastric epithelial cells is required for prolonged persistence in these to mach (18a). Initial studies of potential target cell receptors for *H. pylori* demonstrated the binding of certain strains of this bacterium to the Lewis b blood group (4), and subsequently the *H. pylori* Lewis b-binding adhesion, blood group antigen-bindingadhesin (BabA) was identified (12). *H. pylori* strains expressing BabA together with the vacuolating cytotoxin VacA and the cytotoxin-associated antigen CagA strains are associated with severe gastric diseases such as peptic ulcer and gastric adenocarcinoma (9, 26). Subsequent studies demonstrated that the BabA adhesion has adapted to the fucosylated blood group antigensmostprevalentin the local population (2). Two bacterial adhesins, located on the outer membrane proteins, have so far been identified, BabA, which recognizes Lewisb antigen (12), and SabA, which binds to sialylated glycoconjugates (20).

In the present study, we have focused on the *H. pylori* binding monoglycosylceramide due to cell membrane close-located of this receptor using TLC and liposomes binding assay systems. The aim of this research was to focus on the importance of the short and simple sugared-glycolipids as receptors for *H. pylori* and the role of the these bacteria to those lipids.

Materials and Methods

H. pylori strains, culture condition and labeling.

The *H. pylori* strains were isolated from patients at the Sahlgrenska Hospital in Gothenburg, Sweden as well from obtained from Culture Collection of Gothenburg University (CCUG).

Bacterial strains cultured for chromatogram binding experiments (overlay assay), were grown in a microaerophilic atmosphere at 37 °C on Brucella agar medium (Difco) containing 10% fetal calf serum (Harlan

Sera-Lab, Lough borough, UK) inactivated at 56 °C and BBL IsoVitaleX Enrichment (BD Biosciences). Bacteria were radiolabeledby the addition of 50 µCi^{35}[S]-methionine (Amersham Biosciences) diluted in 0.5 ml of phosphate-buffered saline (PBS), pH 7.4 to the culture plates. After incubation for 48–72 h at 37°C under microaerophilic conditions, the bacteria were harvested, washed twice in PBS (pH7.4), and thereafter suspended to1x10^8 cfu/ml in PBS. The radioactive count of the suspensions was estimated to be 1 cpm/100 *H. pylori* organisms (21).

TLC binding assay.

The chromatogram binding assay was conducted as described elsewhere (10). Two similar glycolipid chromatograms, developed in parallel on Silica Gel 60 coated on aluminum sheets (HPTLC nanoplates; E. Merck AG, Darmstadt, Federal Republic of Germany), were used. The reference chromatogram was sprayed with anisaldehyde for visualization of the glycolipid bands. The other chromatogram was treated with 0.5% (wt/vol) of polyisobutylmethacrylate in diethyl ether (P28;Rohm, Darmstadt, Federal Republic of Germany) for 1 min and soaked in 2% bovine serum albumin in PBS for 2 h. The plate was then overlaid with 2 ml of ^{35}[S] methionine-labeled bacteria (10^8 cells per ml) for 2 h, subjected to five consecutive washes, dried, and exposed to X-ray film (XAR-5, Eastman Kodak Co., Rochester, N.Y.) for 20 to 70 h. The microtiter well assay was performed as described previously (18) used to support the TLC binding strength. Serial dilutions of glycolipids in methanol (50 ul) were dried onto 96-well polyvinyl chloride plates (Cooks M24; Nutacon, Amsterdam, The Netherlands), and the wells were incubated with 2% bovine serum albumin in PBS. Each well was then incubated with 50 ul of radio labeled bacteria (5 x 10^6 cells and 10^5 cpm) for 4 h, washed five times with PBS, and dried, and bound radioactive bacteria in the wells was measured with a scintillation counter.

Liposome binding assay

According to the reverse phase evaporation procedure as previously described (30). Unilamellar liposomes were prepared by using 20 µmol of lipids (glycolipid/egg phosphate idylglecerol/cholesterol/phosphatidyl choline 0.2/0.5/10/10 (mol/mol) in chloroform were dried under nitrogen and red is solved in 15 microliter chloroform: diethyl ether (1:1, v:v), thereafter 4 ml PBS was added and sonicated for 3 min at room temperature using a sonicator bath. The liposomes were formed after evaporation of the organic phase from the emulsified solution using rotary evaporator. Aggregation of bacteria was carried out in a microtiter plate by adding one part of liposome suspension to two parts of bacterial suspension(1x10^9 CFU), mixed well and the plates were incubated at room temperature for one hour. Evaluation of bacteria-liposomes aggregation was carried using the phase contrast in light microscopy.

Acid and non-acid fractions of glycolipids

The acid and non-acid glycosphingolipid fractions were isolated by standard methods (18). Briefly, the material was lyophilized and then extracted in two steps in a Soxhlet apparatus with chloroform and methanol (2:1 and 1:9 by volume, respectively).

Thematerial obtained was subjected to mild alkaline hydrolysis and dialysis followed by separation on a silicic acid column. Acid and non-acid glycosphingolipid fractions were obtained bychromatography on a DEAE-cellulose column. To separate the non-acid glycolipids from alkali-stable phospholipids, this fraction was acetylated and separated on a second silicic acid column followed by deacetylation and dialysis. Final purifications were done by chromatography on DEAE-cellulose and silicicacid columns. The non-acid glycosph in go lipid fractions were separated by repeated silicic acid chromatography, and final separation was achieved by HPLC or by chromatography on Iatrobead (Iatrobeads 6RS-8060, Iatron Laboratories, Tokyo, Japan) columns and elution with chloroform/methanol/ water (65:25:4 byvolume) followed by chloroform/ methanol/ water (60:35:8 byvolume) and finally chloroform/methanol/water (40:40:12by volume).

Throughout the separation procedures, aliquots of the obtained fractions were analyzed by thin-layer chromatography, and fractions that were colored green by anisaldehydewere tested for binding of H. pyloriusing the chromatogrambinding assay. The fractions were pooled according to the mobility on thin-layer chromatograms and their H. pylori binding activity.

Preparation of gangliosides.

Gangliosides used in these studies were isolated according to a standard procedure (18) that included extraction of tissues with mixtures of chloroform, methanol, and water, mild alkaline hydrolysis, dialysis, ion exchange chromatography (using DEAE-cellulose) and silica gel chromatography.

Total ganglioside fractions of human granulocytes, obtained after DEAE chromatography was used in TLCoverlay studies. A crude S-3-PG fraction (human erythrocytes), obtained after the silica gel separation was used for preparation of lyso S-3-PG and its derivatives. Highly purified fraction of S-3-PG (human erythrocytes) prepared by high-performance liquid chromatography (HPLC)was used in de-*N*-acetylation/*N*-propionylation studies.

Results

Binding of H. pylori to various glycolipids of acid and non-acid nature and phospholipids separated on TLC plate.

It have been tested a number of glycolipids isolated from acid and non-acid fractions containing 1-5 sugar residue on TLC plate. Results demonstrated in Table1 indicated that almost all *H. pylori* strains positively recognized monoglycosylceramide, Glcβ1Ceron TLC plate, except clinical isolate 782. *H. pylori* semi-rough lab strain as well as R strain, with incomplete LPS, recognized variably Glcβ1Cer. Galβ1Cer was completely detected with all tested *H. pylori* strains. Lactosylceramide, Galβ1-4Glcβ1Cer, on the other hand, has been bound by bacterial isolates including semi-rough lab strain. Rough strain failed, in this research, to bind LacCer, Galβ1-4Glcβ1Cer. Globoseries glycolipids; globotriaosylceramide, G3, and globotetraosylceramide (globoside) G4, from non-acid fraction showed no binding activity to any of the tested *H. pylori*

strains, but gangliosides from the same fraction like GgO3 and GgO4, ganliotriaosylceramide and gangliotetrosylceraosylceramide, respectively, were comcepletely detected by *H. pylori* strains on thin-layer chromatogram, TLC. Acid glycolipids from acid-fraction including NeuAc-GM3, NeuAc-GM1, GD3, GD1a and GD1b revealed negative bindings by *H. pylori* strains. *H. pylori* strains recognized the phosphtidylethanolamine , PE, but failed to bind phosphatidylserine, PS, and phosphatidylcholine, PC. The negative control, ceramide, was not detected by any of the tested strains.

Binding of H. pylori strains to GalβCer with hydroxylated and non-hydroxylated fatty acid of ceramides on TLC as well as incorporated into liposomes.

Results presented in table 2 showed clear affinity of binding of wild type isolates of H. pylori to GalCer with non-hydroxylated fatty acid of the ceramide. Binding of these isolates to hydroxylated GalCer(OH) wasweakerr than binding of the same isolates to the receptor of non-hydroxylated form, when using thin-layer chromatogram. Culture collection as well as rough and semi-rough strains, on the other hand, revealed weak and variable binding activity to GalCer with non-hydroxylated ceramide, but strong binding was detected with binding to hydroxylated GalCer separated on TLC plate. In liposome-binding data in table 3, demonstrated almost similar binding of the H. pylori strains to the receptor incorporated into liposomes with some exception, that the culture collection strains were strong binder to the receptor with either of forms of ceramide, but weaker to GalCer with non-hydroxylated ceramide.

Binding of H. pylori strains to GlcCer with hydroxylated and non-hydroxylated fatty acid of ceramides on TLC as well as incorporated into liposomes.

Results demonstrated in table 2 showed binding data of H. pylori to monoglycosylceramide on TLC plate, discriminating between hydroxylated and non-hydroxylated forms of ceramides. Hydroxylated GlcCer was strongly detected by wild type clinical isolates, whereas culture collection, rough and semi-rough strains weakly and variably bound the receptor. No binding was detected to non-hydroxylated GlcCer . In liposome-aggregation assay, binding data presented in table 3, indicated that GlcCer with no hydroxylated ceramide has been weakly recognized by some bacterial strains. Rough and semi-rough strains revealed no binding to the receptor in the liposomeaggregation assay.

Thin-layer cohromatogram binding assay demonstrates binding of H. pylori to hydroxylated and non-hydroxylated GlcCer.

In figure 1, two identical TLC plates with four samples including GlcCer(OH) hydroxylated and GlcCer(non-OH) non-hydroxylated form, gangliotetraosylceramide as positive control and ceramide as negative control were applied in overlay binding assay using radio-labeled *H. pylori* strains. Binding result showed clear positive binding to *hydroxylated* GlcCer, whereas GlcCer-containing non-hydroxylated ceramide was not detected by bacteria.

Gangliotetraosylceramide, Galβ1-3GalNAcβ1-4Gaβ1-4Glcβ1Cer, strongly recognized by bacteria, while no binding activity was shown to ceramide.

Discussion

The task of the present study was to evaluate binding of various *H. pylori* wild-type and culture collection strains to a number of simple and complex glycolipids of acid and non-acid nature separated on thin-layer chromatogram. Our research demonstrates a new and simple carbohydrate receptor positively recognized by *H. pylori*. Glucosylceramide, Glcβ1Cer and galactosylceramide, Galβ1Cer with hydroxylated fatty acid of ceramide, were the first observed activities detected in bacterial binding to monoglycosylceramide. Very interesting observation,that *H. pylori*bacteria bound with preference Galβ1Cer with non-hydroxylated ceramide and Glcβ1Cer with hydroxylated ceramide using TLC overlay assay. Galβ1Cer(non-OH) was weakly and variably recognized by rough and semi-rough strains, variability of binding might be correlated to incomplete LPS of these strains. The O-side chain of LPS of rough or semi rough may be reduced ormissing, therefore probably the missingpart might be involved in Glcβ1Cer(non-OH) binding. In contrarily, the strains with incomplete LPS strongly recognized the non-hydroxylated GalCer. This phenomenon might be correlated to the epitope presentation recognized by rough strains. Diglycosylceramide, lactosylceramide, Galβ1-4Glcβ1Cer isolated from non-acid fraction with hydroxylated type of ceramide,revealed also binding activity. It have been reported that H. pylori recognized lacer with hydroxylated ceramide (1, 28).

Binding was retained in spite of presence of Galβ1-4 moleculewith the basicGlcβ1Cer. Hence, the terminal Galβ1-4 molecule can be part of binding or had no effect on epitope presentation of lacCer glycolipid. Gangliotriaosylceramide, GgO_3, GalNAcβ1-4Galβ1-4Glcβ1Cerfrom non-acid fraction gangliosides,showed increasedbindingaffinity in comparison withthe hydroxylated Glcβ1Cer alone. In the same manner, binding to silaic acid-containing glycolipids,presented in this research,was negative.We have previously reported that bacterium recognized glycoconjugates in sialic acid-dependent manner (21, 22). In this study, receptor-active glycolipids recognitized by wild type *H. pylori* bacteria, revealed almost similar binding profile using TLC overlay assay except rough, semi rough and culture collection strains. Weak and variable binding of these strains may be correlated to the form of LPS .The rough and semi rough strains usually characterized by incomplete LPS, where the O-side chain is missing or incomplete, that may play a crucial role in the binding variationshowed by these strains. We have reported (in press), that MAbs raised against LPS inhibited strongly binding of wild type strains to tissue culture, while weaker inhibition was observed when applied rough or culture collection strains.

Regarding liposomes aggregation, the presentation of receptor, especially the binding epitope on carbohydrate part of glycolipid, can be more or less available to binding adhesindepending onwhether it separated onartificial surface likeTLC plate or incorporated into liposomes. The glycolipid receptors incorporated into liposomes were practically used as a model of bilayer membrane as *in vivo*

environment to evaluate the influence of the membrane on the epitope presentation.

The minimal binding epitope for P-fimbriae in binding of *E. coli* to globoseries is Galα1-4Gal disaccharide in the carbohydrate chain of the glycolipids (Abul-Milh, in press). Galβ1Cr or Glcβ1Cer may deal with a basic and minimal binding epitope recognized by LPS adhesin. In the*in vivo* environment, the steric interference from neighboring saccharides outside receptor-binding sitemay affect the presentation of binding epitope. In this study, the liposome-glycolipid model was demonstrated with one incorporated receptor, therefore it will be useful to design a multi-incorporated receptor glycolipids, glycoproteins and proteins. More, a parallel adhesin-analysis study together with binding specificity will be useful in understanding the mechanism of binding specificity.

Table 1

Binding of various *H. pylori* stains to glycolipids and phospholipids separated on thin-layer chromatogram

	Binding strain											
Glyco-orsphingolipid structures (Trivial name)	**LC11**	**95**	**431**	**475**	**576**	**782**	**832**	**877**	**909**	**949**	**955**	**R2**
Non-acid glycolipids												
1.Ceramide	-	-	-	-	-	-	-	-	-	-	-	-
2.Gleβ1Cer (glucosylceramide)[3]	+/-[4]	+	+	+	+	-	+	+	+	+	+	+/-
3.Galβ1Cer (Galactosylceramide)[3]	+	+	+	+	+	+	+	+	+	+	+	+
4.Galβ1-4Glcβ1Cer (Lactosylceramide)	+	+	+	+	+	+	+	+	+	+	+	-
5.Galα1-4Galβ1-4GlcB1Cer (Globotriaosylceramide) G3	-	-	-	-	-	-	-	-	-	-	-	-
6.GalNAcβ1-3Galα1-4Galβ1-4Glcβ1Cer (Globotetraosylceramide) G4	-	-	-	-	-	-	-	-	-	-	-	-
7.GlNAcB1-4GalB1-4GleB1Cer (Gangliotriaosylceramide) GgO3	+	+	+	+	+	+	+	+	+	+	+	+
8.Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1Cer (Gangliotetraosylceramide) GgO4	+	+	+	+	+	+	+	+	+	+	+	+
Acid-glycolipids												
9.NeuAcα 2-3Galβ1-4Glcβ1Cer (NeuAc-GM3)	-	-	-	-	-	-	-	-	-	-	-	-
10.Galβ1-3GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ1Cer (NeuAc-GM1)	-	-	-	-	-	-	-	-	-	-	-	-
11.NeuAcα 2-8NeuAcα2-3Galβ1-4Glcβ1Cer (GD3)	-	-	-	-	-	-	-	-	-	-	-	-
12.NeuAcα 2-3Galβ1-3GalNAcB1-4 (NeuAc α2-3)Galβ1-4Glcβ1Cer(GD1a)	-	-	-	-	-	-	-	-	-	-	-	-
13.Galβ1-3GalNAcβ1-4(NeuAcα2-8NeuAcα2-3)Galβ1-4Glcβ1Cer (GD1b)	-	-	-	-	-	-	-	-	-	-	-	-
Phospholipids												
14.Phosphatidylethanolamine (PE)	+	+	+	+	+	+	+	+	+	+	+	+
15.Phosphatidylserine (PS)	-	-	-	-	-	-	-	-	-	-	-	-
16.Phosphatdylcholin (PC)	-	-	-	-	-	-	-	-	-	-	-	-

1) Lab strain with semi-rough LPS; 2) R:Rough strain (with incomplete LPS).; 3) Ceramide with hydroxylated fatty acid; 4)+/- variable binding.

Table 2

Binding of H. pylori to hydroxylated and non-hydroxylated fatty acids of glucosylceramide and galactosylceramed on thin-layer chromatograms

H. pylori strain	Glycolipid			
	GalCer (OH)	GalCer (non-OH)	GlcCer (OH)	GlcCer (non-OH)
LC31	+/-	+/-	+/-	-
95	+	++	++	-
431	+	++	++	-
475	+	++	++	-
576	+	++	++	-
782	+	++	+	-
832	+	++	++	-
877	+	++	++	-
909	+	++	++	-
949	+	++	++	-
955	+	++	++	-
CCUG 17874	++	(+)/-	+	-
CCUG 17875	++	(+)/-	+	-
R	++	(+)/-	+/-	-

++: strong binding; +: weak binding; +/- :variable binding;(+)/- :weak & variable binding and - negative binding.

Table 3

Binding of H. pylori to hydroxylated and non-hydroxylated fatty acids of glucosylceramide and galactosylceramed incorporated into liposomes

H. pylori strains	Glycolipid			
	GalCer (OH)	GalCer (non-OH)	GlcCer (OH)	GlcCer (non-OH)
LC11	++	+	++	-
095	+	++	-	(+)
832	+	++	++	-
909	+	++	++	(+)
955	+	++	++	-
CCUG 17874	++	++	+	(+)
CCUG 17875	++	++	++	+
R	++	+	++	-

1) ++: strong binding(strong aggregation)
2) +: weak binding
3) (+) : very weak binding
4) - : negative binding

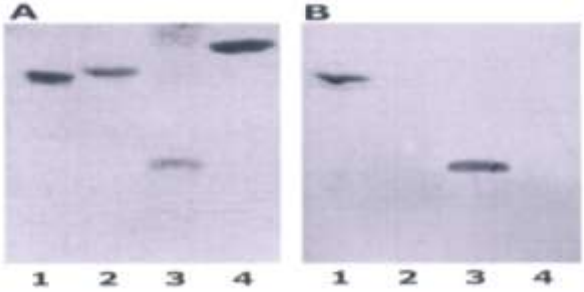

Figure 1

Binding of *H. pylori* to glucosylceramide on thin-layer chromatogram

A. Anisaldehyde **B. OL with bacteria**
1. GlcβCer(OH)
2. GlcβCer (non-OH)
3. Gg4
4. Ceramide(OH)

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الخلاصة

"اختلاف التصاق بكتريا الهيليكوبكتر بيلوري بالمستقبل الكلايكوليبيد ذو السكر الاحادي باختلاف مكوناته الدهنية "البحث الحالي يقدم دراسة عن التصاق بكتريا قرحة المعدة والاثنى عشري (الهيليكوبكتر بيلوري) بالمركبات الكربوهيدراتيه الدهنية (الكلايكوليبيدات) المنشوره على صفائح السيليكا وتلك الداخله في تركيب الايبوسومات الدهنية كمستقبلات. لقد استعملنا في هذه الدراسة قابلية التصاق بكتريا الهيليكوبكتر بنوعها الوحشي وكذلك بعض انواعها المختبريه او المصنفه عالميا بالكلايكوليبيدات احادية السكر. البكتريا من النوع الوحشي التصقت بشكل واضح بالكالاكتوسيل سيرمايد (كلايكوليبيد احادي السكر) الغير الحاوي على مجموعة الهدروكسيل في الجزء الدهني منه (السيرامايد). وبنفس الوقت التصقت نفس البكتريا الوحشية بشكل اضعف بنفس الكلايكوليبيد الاحادي السكر في حالة احتوائه على مجموعة الهدروكسيل في السيرامايد. النوع المختبري او المصنف (الغير الوحشي) اعطى سلوكية التصاق جيده بكلايكوليبيد احادي السكر بنوعيه الحاوي وغير الحاوي على الهدروكسيل معاكسه تماما للنوع الوحشي من البكتريا.

بخصوص الكلوكوسيل سيرمايد (كلايكوليبيد احادي السكر-الكلوكوز) الخالي من مجموعة الهدروكسيل فقد وجد انه لا يوجد اي نوع من البكتريا التي اجري البحث عليها تلتصق به، اي انه مستقبل سالب لبكتريا التهاب المعدة والاثني عشري، بينما نوع الكلوكوسيل سيرمايد الحامل لمجموعة الهدروكسيل اظهر نشاطا واضحا بالتصاق البكتريا به اشبه ما يكون للكالاكتوسيل سيرامايد المجرد من مجموعة الهدروكسيل عند استعمال النوع الوحشي او المرضي من البكتريا. أن التصاقا متغيرا او ضعيفا قد ظهر عند استعمال البكتريا المختبريه او المصنفه وليست الوحشيه منها بالمستقبل الاخير. الكلايكوليبيد الثنائي السكر(لاكتوسيل سيرامايد)، المكون من الكلوكوز والكالاكتوزوالحاوي على مجموعة الهدروكسيل اظهر ايضا نشاطا التصاقيا جيدا. في اختبار الايبوسومات المكونه من الكلايكوليبيدات مع او بدون مجموعة الهدروكسيل فقد ظهر ان الكلوكوسيل سيرمايد الحامل لمجموعة الهدروكسيل انه مستقبلا ايجابيا للنوع الوحشي من البكتريا بينما النوع الغير الحامل للهدروكسيل كان مستقبلا ضعفيفا ومتذبذبا بالتصاق لجميع نواع البكتيري. نتائج الالتصاق الايجابية لهذه البكتريا على سطح السيليكا حددت مستقبلات اخرى ايجابيه من الكلايكوليبيدات-الحامضية المستحصله من المكون الحامضي والتصقت به ومنها الكانكليوسيدات الثلاثيه والرباعية. لقد اجري البحث عن مستقبلات فعاله وسط الفوسفوليبيدات بعد فحص عدد منها فوجد الفوسفاتديل ايثانول امين (بي اي) هو المستقبل الوحيد والفعال.