SPECIFIC BINDING OF *BURKHOLDERIA PSEUDOMALLEI* CELLS AND THEIR CELL-SURFACE ACID PHOSPHATASE TO GANGLIO-TETRAOSYLCERAMIDE (ASIALO GM1) AND GANGLIOTRIAOSYLCERAMIDE (ASIALO GM2)

Koomi Kanai[1], Yasuo Suzuki[2], Eiko Kondo[4], Yasuhiro Maejima[4] Daisei Miyamoto[4], Takashi Suzuki[2] and Takeshi Kurata[3]

[1]National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Tivanond Road, Nonthaburi 11000, Thailand; [2]Department of Biochemistry, University of Sizuoka School of Pharmaceutical Sciences, 52-1 Yada, Sizuoka 422, Japan; [3]Department of Pathology, National Institute of Health, Toyama 1-23-1, Shinjuku-ku, Tokyo 162, Japan

Abstract. Specific binding between bacterial cells and host tissue is an early step of the pathogenesis of infection. *Burkholderia pseudomallei* cells, the causative micro-organisms of melioidosis, were demonstrated to bind specifically to tissue glycolipids (asialo GM1 and asialo GM2) by solid phase binding assay on thin layer chromatograms. The detection limit was around 400 pmol of the glycolipids. Acid phosphatase purified from the culture filtrate of *B. pseudomallei* was tested for such binding properties, and the same results were obtained. According to our previous studies, the enzyme is a glycoprotein located on the cell surface, and hydrolysed tyrosine phosphate most actively among the substrates so far tested. The mode of binding between the enzyme and the glycolipids was analyzed by comparison of binding levels among three samples different in protein content, sugar content and specific phosphatase activities per protein and sugar residue. The results suggested the possibility of a receptor-ligand relationship between the bacterial enzyme and the host-cell glycolipids (asialo GM).

INTRODUCTION

B. pseudomallei is the causative agent of melioidosis, a tropical or subtropical infectious disease, whose endemic areas are mainly Southeast Asia and northern Australia (Leelarasamee and Punyagupta, 1988). Though this species of bacteria is usually living in the natural environment (soil and water), it causes opportunistic infections with a wide spectrum of clinical course from acute or subacute septicemic type to chronic or asymptomatic latent case (Dance, 1991).

A multifactorial mechanism has been proposed for the pathogenesis of melioidosis (Wongwanich *et al,* 1996). In the present paper, an additional aspect of the pathogenesis will be introduced, a specific receptor-ligand system which may be operating to mediate adhesion between *B. pseudomallei* cells and host tissue.

Correspondence: Dr Eiko Kondo, 1-5-11-906, Igusa, Suginami-ku, Tokyo 167-0021, Japan.

For the past several years, we have been engaged in studying acid phosphatase activity of *B. pseudomallei* and have come to a conclusion that the enzymatic activity is mainly due to the presence of tyrosine phosphatase of glycoprotein nature located on the cell surface or secreted therefrom (Kondo and Kanai, 1994). During the course of this study, we received information that cell-cell adhesion can be mediated by a receptor-like protein tyrosine phosphatase (Gebbink *et al,* 1993).

Meanwhile, *B. cepacia,* which is a near relative of *B. pseudomallei* taxonomically, was reported to bind specifically to gangliotetraosylceramide (asialo GM1) and gangliotriasylceramide (asialo GM2), possibly in a receptor-ligand relationship (Krivan *et al,* 1988).

Taking together the information, we were interested in the possibility that tyrosine phosphatase of *B. pseudomallei* may function as a receptor to bind asialo GM1 and asialo GM2. The results of our study described here are favorable to our assumption.

MATERIALS AND METHODS

Microorganisms

Test strains (UB12, UB26, UB165) were selected from our collection of fresh isolates of *B. pseudomallei* donated by Sappasittiprasong Hospital, Ubon Rajthani, Thailand. The strains were identified in the hospital laboratory on the basis of routine identification tests. After receiving them, further confirmation was made in our laboratory by fatty acid pattern analysis by GLC and also by assay for pH-activity pattern of acid phosphatase (Kondo *et al,* 1991). *B. cepacia* (UB 146) was employed for control tests.

Culture of microorganisms

A stock culture of each strain was plated directly onto blood agar of 9-cm diameter. After overnight incubation at 37°C, one colony on the plate was selected and transferred to triptic soy broth. The overnight growth was then plated with a cotton swab onto triptic soy agar (16cm diameter). The overnight growth was used for preparation of a bacterial suspension for the binding experiment and also as the inoculum to two flasks each containing 1.5 liter of Mueller-Hinton liquid medium. These flasks were incubated at 37°C for 10 days and the culture filtrate was subjected to fractionation.

Glycolipid standards

Eight glycolipid standards employed in this study are listed in Table 1.

Preparation of glycolipids

All natural glycolipids used were purified in the University of Shizuoka by Y Suzuki and his associates from the following sources. GM1a, GD1a, GD1b, GT1b and Ga1Cer were obtained from bovine brain (Hirabayashi *et al,* 1978; Suzuki *et al,* 1986). GlcCer, LacCer, and Gb_3Cer were from porcine erythrocytes (Clarke *et al,* 1971). GM3 was from human liver (Seyfried *et al,* 1978), and GM2 was from Tay-Sachs brain (Suzuki *et al,* 1985). Gg_4Cer (asialo-GM1) and Gg_3Cer (asialo-GM2) were from guinea pig erythrocytes (Svenrerholm *et al,* 1973; Seyama and Yamakawa, 1974). Concentrations of gangliosides and glycolipids were quantified by densitometric analysis using oricinol-HCl or resorcinol-H_2SO_4 staining as described previously (Suzuki *et al,* 1992).

Thin-layer overlay method for detection of the binding of bacterial cells to glycolipids

Thin-layer chromatography of glycolipids was carried out on silica gel high performance plates (Merck, West Germany) of 5 cm x 10 cm. After spotting, the glycolipids were developed with chloroform; methanol; 0.25% aqueous KCl (50:40:10). The plates were dried, dipped in hexane containing 0.1% poly-isobutylmethacrylate, and air dried. They were then sprayed with PBS containing egg-white albumin (EWA) for 2 to 3 hours. After excess buffer was drained from the plates, the plates were overlayed for 4 hours with a bacterial suspension of 10^7-10^8 cells per ml and left to stand overnight in a refrigerator. The plates were then washed with PBS several times to remove unbound becteria, and blocked again by EWA solution and washed with PBS three times for 20 to 30 minutes by occasional shaking. The plates were subjected to treatment with a 1:500 dilution of mouse antibodies against the bacteria or their components for 1 to 2 hours and washed with EWA and PBS. To make the location of glycolipid-bound bacteria visible, the plates were then treated with a 1:1000 dilution of anti-mouse lgG labeled with peroxidase, washed three times with PBS for 1-2 hours, and finally exposed to a mixture consisting of tris-HCl buffer, 0.3% 4-chloro-1-naphthol and H_2O_2 in the ratio of 5:1:0.02. After being washed with water and dried, the plates were monitored by a densitometer set at 578 nm. All the steps in this procedure were carried out at 4°C excepting the last step at 37°C.

Isolation of phosphatase-active fractions from the culture filtrate of *B. pseudomallei*

After termination of incubation for 2 days, each culture was added with formalin to a concentration of 0.35% and left to stand overnight at 37°C. The sterility test showed no detectable living bacilli at this time. The bacterial cells were removed therefrom by centrifugation at 10,000 rpm for 30 minutes. The supernatant was concenrated to 0.1 volume in a rotary evaporator. The concentrate was mixed with an equal amount of a saturated ammonium sulfate solution and left to stand overnight at 4°C. The resulting pricipitate was collected by centrifugation at 4,000 rpm for 30 minutes. The precipitate was dissolved in 150 ml of 0.067 M phosphate buffer, pH 7.0, and the half saturation

Table 1

Structure of glycolipids tested for binding to *B. pseudomallei* cells.

Glycolipids		Structure
Gg_4Cer (Asialo-GM1)	:	Gal β1-3Gal NAc β1-4Gal β1-4Glc β1-Cer
Gg_3Cer (Asialo-GM2)	:	GalNAc β1-4Gal β1-4Glc β1-Cer
GM1a	:	Gal β1-3GalNAc β1-4 (Neu5Ac α 2-3) Gal β1-4Glc β1-Cer
GM2	:	GalNAc β1-4 (Neu5Ac α 2-3) Gal β1-4Glc β1-Cer
GM3	:	Neu5Ac α 2-3Gal β1-4Glc β1-Cer
GD1a	:	NeuAc α 2-3Gal β1-3 GalNAc β1-4 (Neu5Ac α 2-3) Gal β1-4Glc β1-Cer
GD1b	:	Gal β1-3GalNAc β1-4(Neu5Ac α2-8Neu5Ac α 2-3) Gal β1-4Glc β1-Cer
GT1b	:	Neu5Ac α 2-3Gal β1-3Gal NAc β1-4 (Neu5Ac α 2-8Neu 5Ac α 2-3) Gal β1-4Glc β1-Cer

procedure with ammonium sulfate was conducted twice more. The final precipitate was dissolved in 100 ml of distilled water and dialysed against water at 4°C for 4 to 7 days. Then the dialysis bag was placed in Ficol powder to concentrate the inside solution to about 60 ml.

The above ammonium sulfate precipitated fraction was then applied to a Sephadex G-75 column (2 x 30 cm) equilibrated and eluted with water. The loaded amount was 20 ml, and the eluates were collectd in 3g amounts. A 20 µl portion from each tube was employed for determination of protein and sugar. Pooled eluate fractions of each peak were lyophilized.

The Sephadex fractions were then applied to a DEAE-cellulose column (preswollen Microgranular Anion Exchanger DE 52, Whatman Bio Systems Ltd, England) of 1.5 x 11 cm equilibrated with 0.025 M Tris-HCl buffer pH 7.1. The loaded amount of the Sephadex fractions was 100 mg dry weight each. Elution was made stepwise, first with 100 ml of 0.025 M Tris-HCl buffer pH 7.1, then 0.1 M NaCl and finally with 0.5 M NaCl in 0.025 M Tris-HCl buffer. The fraction size was 4 g. The eluates in each peak depicted by protein and sugar determinations were pooled, dialyzed, and lyophilized.

Determination of protein and sugar content

Protein levels were determined by the method of Lowry *et al* (1951) or by Bio Rad protein assay reagent (Bio Rad Chimical Division, Richmond, CA, USA). Bovine serum albumin (fraction V, Sigma) was used as protein standard. Sugar determination was made by the phenol-H_2SO_4 method as follows. A sample of 20 µl was placed in a test tube of 1.2 x 7 cm (Corning) with 780 µl of water, to which was added 20 µl of 80% phenol. This mixture was then treated with 2 ml of concentrated sulfuric acid. After the temperature dropped to 25-30° C, the developed color was read with a Coleman spectrophotometer set at 490 mµ. Glucose was used as sugar standard. The anthrone test was also employed for sugar determination in some experiments.

Phosphatase assay

Non-specific phosphatase activity was assayed in a reaction mixture consisting of 0.8 ml of buffer, 0.1 ml of the enzyme sample, and 0.1 ml of p-nitrophenyl phosphate (PAP) solution (0.2%). For pH range from 3.19 to 6.22, acetate buffer solutions (0.1M) were prepared. For pH range higher than 6.5, 0.1M Tris-HCl buffer solutions were employed.

The reaction mixture was incubated in a water bath at 40°C for 30 minutes. To stop the reaction and develop the yellow color of released p-nitrophenol, 1.0 ml of 0.5M NaOH was added. After addition of one ml distilled water the reaction mixture was subjected to colorimetric determination with a Coleman spectrophotometer set at 420

mμ. To the blank tube NaOH solution was added at zero time.

In our previous studies (Kanai and Kondo, 1991; Kondo *et al*, 1994) the substrate specificity of this phosphatase activity was examined in the whole bacterial cells and the enzyme preparation isolated from the culture filtrate. In both cases, tyrosine phosphate was the most efficient substrates among those so tested, showing 120 to 180% higher reaction than that with PNP, thus suggesting that the screening assay of phosphatase activity with PNP prevides a meaure of the level of tyrosine phosphatase.

Immunofluorescence staining and microscopy

Coloies of *B. pseudomallei* on tryptic soy agar were suspended in PHS to a concentration of approximately 109 cells per ml. This suspension was made by turbidity comparison with a McFarland standard tube. It was then treated with 0.35% formalin to kill the cells. One drop of the suspension was placed on a glass-slide and air-dried. The cells were fixed with acetone for 15 minutes. The fixed smears were coverd with a 1:20 dilution of the pooled monoclonal antibodies to the phosphatase fraction provided from the Department of Pathology, NIH, Tokyo and incubated in a moist chamber at 37°C for 45 minutes. The smears were washed 3 times with fresh PHS each-time, and air-dried. Finally, the smear was treated with 20 μl of a 1:20 dilution of fluorescein isothiocyanate-conjugated anti-IgG of homologous species (ICN Immunobiologicals, IL, USA) and incubated in a moist chamber at 37°C for 45 minutes. Fluorescence microscopy was conducted with a fluorescence microscope Olympus BH).

RESULTS

Binding of whole bacterial cells to glycolipids

A thin-layer plate of silica gel was spotted with ten kinds of glycolipids (10 μg/lane each) as shown

Table 2

Glycolipids tested for binding *B. pseudomallei*.

Glycolipids	*B. pseudomallei* strains		Cell-fractions*		*B. cepacia* strain
	UB165	UB26	UB26-C	UB26-E	UB146
Asialo GM1	+++	+++	+++	+	–
Asialo GM2	+	++	++	+	–
Galactosylceramide (CaICer)	–	–	–	+	–
Lactosylceramide (CDH)	–	–	–	+	–
Globotriapsylceramide (Gb_3)	–	–	–	+	–
GM1a	–	–	–	+	–
GM2	–	–	–	+	–
GM3	–	–	–	+	–
GD1a	–	–	–	+	–
GD1b+GT1b	–	–	–	+	–

* UB26-C is a membrane-rich fraction and UB26-E is the cell-free supernatant fraction each obtained by differential centrifugation of the homogenate of UB26 *B.pseudomallei* cells (Kondo and Kanai, 1994).

+++, ++, + indicate the level of binding intensity graded arbitrarily.

in Table 1 and developed with the solvent system as described before. The plate was overlayed with a formalin-killed bacterial suspension of *B. pseudomallei* (strain UB165, UB26) and of *B. cepacia* (UB146) of 4 day growth, then treated by mouse sera to whole bacterial cells, and finally by peroxidase-labeled anti-mouse IgG. During this procedure, the blocking of the plate with EWA solution and the washing with PBS were done between each step. Detection of the binding sites of bacterial cells was monitored by treatment the plate with 0.3% 4-chloro-1-naphthol and H_2O_2 to develop blue color, and the level of reaction was read by a densitometer set at 578 mμ. The results shown in Table 2 indicate that only asialo GM1 and asialo GM2 bound to *B.pseudomallei* cells.

Being encouraged by this observation, we proceeded to a further experiment with the graded amounts of asialo GM1 and asialo GM2 from 2,000 pmol down to 400 pmol. The results are given in Fig 1, which shows that asialo GMl had a stronger affinity to the bacterial cells and 400 pmol is sufficient to produce a detector response.

The test with living *B. pseudomallei* cells (strain UB12, UB26) was also conducted to examine the dose-response of binding to four-graded amounts (2.0 nmol to 0.5 nmol) of asialo GM1 and GM2. Asialo GM1 gave a stronger affinity to living cells than asialo GM2. An example of the results is shown in Fig 2, where the difference in the spot-size of migrated lipids at the same Rf site is clearly revealed on the TLC plate.

Finally, the binding expriment was carried out

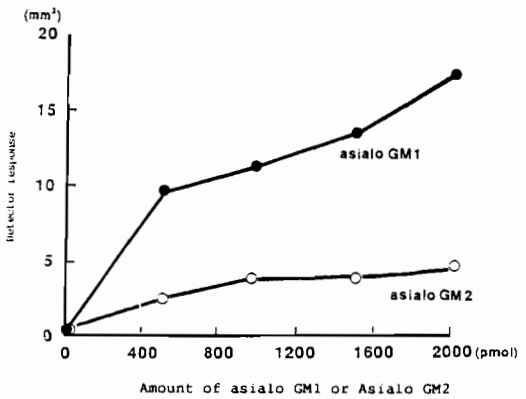

Fig 1–Binding of three day-culture-cells of *B.pseudomallei* to asialo GM1 and asialo GM2.

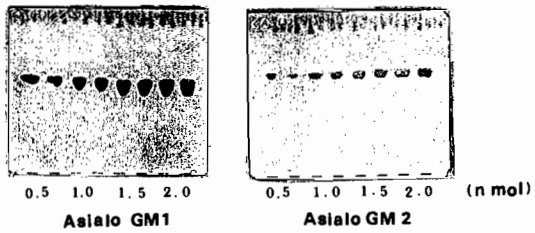

Fig 2–Binding of one day-culture-living cells of *B.pseudomallei* to asialo GM1 and asialo GM2.

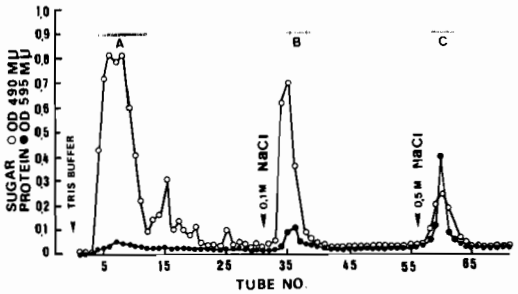

Fig 3–Three fractions (A, B, C) separated by DEAE cellulose-column chromatography of the component obtained from the culture filtrate of *B.pseudomallei* by precipitation with ammonium sulfate and gel-filtration with sephadex G-75 column.

with cell-fractions a membrane-rich fraction and cell-free supernatant (Kondo and Kanai, 1994), separated from the homogenate of mechanically disrupted *B. pseudomallei* cells to identify the main site of binding to the glycolipids. The results are included into Table 2. The membrane fraction was found to bind more effectively asialso GM1 and GM2.

Binding of isolated acid phosphatase fraction to glycolipids

On the assumption that the binding of *B. pseudomallei* to host tissue glycolipids may be due to the presence of some cell-surface component of this species of bacteria as receptor, we employed acid phosphatase fractions (tyrosine phosphatase) separated from the culture filtrate as a candidate for such receptors since we have been working for the past several years on the characterization of this enzymatic activity. Several pieces of evidence indicated that the enzyme is a heat-stable glycoprotein located on and/or secreted from the cell surface (Kondo and Kanai, 1994).

Table 3

Specific activity of acid phosphatase in DEAE-column fraction (A, B, C) of the culture filtrate of *B. pseudomallei* (UB12).

Fraction	Contents (μg) per 100mg sample		Specific activity of acid phosphatase (PNPμM/ 30min/ 100μg of the below)		
	Protein	Sugar	Sample	Protein	Sugar
A	5.0	77.5	0.004	0.08	0.005
B	23.0	75.0	0.017	0.074	0.023
C	40.0	40.0	0.035	0.087	0.085

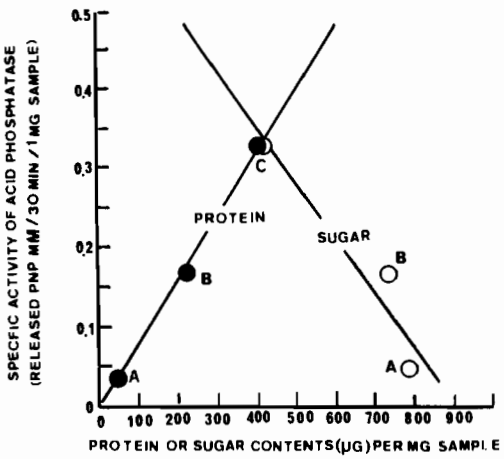

Fig 4–Specific activity (PNP mM/30 min/ mg fraction) of acid phosphatase in DEAE column-fractions (A, B, C) of the culture filtrate in relation to protein and sugar content.

Instead of whole bacterial cells, we employed three fractions obtained from the culture filtrate by ammonium sulfate precipitation, gel-filtration with Sephadex G-75 and column chromatography with DEAE-cellulose (Fig 3). These three fractions (A, B C) were different in the level of enzymatic activity, sugar content, and protein content.

Fraction A was the pooled eluate of the first peak (void volume) from the DEAE-cellulose column, Fraction B was the second peak eluted by 0.1M NaCl, and Fraction C was the third peak eluted by 0.5M NaCl. Protein and sugar contens of each fraction were determined and the specific activity of acid phosphatase was calculated as released PNP μM/30 minutes/100 μg sample (Table 3).

With these data in mind, we proceeded to the binding experiment with the three fractions (A, B, C) using the same procedure as before, except that the antisera were monoclonal antibodies to the isolated enzymatic fraction. As a negative control, whole cells of *B. cepacia* were employed to demonstrate that the antigenic specificity of *B. pseudomallei* does not cross with *B.cepacia.* The results are shown in Fig 4.

Table 3 and Fig 4 show that fraction A had the highest sugar content per weight but the lowest protein content. Fraction C was the lowest in sugar content and the highest in protein content. Fraction B was of intermediate level in sugar and protein content between A and C.

Accordingly, it is natural that the specific phosphatase activity per fraction weight is the highest in fraction C, since the enzyme is a protein. However, it is remarkable that the specific activity per protein was almost the same among these three fractions, thus suggesting that the phosphatase contained in each fraction (A, B, C) may be due to the distribution of the identical enzyme.

Most interesting is the result that the specific activity per sugar was increasingly higher in the order A, B and C, and that the specific activities per protein and sugar were almost the same as in fraction C. This will be explained reasonably if we admit that the enzyme is a glycoprotein separated essentially pure in fraction C.

Fig 5 is the result of the binding experiments with fractions A, B and C to asialo GM1 and asialo GM2. Detector responses indicated the highest level of affinity of fraction C to both glycolipids. To illustrate the characteristics of this binding, we prepared Figs 6 and 7 from the data shown in Table 3 and Fig 5.

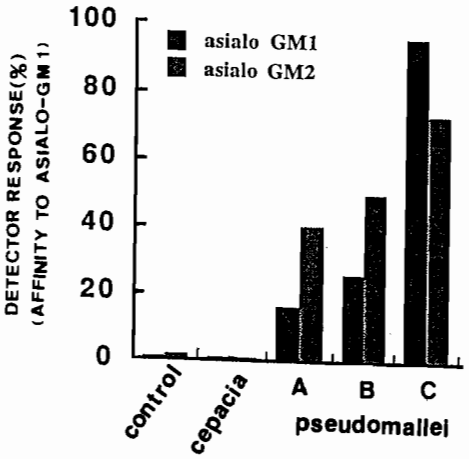

Fig 5–Adhesion to asialo GM1 and asialo GM2 of phosphatase-active fractions (A, B, C) separated by DEAE-column chromatography from the culture filtrate of *B. pseudomallei* (UB12).

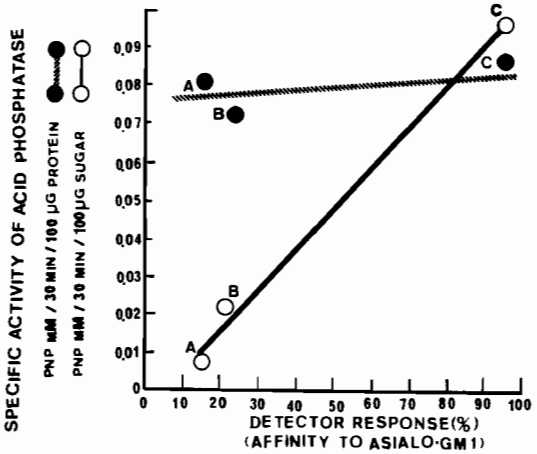

Fig 6–Affinity to asialo GM1 of three DEAE-column fractions (A, B, C) of the culture filtrate of *B. pseudomallei* (UB12) is associated with specific activity of acid phosphatase per sugar, not the per protein activity.

The data here, that fraction C showed the highest level of detector response, suggest two possibilities: 1) the sugar moiety of the enzyme is responsible for the binding, since the level of specific phosphatase activity per sugar is the highest in fraction C; 2) the binding occurs with the protein moiety of the enzyme, since fraction C has the highest amount of enzyme protein per sample weight.

The cells of *B. cepacia* did not reveal a positive

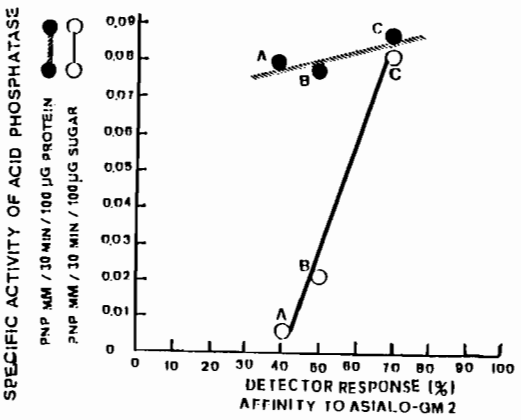

Fig 7–Affinity to asialo GM2 of three DEAE-column fractions (A, B, C) of the culture filtrate of *B. pseudomallei* (UB12) is associated with specific activity of acid phopsphatase per sugar, not the per protein activity.

adhesion reaction in this system, as shown in Table 2. This may be because that the antiserum is specific to *B. pseudomallei* but not to *B. cepacia* as we experienced in our previous experiments (Naigowit, 1993).

Indirect immunofluorescence staining of *B. pseudomallei* cells employing the antisera to isolated phosphatase fraction

To confirm the positive staining as additional evidence to support the concept that the phosphatase of *B. pseudomallei* is associated with the cell-surface, the authors repeated such experiments using clinical specimens (pus, blood, sputum) of melioidosis patients. The staining was successful in more than 90% of the samples. The photoillustrations were presented previously (Naigowit, 1993, 1994).

DISCUSSION

In 1988, Krivan, Ginsburg and Roberts demonstrated that *P. aeruginosa* and *B. cepacia* have receptor(s) to bind specifically tissue glycolipids (asialo GM1 and asialo GM2). Their study was to examine the possibility that the carbohydrate moiety of these tissue glycolipids may mediate adhesion of the bacteria to host tissue as the initial step of the pathogenesis leading to cystic fibrosis.

In their experiments, such binding was demonstrated by detection of isotopically labeled bacterial cells on the thin-layer chromatograms impregnated with asialo GM1 and asialo GM2. From the comparative observations of the stractualy related glycolipids with asialo GM1 and asialo GM2, they suggested that at least terminal or internal GalNAc β 1-4Gal sequences in glycolipids may be critical for the binding of these two pathogenic species of bacteria.

Iwamori *et al* (1993) also reached the same conclusion working on recombinant *E. coli* which has the gene encoding the adhesion factor of *P. aeruginosa.*

Once cystic fibrosis is established it is difficult to eradicate *P. aeruginosa* cells completely from lung tissue. The same is true with the case of pnuemonia due to *B. pseudomallei,* even more serious than the case of *P. aeruginosa.* In the detail of pathogenic morphology, there would be some differences between pneumonia caused by *B. pseudomallei* and cystic fibrosis caused by *P. aeruginosa.* However, the binding of the causative microorganisms to target tissue cells is undoubtedly the crucial initial step of the pathogenesis of infection.

Krivan *et al* (1988) reported also that *P. cepacia* has the receptors for asialo GM1 and asialo GM2. *P.cepacia* is now grouped into the Genus *Burkholderia* according to the recent taxonomy proposed by Yabuchi *et al* (1992). *B. cepacia is* very similar to *B.pseudomallei* in many biochemical characteristics, though less pathogenic and different in the antigenicity. Therefore, we have been concerned with a question whether or not *B. pseudomallei* binds to asialo GM1 and asialo GM2. As reported above, the answer was positive. Besides, an additional finding was obtained that cell-surface acid phosphatase may be one of the candidates for the ligand molecules in *B. pseudomallei.*

As stated, acid phosphatase activity of *B. pseudomallei* as screened using the nonspecific substrate PNP is actually tyrosine phosphatase, most probably protein tyrosine phosphatase (PTP) which is considered to be a two-component system playing an important role in the signal transfer mechanism. In our previous paper (Kanai *et al,* 1996), we reported that PTP in *B. pseudomallei* has a receptor molecule which interacts with insulin. Just like protein tyrosine kinase which is thought to work as a regulator of cell growth, differentiation, and metabolism (Jin and Inoue, 1993), PTP is also responsible for such regulation participating in the dephosphorylation of phosphorylated materials (Hashimoto *et al,* 1992; Bourret *et al,* 1991).

We have new information that the phosphatase of *B. pseudomallei* has a strong affinity to asialo GM1 and asialo GM2. In the present state of our knowledge, however, this binding remains only as a mechanical event for the bacteria to attach to host tissue. Our future efforts should be directed to the analysis of the functional aspects of this binding, especially in relation to the signal transduction of environmental changes into cell-inside.

Our last question is whether or not the phosphatase of *B.cepacia* can bind to the glycolipids. This question arises from our findings that the phosphatase has almost the same nature of enzymatic activity (Kondo *et al,* 1991), but they are not the same in the antigenicity test, suggesting a different antigenic structure of the sugar chain of the enzyme. This question must be answered by the future experiment with tyrosine phosphatase purtified from *B. cepacia* for binding assay. It is generally accepted that the binding in question occurs as the result of interaction between sugar chains of the enzyme and the glycolipids. We have also a concern about the information that PTP of *Yersinia* is a virulence factor of this species (Bliska *et al,* 1991; Guan and Dixon, 1990). Interaction of insulin with *Pseudomonas pseudomallei* in connection with the infection also attracted recent interest (Woods *et al,* 1993).

REFERENCES

Bliska JB, Guan K, Dixon JE, Falkow S. Tyrosine phosphate hydrolyses of host proteins by an essential *Yersinia* virulence determinant. *Proc Natl Acad Sci USA* 1991; 88: 1183-91.

Bourret AB, Borkovich KA, Simon MI. Signal transduction pathways involving protein phosphorylation in prokaryotes. *Ann Rev Biochem* 1991; 60: 401-44.

Clarke JT, Wolfe LS, Perlin AS. Evidence for a terminal-D-galactopyranosyl residue in galactosylalactosyl-glucosyl ceramide from human kidney. *J Biol Chem* 1971; 246:5563-9.

Dance DAB. Melioidosis: The tip of the ice berg? *Clin Microbiol Rev* 1991; 4: 52-60.

Gebbink MFG, Zondag GCM, Wubbopts RW, Beijerobergen RL, Van Etten I, Woolenear WH. Cell-cell adhesion mediated by a receptor-like protein Tyrosine phosphatase. *J Biol Chem* 1993; 268: 16101-4.

Guan K, Dixon JE. Protein tyrosine phosphatase activity of an essential virulence determinant in *Yersinia*. *Science* 1990; 249: 553-5.

Hashimoto N, Feener EP, Zhang WR, *et al.* Insulin receptor protein-tyrosine phosphatases. *J Biol Chem* 1992; 267: 13811-4.

Hirabayashi Y, Nakao Y Kato H, *et al.* Improved method for large-scale purification of brain gangliosides by Q-sepharose column chromatography. Immunochemical detection of c-series polysialogangliosides in adult bovine brains. J *Chromatogr* 1988; 445: 337-84.

Iwamori M, Fukasawa K, Suzuki N, Nagai Y. Reaction to glycolipids of *E. coli* to which the gene to code the adhesion factor of *P. aeruginosa*. *Biochem* 1993; 65: 695 (In Japanese).

Jin T, Inoue M. Ligand binding to the receptor domain regulates the ratio of kinase to phosphatase activities of the signaling domain of the hybrid *Escherichia coli* transmembrane receptor, Taz 1. *J Mol Biol* 1993; 232: 484-92.

Kanai K, Dejsirilert S. *Pseudomonas pseudomallei* and melioidosis, with special reference to the status in Thailand. *Jpn J Med Sci Biol* 1988; 41: 133-7.

Kanai K, Kondo E. Substrate response in acid phosphatase activity of *Pseudomonas pseudomallei* and *Pseudomonas cepacia*, with special reference to tyrosine phosphatase. *Jpn J Med Sci Biol* 1991; 44: 225-37.

Kanai K, Kondo E. Review: A recent advances in biomedical sciences of *Burkholderia pseudomallei* (Basonym: *Pseudomonas pseudomallei). Jpn J Med Sci Biol* 1994; 47: 1-45.

Kanai K, Kondo E, Kurata T. Affinity and response of *Burkholderia pseudomallei* and *Burkholderia cepacia* to insulin. *Southeast Asian J Trop Med Public Health* 1996; 27: 584-91.

Kondo E, Kanai K. Demonstration of cell-surface glycoprotein fractions with tyrosine phosphatase activity in *Pseudomonas pseudomallei* and their putative pathological significance. In: Puthuchevy SD, Malik YA, eds. Melioidosis 1994; 84-101.

Kondo E, Naigowit P, Phamichruttiwong P, *et al.* Fatty acid profile and acid phosphatase activity of fresh isolates of *Pseudomonas pseudomallei. Jpn J Med Sci Biol* 1991; 44: 195-211.

Kondo E, Wangroonsub P, Naigowit P, *et al.* Separation of antigenic glycoprotein fractions from cell-free homogenate of *Pseudomonas pseudomallei* and characterization as tyrosine phosphatase. *Southeast Asian J Trop Med Public Health* 1994; 25: 437-42.

Krivan HC, Ginsburg V, Roberts OD. *Pseudomonas aeruginosa* and *Pseudomonas cepacia* isolated from cystitis fibrosis patients bind specifically to gangliotetraosylceramide (asialo GM1) and gangliotriaosylceramide (asialo GM2). *Arch Biochem Biophys* 1988; 260: 493-6.

Leelarasamee A, Punyagupta S, Melioidosis. Epidemiology with Thai experience. In: Punyagupta S, Siresanthara T, Stapatayavong B, eds, Melioidosis. Bangkok: Medical Publisher, 1988; 238-64.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193: 265-75.

Naigowit P, Kurata T, Wangroongsub P, *et al.* Application of indirect immunofluorescence microscopy to colony identification of *Pseudomonas pseudomallei. Asian Pacific J Allergy Immunol* 1993; 11: 149-54.

Naigowit P, Kurata T, Kondo E, Kanai K. Application of immunofluorescent method for detection of *Pseudomonas pseudomallei* cells in smears of clinical specimens and for identification of their colonies on media. In: Puthuchesry SD, Malik YA, eds. Melioidosis 1994: 84-101.

Seyama Y, Yamakawa T. Chemical structure of glycolipid of guinea pig red blood cell membrane. *J Biochem* 1974; 75: 837-42.

Seyfried TN, Ando S, Yu RK. Isolation and characterization of human liver hematoside. *J Lipid Res* 1978; 19: 538-43.

Suzuki Y, Matsunaga M, Matsumoto M. N-acetylneuraminyl-lactosyl ceramide GM3-NeuAe, a new influenza A virus receptor which mediates the adsorption-fusion process of viral infection: Binding specificity of influenza virus a A/Aichi/2/68 (H3N2) to membrane-associated GM3 with different molecular species of sialic acid. *J Biol Chem* 1985; 97: 1189-99.

Suzuki Y, Nagao Y, Kato H, *et al.* Human influenza a virus hemagglutinin distinguishes sialyloligusaccharides in membrane-associated gagliosides as its receptor which mediates the adsorption and fusion processes of virus infection: Specificity for oligosaccharides and sialic acids and the sequence to which sialic acid is attached. *J Biol Chem* 1986; 261: 17057-61.

Suzuki Y, Nakao T, Ito T, *et al.* Structural determination of gangliosides that bind to influenza A, B, and C viruses by an improved binding assay: strain-specific receptor epitopes in sialo-sugar chains. *Virology* 1992; 189: 121-31.

Svennerholm L, Mannson JE, Li YT, Isolation and structural determination of a novel ganglioside, a disialosylpentahexosylceramide from human brain. *J Biol Chem* 1973; 248: 740-2.

Wongwanich S, Kondo E, Kanai K. Putative pathogenic factors of *Burkholderia pseudomallei* as suggested from the comparison with *Burkholderia cepacia*. *Southeast Asian J Trop Med Public Health* 1996; 27: 111-8.

Woods DE, Jones AL, Hill PA. Interaction of insulin with *Pseudomonas pseudomallei*. *Infect Immun* 1993; 61: 4045-50.

Yabuuchi E, Kosako Y, Oyaizu H, *et al.* Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species of *Burkholderia cepacia* (Palleroni and Holmes, 1981) comb. vov. *Microbiol Immunol* 1992; 36: 1251-75.