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Monoclonal Antibody that Neutralizes Pertussis Toxin Activities

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SUMMARY Pertussis or whooping cough is a disease with high mortality among infants and small children. The disease is caused by infection of the respiratory tract by a gram negative bacterium, *Bordetella pertussis*. The superficial colonized bacteria produce a myriad of toxins which enter the circulation causing various pathophysiologicalal changes in the host. Although antimicrobial therapy reduces the number of the coughed out bacteria and also the infectious time of the infected host, but it is not effective in amelioration of the clinical manifestations as the pertussis morbidity is due principally to the pertussis toxin (PT). Antibody based-therapy is frequently practiced in conjunction with other supportive measure to resuscitate the patient. Nevertheless, human derived antiserum against PT is of the limited supply and the ethical concern. Thus in this study a hybridoma clone, *i.e*. clone PT6-2G6, secreting monoclonal antibody (MAb) specific to the S1 subunit, the active enzyme of the PT that intracellularly ADP-ribosylates the host Gi-protein, was produced. The MAbPT6-2G6 inhibited the *in vitro* hemagglutination of chicken erythrocytes which is the activity of the B oligomer of PT; thus we hypothesize that the MAb bound to its epitope on the S1 subunit and stereologically hinders the binding sites of the B subunits. The MAb also inhibited *ex vivo* Chinese hamster ovarian cell clustering and neutralized the *in vivo* leucocytosis- promotion in mice which are usually mediated by intracellular S1 subunit. The large molecular nature of the intact MAb and its molecular hydrophilicity led us to speculate that the observed PT neutralizing activities of the MAb were due to interfering with the cellular entry of the S1 rather than the intracellular enzyme neutralizing activity *per se.* While further experiments are needed to pinpoint the MAb neutralizing activity and to identify the amino acid sequence and location of the MAbPT6-2G6 epitope, our findings indicate that this murine MAb, in its humanized-version, should have high therapeutic potential for pertussis.

Pertussis or whooping cough is a worldwide fatal disease of humans especially among infants and young children. Before the introduction of the vaccine in 1950s,[1] pertussis is one of the major causes of childhood mortality. The disease is caused by a gram-negative bacterium named *Bordetella pertussis.* Human is the only reservoir of the infection and human-to-human transmission occurs by direct inhalation of the aerosol droplets containing the causative bacteria coughed out from the infected individual.[2] *B. pertussis* bacteria initially adhere to the respiratory ciliated epithelium using constitutively expressed adhesins on the bacterial surface, *e.g.* filamentous hemagglutinin, fimbrial agglutinogens, pertactin (an outer membrane protein) which bind to several host components, *i.e.* various sulfated sugars (chondroitin, dextran, heparan), integrin (CR3) and fibro-nectin.[3,4] The attached bacteria then multiply rapidly (colonizaion) and produce various virulence factors for host immune evasion and bacterial survival and pathogenesis. Clinical pertussis has two distinct stages: the catarrhal phase started about 7-10

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days after the superficial attachment of the *B. pertussis* at the respiratory epithelium and is characterized by common cold-like symptoms with mild intermittent cough and low grade fever. The cough is later intensified, becomes paroxysmal and spasmodic which are the characteristics of the second phase. During the paroxysmal phase thick mucus plugs may block the respiratory tract and infants may have sustained apnea, hypoxia and collapse during the coughing spells. Pneumonia is not uncommon while neurological complications, ranging from tremor and seizure to severe neurological problems, are frequently observed. Among the *B. pertussis* virulence factors, pertussis toxin (PT), a 105 kDa hexamer, is the principal toxic component. PT is an A-B_5 toxin consisting of A or S1 subunit which is an ADP-ribosyltransferase and a B pentamer made up of two dimers, *i.e,* S2S4 and S3S4, and one S5, which is responsible for binding of the holotoxin to the host cell surface. PT exists in two forms, cell-bound and secreted. The cell-bound PT functions as one of the bacterial colonizing factors.[5,6] Once the PT bound to the target host cell *via* its B subunits, the A (S1) subunit is cleaved-off and traverses the plasma membrane into the cytoplasm where it transfers ADP-ribose moiety from NAD to a cysteine residue at position 352 of the α-subunit of the membrane-bound inhibitory guanosine-binding protein (Gi-protein). The normal function of this Gi protein [adenylate cyclase inhibition by means of guanosine triphosphate (GTP) hydrolysis to guanosine diphosphate (GDP)], is disrupted. The result is a sustained adenylate cyclase activation and, in the effect, the massive increment of intracellular cyclic adenosine mono-phosphate (cAMP).[7] The increase of intracellular cAMP causes cellular function disruption including defect in all steps of phagocytic activity and/or cytotoxicity of the host cells.[8] PT *via* the S1 subunit causes also systemic leucocytosis, termed leucocytosis-promotion (LP), histamine sensitization and islet cell activation with massive release of insulin.[9] The S1 subunit of PT was found to mediate characteristic rounding and clustering of the Chinese hamster ovarian (CHO) cells *ex vivo.*[10] The PT-B subunit not only binds to the target cell for S1 intracellular delivery but was found also to have immuno-(pathological) potentiating effects, *e.g.* T-lymphocyte mitogenicity.[11] The B pentamer *via* its S2S4 and S3S4 dimers causes chicken erythrocyte agglutination *in vitro*.[10]

Treatment of *B. pertussis* infection by using the antimicrobial agents may reduce the number of bacteria coughed out by the patient and the patient's infectious time, which is important in reducing the bacterial spread. However, the drugs are not effective in amelioration of the pertussis during the catarrhal and paroxysmal stages as the clinical manifestations and the pathophysiology in the patients are caused by the circulating toxins readily secreted by the *B. pertussis* confined in the respiratory tract. Frequently, human immune globulin is used in conjunction with supportive measures such as artificial respiration for treatment of pertussis. The therapeutic antibodies are, however, derived from plasma pool of donors immunized with pertussis toxoid which often raises practical problem (inadequate supply) and ethical issue. Murine monoclonal antibodies specific to the PT in their humanized-form would serve as an alternative of the human therapeutic antibody. Thus, in this study we produced murine hybridomas secreting monoclonal antibodies (MAb) that neutralize activities of PT.

MATERIALS AND METHODS

PT, pertussis toxoid and other antigens

Purified pertussis toxin (PT) was a gift from BIKEN Institute, The Research Foundation of Microbial Disease of Osaka University, Japan. Pertussis toxoid was from the Division of Biological Products, Department of Medical Sciences, Ministry of Public Health, Thailand. Tetanus and diphtheria toxins were from the Government Pharmaceutical Organization (GPO), Thailand. Cholera toxin and streptolysin O were purchased from Sigma Chemical Co., USA. Whole cell homogenates of microorganisms commonly found in the human respiratory tract were prepared as previously described.[12] List the antigens used in this study are shown in Table 1. Murine MAb specific to the A subunit of cholera toxin produced in our laboratory was used as a negative MAb control.

Hybridomas and monoclonal antibody (MAb) production

Hybridomas secreting specific monoclonal antibodies to PT protein were produced as previously described.[13] Female BALB/c mice (8 weeks old)

Table 1 Reactivity of monoclonal antibodies secreted by the established hybridomas to homologous and heterologous antigens when tested by the indirect ELISA

Antigenic type	Toxin/organism	PT1-2E6	PT1-3B10	PT5-2C11	PT6-2H7	*PT6-2G6	PT7-1H11	PT7-2F6
Toxins								
	Pertussis toxin	+	+	+	+	+	+	+
	Tetanus toxin	+	+	-	-	-	-	-
	Diphtheria toxin	+	+	-	-	-	-	-
	Cholera toxin	+	+	-	-	-	-	-
	Streptolysin O	-	-	-	-	-	-	-
Bacteria								
	Acenetobacter spp.	+	+	-	-	-	-	-
	Bordetella pertussis	+	+	+	+	+	+	+
	Corynebacterium diphtheriae	+	+	-	-	-	-	-
	Haemophilus influenzae	+	+	-	-	-	-	-
	Klebsiella pneumoniae	+	+	-	-	-	-	+
	Pseudomanas aeruginosa	+	+	-	+	-	-	+
	Staphylococcus aureus	+	+	-	-	-	+	+
	Staphylococcus coagulase negative	+	+	-	-	-	-	-
	-Streptococci Gr. A	+	+	-	-	-	-	-
	-Streptococci Gr. B	+	+	-	-	-	-	-
	Streptococcus pneumoniae	+	+	-	-	-	-	-
Yeast								
	Candida albicans	+	+	-	-	-	-	-

* MAb secreted by this clone was further used in the subsequent experiments

were kindly provided by the Armed Forces Research Institute of Medical Sciences (AFRIMS), U.S. Component, Bangkok, Thailand. The mice were pre-bled before being injected intraperitoneally with 1 μg of PT in 200 μl NSS emulsified in equal volume of Freund's complete adjuvant. The mice were given two booster doses at two week-intervals with the same dose of the antigen in Freund's incomplete adjuvant. Fourteen days after the last booster, all mice were bled and their sera were assessed for antibody titers against the PT by an indirect ELISA. An immune mouse showing the highest serum titer was used as a spleen cell donor in the hybridoma production. The selected mouse was given an intravenous injection with 15 μg of pertussis toxoid in 200 μl NSS. Three days later, the mouse was bled to collect the immune serum (IS) then it was sacrificed. Spleen cells of this mouse were fused with P3x-63-Ag8.653 myeloma cells by using polyethylene glycol 4,000 as a fusogen at the ratio of ~10 spleen cells to one myeloma cell. Spent culture medium in wells containing growing hybrid cells were tested for antibodies against the homologous antigen by indirect ELISA. Cells from the antibody-positive wells were subjected to monocloning by limiting dilution method using spleen cells of a naïve BALB/c mouse as feeder cells. Culture fluids of individual hybridoma clones were retested against homologous and heterologous antigens by the indirect ELISA. Clones secreting monoclonal antibodies (MAb) reactive only to PT were selected. Antigenic specificities of the MAb secreted by individual clones were determined by Western blot analysis (WB) against the SDS-PAGE separated-PT. Immunoglobulin types and subtype(s) of the MAb were identified using Mouse Typer Sub-isotyping kit (Bio-Rad, CA, USA).

A hybridoma secreting MAb specific to PT was grown in serum free medium to late log phase.

The cell spent medium was collected, concentrated by pressure filtration method (Amicon, MA, USA) and purified by using a protein G affinity column. Protein concentration of the IgG preparation was determined.[14]

Indirect ELISA

An indirect ELISA was used for determining antibody titers of sera from immunized mice and for detecting antibodies in the cell spent culture media for screening of positive hybrids. The technique was also used for determining specificity *versus* cross-reactivity of the MAbs. Wells in polystyrene microtiter plates (Costar, Corning, NY, USA) were individually coated (100 µl per well) with appropriate antigens (1 µg of bacterial toxin per ml or 50 µg of bacterial homogenate per ml of carbonate-bicarbonate buffer, pH 9.6). The plates were incubated at 37°C in a humid chamber for 1 hour then all wells were washed three times with phosphate buffered saline (PBS) containing 0.5 % Tween-20 (PBS-T) to remove the unbound antigens. The remaining unoccupied sites in each well were blocked by incubating with 200 µl of a blocking buffer [1% bovine serum albumin (BSA) in PBS] at 37°C for 1 hour. The excess BSA was washed off, and 100 µl of antibody preparation was added to the well. The antigen-antibody reaction was allowed to take place at 37°C for 1 hour. After washing with PBS-T, 100 µl of a 1:1,000 dilution of rabbit anti-mouse Ig horseradish peroxidase (Dakopatts, Glostrup, Denmark) in PBS containing 0.2% BSA and 0.2% gelatin was added to each well and incubated as above. The unbound conjugate was removed by washing with PBS-T. The enzyme substrate was added to all wells (100 µl per well). The reaction was allowed to take place in the dark for 10 minutes and was then stopped by adding 50 µl of 1 N NaOH per well. The optical density of the content in each well was determined at 492 nm using an ELISA reader (Titertek Multiskan, Flow Laboratory, Switzerland). The ELISA titer of an antibody preparation was the highest dilution of the preparation giving an optical density at or higher than 0.05 above the blank (content of well that PBS was added instead of the antibody). One indirect ELISA unit was defined as the least amount of the antibody preparation which gave a positive indirect ELISA reaction.

SDS-PAGE and Western blot analysis (WB)

SDS-PAGE was carried out in a vertical slab gel apparatus (Mini-PROTEAN-III, Bio-Rad, USA) according to the previously described method.[15] PT in the sample buffer was heated at 56°C for 30 minutes before loading onto a 4% stacking gel and 15% acrylamide separating gel were used in the procedure. WB was performed by electro-transblotting the SDS-PAGE separated-PT from the gel to a nitrocellulose (NC) membrane. The unoccupied sites on the NC membrane were blocked by placing the membrane in a solution of a blocking buffer (3% BSA, 5% gelatin in PBS) at 25 °C with gentle rocking for 1 hour. The membrane was washed with PBS-T and incubated in an MAb solution for 1 hour, washed and placed in a solution of rabbit anti-mouse immunoglobulin-horseradish peroxidase conjugate (Dakopatts) at a dilution of 1:1,000 in PBS containing 1% BSA and 1% gelatin at 25°C for 30 minutes. After excessive washing with the washing buffer, the membrane was placed in a freshly prepared substrate solution for 5 minutes, washed with distilled water, and air dried.

Hemagglutination assay (HA) and hemagglutination-inhibition (HI) test

Hemagglutination assay (HA) was performed as previously described.[16] Briefly, 50 µl of 0.7% chicken erythrocytes in PBS (v/v) was mixed with serially diluted PT. The preparation was incubated at 25°C for 30 minutes. The least amount of PT that cause complete agglutination of the red blood cells (hemagglutination dose 100; HD100) was determined by observing the hemagglutination by light microscopy at 100x magnification. For the HI test, one HD100 of PT in 25 µl of PBS was incubated with various amounts of MAb at 25°C for 30 minutes. Fifty µl of 0.7% chicken erythrocyte suspension was added to each PT-MAb mixture and the preparation was kept at 25°C for 30 minutes. The lowest amount of the MAb that could inhibit the hemagglutination caused by the HD100 of PT was taken as an HI dose.

Chinese hamster ovarian (CHO) cell assay

CHO cells cultured in the presence of PT display a characteristic clustering pattern.[10] In this

study, the MAb specific to S1 was tested for its ability to inhibit the CHO cell clustering (CC). CHO cells (strain K1) were cultured in a 25 ml-tissue culture flask (Corning) in a 10% fetal bovine serum supplemented-RPMI-1640 medium containing 1% antibiotics, *i.e.* 100 units penicillin and 100 μg streptomycin per ml (complete RPMI medium) in a 5% CO_2 incubator at 37°C until their confluent growth was obtained which took about 2-4 days. The cells were trypsinized, harvested from the flask and adjusted to 2 x 10^5 cells/ml with the serum supplemented-RPMI medium. Wells in the 96-well tissue culture plate (Costar) were added with 100 μl of the medium containing various amounts of PT. CHO cells (100 μl) were added to individual PT containing wells (test wells) and also to the wells containing only the RPMI medium (negative control wells). The plate was incubated in the 5% CO_2 incubator at 37°C for 16-24 hours. The least amount of PT that caused clustering of ~50% of the cells in the well as compared to the negative control well was taken as one cell clustering dose 50 (CCD50). For the CHO cell clustering inhibition (CCI) assay, various amounts of the MAb in 50 μl of complete medium was added to duplicate wells in a tissue culture plate and one CCD50 of the PT was added to each well. Duplicate wells containing only the PT (positive control wells) were included in the plate. After incubation at 25°C for 30 minutes, CHO cells in 100 μl of the RPMI medium were added to each culture well and the plate was incubated in the 5% CO2 incubator as above. The cells in all wells were inspected every 6 hours under 100x magnification light microscopy. The minimum amount of the MAb that could rescue the cells from clustering when 50% of cells in the positive control wells showed the characteristic clustering was determined.

Leukocytosis-promotion (LP) inhibition test

On day 0, EDTA blood samples were taken individually from 30 female, 6 week old BALC/c mice. The number of white blood cells (WBC) in each sample was counted as the baseline data. The mice were then divided into 5 groups (groups 1-5). Mice of groups 1 and 2 were each injected intravenously with 100 μl PBS alone (negative LP control) or PBS containing 500 μg of MAb specific to the A subunit of cholera toxin plus 100 ng PT (positive LP control), respectively. Mice of groups 3-5 were intravenously injected with the same amount of PT previously incubated with three different concentrations of S1 specific MAb. Four days post-injection (day 3), all mice were bled and their WBC were counted. The WBC count of each mouse on day 3 was subtracted with baseline WBC count of day 0 of that particular mouse to obtain the ΔWBC. The ΔWBC of mice of groups 3-5 were compared with the ΔWBC of group 1 (negative LP control) and group 2 (positive LP control) by using Mann Whitney U test. Difference at $p < 0.05$ was statistically significant.

RESULTS

Hybridomas and specific MAb to PT

The immunized mouse which was used as the immune splenocyte donor had serum ELISA titer of 1:64,000 against PT. A total of 7.8 x 10^7 spleen cells were obtained from this mouse and were fused with about 7.8 x 10^7 myeloma cells. Culture fluids from a total of 370 wells which revealed growing cells were tested for antibodies against the homologous antigens by the indirect ELISA, and the spent culture fluids from 45 wells (12.16%) were positive. Cells of only seven wells were found to retain the antibody producing capability after sub-culturing. The spent culture fluids of cells from these wells (PT1 to PT7) were subjected to WB analysis against the SDS-PAGE separated-PT. It was found that antibodies from cells of PT1 well gave faint bands to S1, S2 and S3; antibodies from PT2 and PT3 wells gave negative Western blot result; antibodies from PT4 well gave weak reaction against S3 subunit; and antibodies from PT5, PT6 and PT7 wells showed intense bands against S1 subunit (data not shown). Cells from all of these seven wells were cloned by limiting dilution method and seven stable hybridromas, designated clones PT1-2E6, PT1-3B10, PT5-2C11, PT6-2G6, PT6-2H7, PT7-2F6 and PT7-1H11, were established. The MAbs from all of these monoclones reacted to PT by the indirect ELISA. However, only two clones, PT5-2C11 and PT6-2G6, secreted MAb reacted only to PT and did not react to any of the tested heterologous antigens (Table 1). The immunoglobulin isotype of both clones was IgG1. Fig. 1 shows results on specificities of the MAb secreted by the seven established hybridomas against SDS-PAGE separated-PT by Western blot analysis.

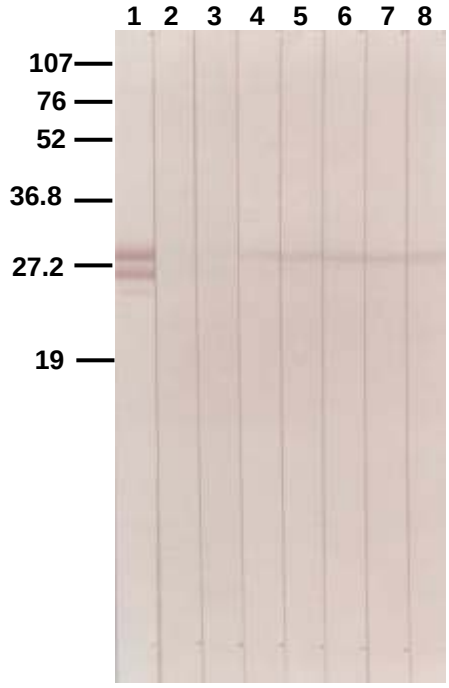

Fig. 1 Western blot analysis (WB) pattern of monoclonal antibodies in spent culture fluids of the seven monoclones against the SDS-PAGE-separated-PT. Lane 1, mouse immune serum; lanes 2-8, MAbPT1-2E6, MAbPT1-3B10, MAbPT5-2C11, MAbPT6-2G6, MAbPT6-2H7, MAbPT7-2F6 and MAbPT7-1H11, respectively. Numbers at the left are relative molecular masses (*Mr*).

Inhibition of PT mediated- hemagglutination by MAbPT6-2G6

Because the cells of the clone PT6-2G6 were healthy, grew fast and secreted high titer of MAb (MAbPT6-2G6) against S1 subunit of PT, this clone was selected for further use.

In the hemagglutination assay (HA), the minimum amount of PT that caused complete hemagglutination of the chicken erythrocytes (HD100) was found to be 125 ng (Fig. 2, A). The hemagglutination mediated by this amount of PT was readily inhibited by 6.25 mg of MAb PT6-2G6 (Fig. 2, B).

Neutralizing activity of MAbPT6-2G6 on PT mediated-CHO cell clustering

The minimum amount of PT that could cause 50% characteristic CHO cell clustering (CCD50) was 5.6 pg (Fig. 3). The MAb PT6-2G6 at 32.5 μg could mediate ~50% inhibition of CC caused by a CCD50 of PT.

Neutralization of PT mediated-leucocytosis-promotion by the MAbPT6-2G6

Fig. 4 shows neutralizing activity of MAbPT6-2G6 against leucocytosis-promotion effect mediated by PT in mice. Mice of group 1 were injected individually with PBS (negative LP controls), mice of group 2 received PT previously incubated with monoclonal antibody to cholera toxin A subunit (positive LP controls), and mice of groups 3-5 were injected with PT incubated with various amounts of MAbPT6-2G6. It was found that the MAbPT6-2G6 could cause statistically significant decrease of LP mediated by the 100 ng of PT in a dose dependent manner (MAb 500 μg > 250 μg >125 μg). However, the decrease of LP mediated by the MAb did not abrogated the PT mediated LP completely as the ΔWBC of groups 3-5 were still significantly higher than that of group 1 (negative LP control).

DISCUSSION

Ability of specific antibody to protect against disease, *i.e.* diphtheria, was first discovered by von Behring and Kitasato in the early 1890s. Since then the antibody based-therapy, called serum therapy, was practiced for treatment of various infectious diseases in human. The discovery of sulfonamides and antibiotics in the mid 1930s, however, made a change in the treatment strategy of infectious diseases and the serum therapy was mostly replaced by the antimicrobial chemotherapy. Nevertheless, the antibody still retains its therapeutic niche for many viral infections as well as for envenomation, *e.g.* snakebite, and bacterial intoxication, *e.g.* tetanus, botulinum and pertussis.[17,18] For pertussis, even though the effector mechanisms of protective immunity against the disease is not completely understood and may involve both humoral and cell mediated immune mechanisms,[19] evidences in the literature have pointed out that specific antibodies to PT play important role in amelioration of the pertussis severe morbidity and in protection against the *B. pertussis* infection.[20, 21]

In this study, murine hybridomas, *i.e.* clones PT5-2C11 and PT6-2G6 secreting monoclonal anti-

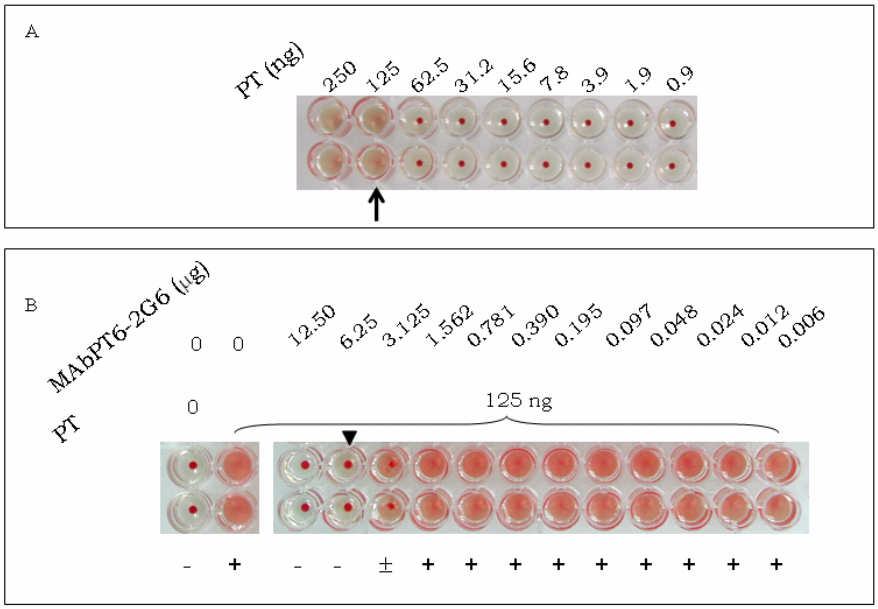

Fig. 2 Hemagglutination assay for determining HA100 of PT (A) and hemagglutination inhibition assay for determining the HI activity of MAbPT6-2G6 (B). The HA100 of PT was 125 ng (arrow in A) and the HI100 of the MAbPT6-2G6 was 6.25 μg (arrow head in B). +, positive HI; -, negative HI; ±, partial HI reaction.

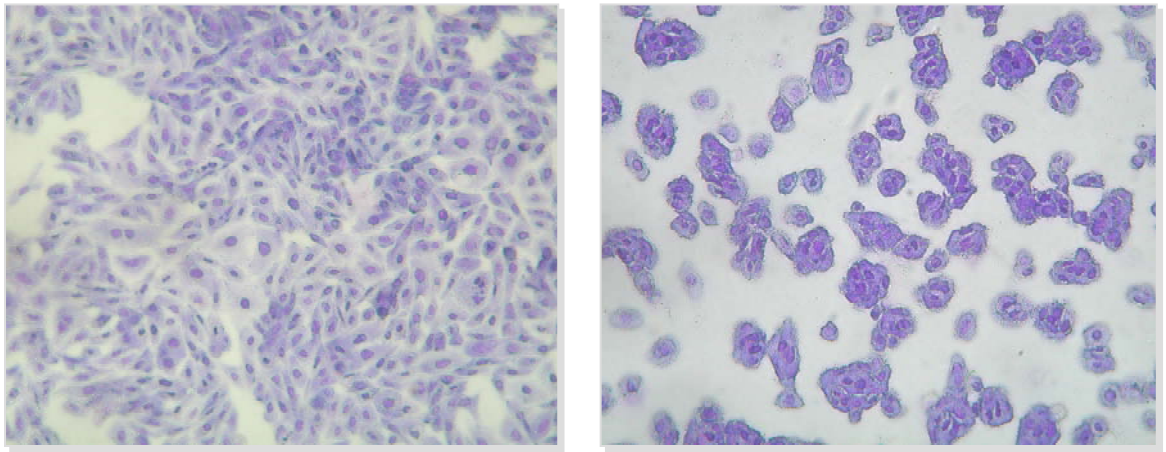

Fig. 3 Chinese hamster ovarian (CHO) cell clustering phenotype. Left, Monolayer of normal CHO cells in tissue culture; Right, characteristic clustering (CC) of CHO cells mediated by PT.

bodies reacted specifically to S1 subunit of PT were produced. The MAbPT6-2G6 was tested for the neutralizing activity against PT mediated-functions *in vitro*, *ex vivo* and *in vivo*. Our finding that intact MAbPT6-2G6 could readily inhibit the *in vitro* effect of the PT mediated chicken erythrocyte agglutination, which is the normal activity of the S2S4 and S3S4 dimers of the B oligomer of PT,[22] implies that the epitope of this MAb on the S1 subunit may be in the juxtaposition with the hemagglutination site(s) of the B subunits; thus binding of the MAb to the epitope would stereologically hinder the hemagglutination sites of the B pentamer.

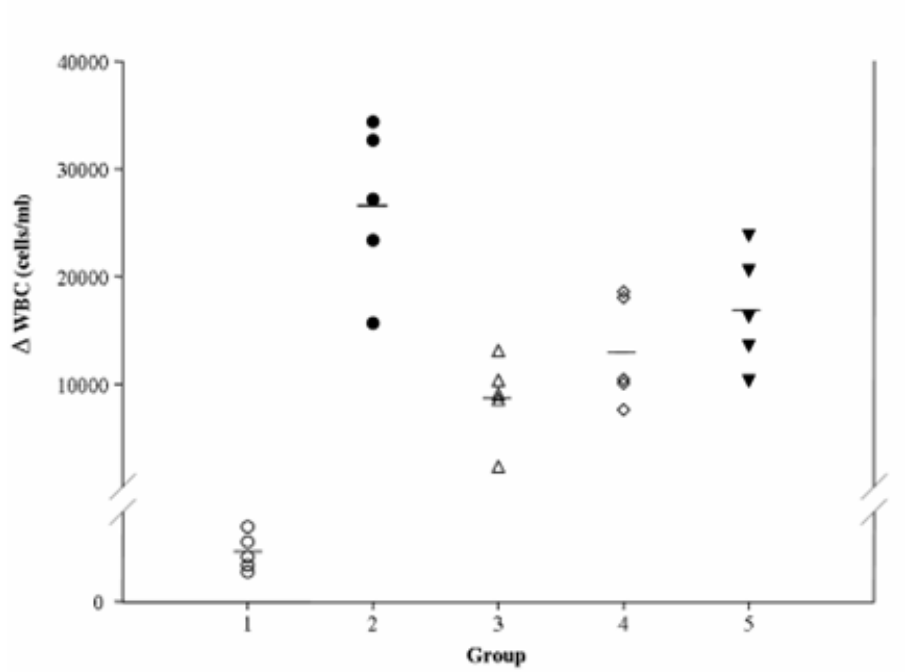

Fig. 4 Results of leucocytosis-promotion (LP) inhibition mediated by MAbPT6-2G6. Group 1 (O), each mouse received intravenous (i.v.) injection with 100 μl PBS (negative LP control); groups 2 (●), each mouse received i.v. injection with 100 ng PT incubated with 500 μg of monoclonal antibody specific to cholera toxin A subunit (positive LP control); group 3 (Δ), individual mice received i.v. injection with 100 ng PT incubated with 500 μg of MAbPT6-2G6; group 4 (◇), individual mice received i.v. injection with 100 ng of PT incubated with 250 μg MAbPT6-2G6; and group 5 (▼), each mouse received i.v. injection with 100 ng of PT incubated with 125 μg of MAbPT6-2G6.

Although the molecular events involved in the cellular entry of PT is not well understood, evidence suggested that after binding of the B oligomer to the host cell membrane, the S1 subunit may traverse across the plasma membrane instead of using the receptor mediated endocytotic pathway.[23] It is also known that intact antibody molecule, because of its large size and molecular hydrophilicity, cannot normally penetrate the hydrophobic plasma membrane of the living cells and thus is inaccessible to its intracellular target. The findings that previous incubation of the PT with the MAbPT6-2G6 which is specific to S1 subunit could inhibit the CHO cell clustering and leucocyte-promotion activities are interesting because these PT functions are mediated by the ADP-ribosyltransferase activity of the intracellular S1.[24] Neutralization of the PT by the intact MAb in this study should not be mediated by interfering the enzymatic function of the S1 by MAb *per se,* but rather preventing the intracellular entry of the S1 by forming PT-antibody complexes and/or streologically interfering with binding of the B oligomer to the host cell membrane. We, therefore, speculated further that the epitope of the MAbPT6-2G6 should be exposed on the surface of the PT holotoxin in order to be accessibly bound by the MAb and near to the membrane binding site(s) of the B pentamer. While further experiments are needed to identify the amino acid sequence and location of the MAbPT6-2G6 epitope, our findings demonstrate that this murine MAb, in its humanized-version, should have high therapeutic potential for pertussis.

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REFERENCES

1. Medical Research Council. The prevention of whooping-cough by vaccination. BMJ 1951; 1: 1463-71.
2. Hewlett EL. Pertussis: current concepts of pathogenesis and prevention. Pediatr Infect Dis J 1997; 16: 78-84.

3. Locht C. Molecular aspects of *Bordetella pertussis* pathogenesis. Int Microbiol 1999; 2: 137–44.
4. Babu MM, Bhargavi J, Singh Saund R, Singh SK. Virulence factors in *Bordetella pertussis*. Current Science 2001; 80: 1512-22.
5. Carbonetti NH, Artamonova GV, Mays RM, Worthington ZEV. Pertussis toxin plays an early role in respiratory tract colonization by *Bordetella pertussis*. Infect Immun 2003; 71: 6358-66.
6. Carbonetti NH, Artamonova GV, Andreasen C, Bushar N. Pertussis toxin and adenylate cyclase toxin provide a one-two punch for establishment of *Bordetella pertussis* infection of the respiratory tract. Infect Immun 2005; 73: 2698-703
7. Bruni P, Burns DL, Hewlett EL, Moss J. Effects of pertussis toxin on cAMP and cGMP responses to carbamylcholine in N1E-115 neuroblastoma cells. Mol Pharmacol 1985; 28: 229-34
8. Meade B D, Kind P D, and Manclark C R. Lymphocytosis-promoting factor of *Bordetella pertussis* alters mononuclear phagocyte circulation and response to inflammation. Infect Immun 1984; 46: 733-9.
9. Katada T, Ui M. Islet-activating protein. Enhanced insulin secretion and cyclic AMP accumulation in pancreatic islets due to activation of native calcium ionophores J Biol Chem 1979; 254: 469-79
10. Hewlett EL, Sauer KT, Myers GA, Cowell JL, Guerrant RL. Induction of a novel morphological response in Chinese hamster ovary cells by pertussis toxin. Infect Immun 1983; 40: 1198-203.
11. Strnad CF, Carchman RA. Human T lymphocyte mitogenesis in response to the B oligomer of pertussis toxin is associated with an early elevation in cytosolic calcium concentrations. FEBS Letters 1987; 225: 16-20.
12. Saengjaruk P, Chaicumpa W, Watt G, Bunyaraksyotin G, Wuthiekanun V, Tapchaisri P, *et al*. Diagnosis of human leptospirosis by monoclonal antibody-based antigen detection in urine. J Clin Microbiol 2002; 40: 480-9.
13. Chaicumpa W, Ruangkunaporn Y, Burr D, Chongsa-Nguan M, Echeverria P. Diagnosis of typhoid fever by detection of *Salmonella typhi* antigen in urine. J Clin Microbiol 1992; 30**:** 2513-5.
14. Bradford MM. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding. Anal Biochem 1976; 72: 248-54.
15. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680-5.
16. Sato, H., A. Ito, J. Chiba, and Y. Sato. Monoclonal antibody against pertussis toxin: effect on toxin activity and pertussis infections. Infect Immun 1984; 46: 422-8.
17. Casadevall A, Scharff MD. Return to the past: the case for antibody-based therapies in infectious diseases. Clin Infect Dis 1995; 21: 150-61.
18. Dolby JM, Dolby DE, Bronne-Shanbury CJ. The effects of humoral, cellular and non-specific immunity on intracerebral *Bordetella pertussis* infections in mice. J Hyg 1975; 74: 85-102.
19. Buchwald UK, Pirofski L. Immune therapy for infectious diseases at the dawn of the 21st century: the past, present and future role of antibody therapy, therapeutic vaccination and biological response modifiers. Curr Pharm Des 2003; 9: 945-68.
20. Sato H, Sato Y. Protective activities in mice of monoclonal antibodies against pertussis toxin. Infect Immun 1990; 58: 3369–74.
21. Sato H, Ito A, Chiba J, Sato Y. Monoclonal antibody against pertussis toxin: effect on toxin activity and pertussis infections. Infect Immun 1984; 46: 422-8.
22. Tamura M, Nogimori K, Murai S, Yajima M, Ito K, Katada T, *et al*. Subunit structure of islet-activating protein, pertussis toxin, in conformity with the A-B model. Biochemistry 1982; 21: 5516–52.
23**.** Kaslow HR, Burns DL. Pertussis toxin and target eukaryotic cells: binding, entry, and activation. FASEB J 1992; 6: 2684-90.
24. Castro MG, McNamara U, Carbonetti NH. Expression, activity and cytotoxicity of pertussis toxin S1 subunit in transfected mammalian cells. Cell Microbiol 2001; 3: 45-54.