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The 70-Kilodalton Pertussis Toxin-Binding Protein in Jurkat Cells

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^{125}I-ASD photoaffinity-labeling derivatives of pertussis toxin (^{125}I-ASD-PT) or lipopolysaccharide (^{125}I-ASD-LPS) labeled similar 70-kDa proteins in Jurkat cells, a cell line derived from human CD4$^+$ T lymphocytes. Labeling of this 70-kDa protein by ^{125}I-ASD-PT was inhibited by underivatized PT but not by underivatized LPS. However, an immunoglobulin M monoclonal antibody with specificity for the p73 LPS receptor in murine splenocytes (S. W. Bright, T.-Y. Chen, L. M. Flebbe, M.-G. Lei, and D. C. Morrison, J. Immunol. 145:1–7, 1990) inhibited ^{125}I-ASD-PT labeling of the 70-kDa species in Jurkat cells. Our results suggested that PT may bind to the same 70-kDa protein as LPS does in Jurkat cells but that PT and LPS bind to different sites on this receptor candidate. ^{125}I-ASD-PT photoaffinity labeling of the 70-kDa protein was also inhibited by underivatized glycoproteins to which PT has been shown to bind, and this inhibition correlated with the relative binding affinities of the glycoproteins for PT. ^{125}I-ASD derivatives of two sialic acid-specific plant lectins, *Maackia amurensis* leukoagglutinin and *Sambucus nigra* agglutinin, with oligosaccharide binding specificities similar to those of PT also labeled a 70-kDa protein in Jurkat cells. This suggests that the 70-kDa PT receptor candidate in Jurkat cells likely contains sialooligosaccharide sequences to which PT, *M. amurensis* leukoagglutinin, and *S. nigra* agglutinin bind. The cross-reacting epitope recognized by monoclonal antibody 5D3 in this 70-kDa species might overlap the PT- and LPS-binding sites.

Pertussis toxin (PT) is one of several important virulence factors produced by *Bordetella pertussis*, the etiologic agent of whooping cough (50). PT is capable of inducing a number of biological responses in eukaryotic cells (26) and is the component best correlated with protection in the whole-cell whooping cough vaccine (6, 27, 35). The toxin is a heterohexamer composed of a catalytic A subunit (denoted S1) and a receptor-binding (B) oligomer. The PT B oligomer consists of four heterogeneous subunits (42, 43) arranged as two dimers (S2-S4 and S3-S4) joined by the smaller (S5) subunit (23, 29). This organization of the B oligomer was recently confirmed by X-ray diffraction analysis of PT holotoxin crystals (39). The S1 subunit has ADP-ribosyltransferase activity that catalyzes the transfer of ADP-ribose from NAD to $G_{\alpha i}$, part of the GTP-binding protein (G-protein) complex that regulates important pharmacological messengers in eukaryotic cells.

The B oligomer of PT displays complex lectin-like binding activity, with specificity for asparagine-linked oligosaccharide receptors on serum glycoproteins, Chinese hamster ovary cells, and erythrocytes from several species (1, 3, 16, 17, 37, 47, 51). The lectin-like properties of PT may also contribute to the adhesion of *B. pertussis* to lactosylceramide sequences on ciliated respiratory epithelial cells (46).

Although the enzymatic activity of the S1 subunit is ultimately responsible for many of PT's biological effects, binding of the B oligomer to cell surface receptors in T lymphocytes, macrophages, and, possibly, other mammalian lymphoreticular cell populations (12, 28, 40, 45, 48, 52) also generates a biological response. Therefore, it is important to identify and characterize these PT receptors to fully understand the toxin's role in the disease process.

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We previously used a photoaffinity labeling technique to identify a 70-kDa PT receptor candidate on human peripheral blood T lymphocytes and in Jurkat cells, a cell line derived from human T-helper lymphocytes (8). Using the same technique, Rogers et al. (34) identified two PT-binding proteins on peripheral blood lymphocytes and Jurkat cells. One of these proteins appeared to be the same as the 70-kDa species described in our earlier report (8). A 70-kDa protein was also found to be the dominant binding site for bacterial cell wall peptidoglycan on mouse B and T lymphocytes and macrophages (11). It has been proposed that this peptidoglycan-binding protein is identical to the 73-kDa (p73) lipopolysaccharide (LPS) receptor identified by Morrison and colleagues (5, 10, 22, 24, 25, 33) on murine lymphocytes and macrophages.

These reports suggested that the same or homologous proteins may bind LPS, peptidoglycan, and PT as well as other mitogenic ligands in mammalian lymphoreticular cell populations. Indeed, Lei and Morrison (20) have shown that underivatized PT or PT B oligomer inhibits the ability of ^{125}I-azidosalicylamido-ethyl-1,3′-dithiopropionate (^{125}I-ASD) photoaffinity-labeling derivatives of LPS (^{125}I-ASD-LPS) to photocross-link the p73 LPS receptor in mouse splenocytes. Moreover, LPS also appears to specifically interact with the PT S2 subunit in the fluid phase, perhaps via a lectin-like interaction between PT and glycan sequences containing *N*-acetylglucosamine (GlcNAc) in the LPS core oligosaccharide (21). However, direct binding of LPS to PT was apparently not responsible for the observed reduction in photoaffinity labeling of the 73-kDa LPS receptor by underivatized PT. These data suggest that PT and LPS probably bind to different domains on the same p73 receptor protein in murine splenocytes.

The mechanism of PT's interaction with the 70-kDa human T-lymphocyte receptor candidate has not been determined and could conceivably occur through protein-protein rather than protein-carbohydrate interactions. Moreover, the relationship between LPS- and PT-binding proteins in Jurkat cells has not been studied. Therefore, we have conducted experiments to more clearly define the relationship between PT- and LPS-

binding proteins in Jurkat cells and also further investigate the biochemical nature of the interaction between PT and this 70-kDa receptor candidate.

MATERIALS AND METHODS

Materials. The following reagents were obtained from Sigma Chemical Co., St. Louis, Mo.: laminin, fibrinogen, transferrin, Trizma base, Trizma hydrochloride, glycine, and diethanolamine. Sulfosuccinimidyl-2-(*p*-azidosalicylamido) ethyl-1,3'-dithio-propionate (SASD) and 1,3,4,6-tetrachloro-3α,6α-diphenyl-glycoluril (IODO-GEN) were from Pierce Chemical Co., Rockford, Ill. Acrylamide, bisacrylamide, *N,N,N',N'*-tetramethylethylenediamine (TEMED), molecular weight standards for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and Coomassie blue stain were from Bio-Rad, Mississauga, Ontario, Canada. *Sambucus nigra* agglutinin (SNA) and *Maackia amurensis* leukoagglutinin (MAL) were from Boehringer Mannheim Canada Ltd., Laval, Quebec, Canada. *Salmonella minnesota* wild-type LPS was from List Biologicals Inc., Campbell, Calif. The anti-p73 LPS receptor monoclonal antibody MAb 5D3 and ^{125}I-ASD-LPS were generously provided by D. C. Morrison, University of Kansas Cancer Center, Kansas City. PT was a generous gift from S. Cockle, Connaught Centre for Biotechnology Research, Willowdale, Ontario, Canada. Ampholytes used in the isoelectric focusing procedure were Pharmalyte pH 3-10 or LKB 3.5-10 ampholytes from Pharmacia LKB Biotechnology, Uppsala, Sweden. Chloramine T and X-Omat AR X-ray film were from Kodak Canada Ltd., Vancouver, British Columbia, Canada.

Preparation of ^{125}I-ASD PT, SNA, and MAL conjugates. ^{125}I-ASD conjugates were prepared by the protocol described previously (8). All reactions were carried out under reduced lighting in foil-wrapped borosilicate culture tubes (12 by 75 mm). One hundred micrograms of SASD was dissolved in 50 μl of 100% dimethyl sulfoxide and then diluted 1:20 in sodium phosphate-buffered physiological saline (PBS, pH 7.2). Next, 100 μl of this SASD solution and 3.7 MBq of Na^{125}I were added to a culture tube coated with 40 μg of IODO-GEN. After 1 min, the reaction mixture was removed from the IODO-GEN tube and immediately added to 1.5-ml microcentrifuge tubes containing approximately 10 to 15 μg of PT or lectin bound to 100 μl (bed volume) of fetuin-agarose. After incubation for 30 min at ambient temperature, the agarose suspensions were transferred to glass wool-plugged Pasteur pipettes, washed with approximately 5 ml of PBS, and eluted with 200 μl of 50 mM diethanolamine (pH 11.5) containing 0.15 M NaCl (7) into a culture tube (12 by 75 mm) containing 200 μl of 100 mM Tris-HCl (pH 4.4). If necessary, the pH of the resulting solutions was adjusted to 7 with 1 N HCl. The amount of ^{125}I-ASD-derivatized PT or lectin recovered was determined by the chymotrypsin-treated goose erythrocyte agglutination assay described previously (2). The specific activity of the preparations was determined by trichloroacetic acid precipitation in the presence and absence of reducing agents (8).

Preparation of ^{125}I-ASD-LPS. The results of Lei et al. (19) indicated that *S. minnesota* wild-type LPS bound to the same receptor as *Escherichia coli* O111:B4 LPS. ^{125}I-ASD-LPS was therefore prepared by the method of Wollenweber and Morrison (53) with *S. minnesota* wild-type LPS. All procedures were performed under reduced lighting with foil-wrapped containers. Sonicated LPS (1 mg in 500 μl of water) was added to 0.4 mg of SASD. Borate buffer (500 μl; 0.1 M, pH 8.5) was then added, and the resulting mixture was incubated at room temperature for 30 min. The LPS mixture was again sonicated, 0.4 mg of fresh SASD was added, and the solution was incubated for an additional 30 min. The ASD-LPS was centrifuged at 2,000 × *g* for 5 min, dialyzed against PBS overnight at 4°C, and, when necessary, stored at −80°C. Approximately 0.1 mg (100 μL) of ASD-LPS was iodinated for 10 min in the presence of 18.5 MBq of Na^{125}I, 10 μl of 0.1-mg/ml chloramine T (dissolved in water), and 10 μl of 0.1 mM KI (dissolved in water). The iodination reaction was terminated by adding 30 μl of 0.1-mg/ml sodium metabisulfite (dissolved in water), and the ^{125}I-ASD-LPS was added to a 10-ml volume (0.8 by 19.5 cm) Sephadex G-50 column preequilibrated with 0.2% gelatin in PBS. The column was eluted with PBS, and 0.5-ml fractions were counted in an LKB model 1270 Rackgamma II gamma counter to identify fractions containing ^{125}I-ASD-LPS. These fractions were pooled, dialyzed overnight against PBS, and stored at 4°C until use.

Photoaffinity labeling of cells. Photoaffinity labeling was performed as described previously (8). Cells were washed with 15 ml of PBS four times to remove medium components, especially serum albumin, which can interfere with photoaffinity-labeling reactions involving ^{125}I-ASD-LPS (53). Approximately 0.1 to 0.4 μg of ^{125}I-ASD-PT, ^{125}I-ASD-LPS, or ^{125}I-ASD-lectins was added to approximately 10^6 Jurkat cells or 10^7 human T lymphocytes. The cell suspensions were then incubated in the dark for 90 min at 4°C with occasional shaking. When present, competitive inhibitors were added 15 min before the ^{125}I-ASD conjugates. After incubation, the cell suspensions were exposed to UV light (254-nm emission maximum, 11-cm distance) for 10 min, and then the photoaffinity-labeled cells were washed twice with 2.5 ml of PBS. The cross-link-labeled cells were immediately used for one-dimensional or two-dimensional SDS-PAGE. One-dimensional SDS-PAGE samples were dissolved in SDS sample buffer containing 50 mM dithiothreitol and analyzed on 12.5% acrylamide separating gels. After SDS-PAGE, the gels were dried under vacuum with a Bio-Rad model 1125B slab gel dryer and exposed at −80°C to X-ray film with Du Pont Cronex Lightning-Plus intensifying screens.

Two-dimensional SDS-PAGE. Two-dimensional SDS-PAGE was performed by the method of O'Farrell (30), as described by Dunbar (9). Separation in the first dimension was accomplished by isoelectric focusing on tube gels (1 by 130 mm) with 2% Pharmalyte (pH 3-10) or 2% LKB ampholyte (pH 3.5-10). After isoelectric focusing, the tube gels were loaded onto 12.5% polyacrylamide–SDS slab gels, and the proteins were separated in the second dimension. Duplicate isoelectric focusing tube gels were cut into 5-mm pieces and incubated for 2 h in 2 ml of degassed water obtained from a Milli Q water treatment system. The pH gradient formed during the isoelectric focusing step was determined by measuring the pH of the resulting solutions (30). Two-dimensional gels were stained with 0.125% Coomassie blue R250 in 50% methanol–10% acetic acid (vol/vol), destained with 10% methanol–10% acetic acid (vol/vol), and subjected to autoradiography as described above.

RESULTS

^{125}I-ASD photoaffinity-labeling derivatives of SNA and MAL were prepared to investigate these lectin receptors in Jurkat cells. The ^{125}I-ASD derivatives of both lectins retained their ability to agglutinate chymotrypsin-treated goose erythrocytes, indicating that their binding properties were not affected by the addition of the ^{125}I-ASD cross-linking groups. SDS-PAGE analysis of Jurkat cells incubated with ^{125}I-ASD-

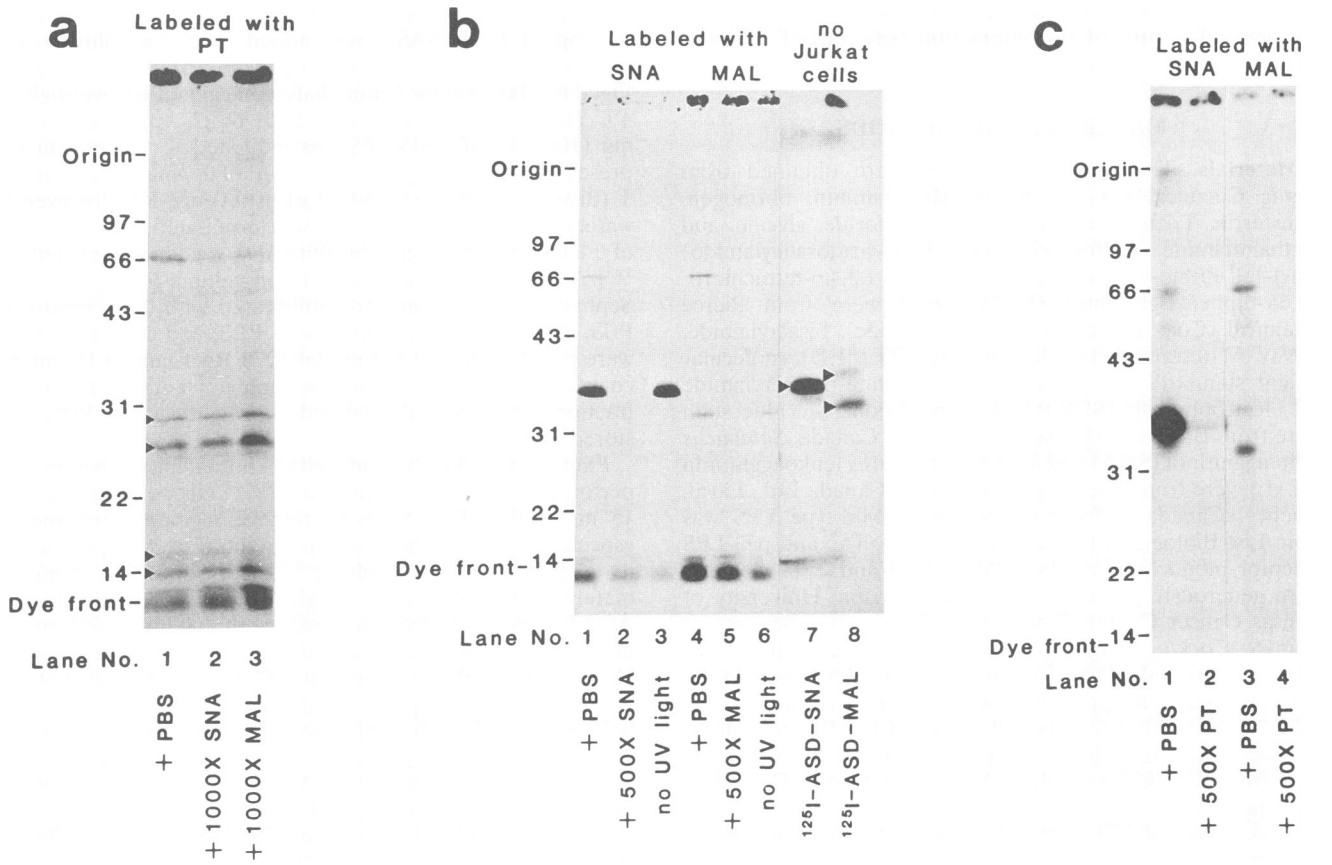

FIG. 1. SDS-PAGE analysis of Jurkat cells photoaffinity labeled with ^{125}I-ASD-PT (a) or ^{125}I-ASD-SNA or ^{125}I-ASD-MAL (b and c). Jurkat cells were photoaffinity labeled with the three ^{125}I-ASD derivatives in the presence and absence of underivatized lectins. After photoaffinity labeling, the PBS-washed Jurkat cells were dissolved in SDS-PAGE sample buffer containing 50 mM dithiothreitol and analyzed on 12.5% polyacrylamide separating gels. The gels were then stained, dried, and exposed to X-ray film to produce the autoradiograms presented in the figure. The positions of protein standards are indicated on the left of each panel (molecular weight in thousands). The dye front and interface between the stacking and separating gels (origin) are also indicated. Arrowheads point to autolabeled PT (a) and SNA and MAL (b) subunits.

SNA or ^{125}I-ASD-MAL and exposed to UV light revealed that the two lectins photoaffinity labeled a protein with the same apparent molecular weight (70,000) as the protein labeled by ^{125}I-ASD-PT (Fig. 1a, lane 1, and 1b, lanes 1 and 4).

A photoaffinity-labeled protein with an apparent molecular weight of 33,000 (Fig. 1b, lanes 1, 3, and 7) represented autolabeled SNA subunits, while the two bands with apparent molecular weights of 32,000 and 36,000 (Fig. 1b, lanes 4, 6, and 8) represented autolabeled MAL subunits. The upper (36 kDa) of these two autolabeled MAL subunits is less apparent but still visible in lanes 4 and 6 of Fig. 1b. Only the autolabeled reaction products were observed when Jurkat cells were omitted from the reaction mixtures (arrowheads, Fig. 1b, lanes 7 and 8).

Autolabeling may result from intersubunit photocross-linking reactions that can occur when oligomeric proteins are used in this procedure (8). Autolabeling may also occur if some ^{125}I, a byproduct in the SASD iodination reaction, is able to react directly with tyrosine groups in proteins being derivatized with the photocross-linking agent. This second potential autolabeling mechanism would explain the labeling of lectin bands in the absence of UV irradiation (Fig. 1b, lanes 3 and 6). The arrowheads beside lane 1 in Fig. 1a indicate the location of autolabeled PT subunits (S1, S2, S5, and S4). For some reason, subunit S3 was less intensely autolabeled than the others.

As predicted, the 70-kDa protein was not labeled by either ^{125}I-ASD-SNA or ^{125}I-ASD-MAL when the Jurkat cells were not exposed to UV light prior to SDS-PAGE analysis (Fig. 1b, lanes 3 and 6). Furthermore, the inclusion of excess underivatized SNA or MAL caused a decrease in the incorporation of label into the 70-kDa protein (Fig. 1b, lanes 2 and 5) when ^{125}I-ASD-SNA or ^{125}I-ASD-MAL was used in the photoaffinity-labeling reactions. The ability of the underivatized lectins to compete for photoaffinity labeling by the ^{125}I-ASD-derivatized lectins indicated that both derivatized and underivatized lectins bound to the same protein.

Densitometric analysis of the autoradiograms (Fig. 1a) revealed that labeling of the 70-kDa protein by ^{125}I-ASD-PT was inhibited by 83% ± 24% (n = 2) by a 1,000-fold excess of underivatized MAL and by 59% ± 3% (n = 2) by a similar excess of underivatized SNA. Underivatized PT inhibited the labeling of the 70-kDa receptor candidate by ^{125}I-ASD-SNA or ^{125}I-ASD-MAL by 95% (Fig. 1c, lanes 2 and 4). These results suggested that PT, SNA, and MAL recognized the same 70-kDa proteins on Jurkat cells. PT, however, appeared to bind to this 70-kDa receptor candidate with a higher affinity than the two lectins. The protein with an M_r of approximately 50,000 in Fig. 1a and c was not labeled consistently.

Sialic acid-containing glycoproteins having known binding affinities for PT (16) were used to compete for ^{125}I-ASD-PT

TABLE 1. Glycoprotein inhibition of photoaffinity labeling of the 70-kDa Jurkat cell protein by ^{125}I-ASD conjugates of PT, SNA, and MAL

Glycoprotein	Molar excess (fold)	% Inhibition of labeling[a]		
		^{125}I-ASD-PT	^{125}I-ASD-SNA	^{125}I-ASD-MAL
Fibrinogen	100	58	53	4
	500	48	78	ND[b]
	1,000	82	100	30
Laminin	50	56	ND	68
	100	65	22	ND
Transferrin	100	0	10	0
	500	31	45	ND
	1,000	63	63	13

[a] Glycoproteins were used to compete for ^{125}I-ASD-PT photoaffinity labeling of the 70-kDa Jurkat cell protein by the protocol described in Materials and Methods. Percent inhibition of labeling was calculated by comparing the intensities (determined by densitometry analysis) of the 70-kDa bands photoaffinity labeled in the presence and absence of inhibitors.

[b] ND, not determined.

photoaffinity labeling of the 70-kDa Jurkat cell protein. When present at a 100-fold molar excess, fibrinogen and laminin reduced the amount of ^{125}I-ASD incorporated into the 70-kDa PT-binding protein by 58 and 65%, respectively, while transferrin had no effect (Table 1). Higher concentrations of fibrinogen further inhibited ^{125}I-ASD-PT photoaffinity labeling of the 70-kDa receptor candidate. At 500- and 1,000-fold molar excesses, transferrin was also capable of inhibiting photoaffinity labeling of the 70-kDa protein. The labeling inhibition data in Table 1 for transferrin correlate with its lower binding affinity for PT than either fibrinogen or laminin (16).

Photoaffinity labeling of the 70-kDa protein by ^{125}I-ASD-SNA and ^{125}I-ASD-MAL was also inhibited by the glycoproteins in a manner consistent with the oligosaccharide specificities of these lectins (Table 1). These results further indicated that the 70-kDa PT-binding protein on Jurkat cells likely possesses sialated oligosaccharide sequences capable of binding PT, SNA, and MAL.

Analysis by two-dimensional SDS-PAGE demonstrated that the 70-kDa protein on peripheral blood monocytic cells (Fig. 2a) and Jurkat cells (Fig. 2b) had a pI of 6.1 ± 0.2 (n = 4). The murine p73 LPS and peptidoglycan receptor candidates identified previously (10, 19) displayed similar isoelectric points, suggesting that they might be homologous to the human lymphocyte 70-kDa PT- and lectin-binding proteins described in this article. To test this hypothesis, we received a sample of ^{125}I-ASD-LPS prepared in the laboratory of David Morrison so that we could directly compare the mobilities of ^{125}I-ASD-LPS and ^{125}I-ASD-PT photoaffinity-labeled Jurkat cell proteins on the same SDS-polyacrylamide gels (Fig. 3).

Samples labeled with ^{125}I-ASD-PT were applied to the lanes on either side of the ^{125}I-ASD-LPS-labeled sample in lane 2 of Fig. 3 to emphasize the similar mobilities of the and Jurkat cell PT- and LPS-binding proteins on SDS-polyacrylamide gels. The slight variation in labeling efficiency of the 70-kDa PT-binding protein in lanes 1 and 3 of Fig. 3 is often observed when the photoaffinity-labeling technique is used. Autolabeled PT subunits can also be seen in lanes 1 and 3 in Fig. 3.

^{125}I-ASD-LPS (*S. minnesota*) prepared in our laboratory also specifically labeled the 70-kDa Jurkat cell membrane protein (Fig. 4a, lane 1), and excess underivatized LPS reduced the amount of label incorporated into this protein (Fig. 4a, lane 2). The presence of excess underivatized LPS did not, however, reduce incorporation of ^{125}I-ASD into the 70-kDa protein when ^{125}I-ASD-PT was used to label the Jurkat cells (Fig. 4b, lanes 2 and 3), suggesting that LPS and PT either bind to different sites on the same 70-kDa protein or bind to different 70-kDa receptors.

Jurkat cells were therefore photoaffinity labeled with ^{125}I-ASD-PT in the presence and absence of excess MAb 5D3, a monoclonal antibody specific for the p73 LPS receptor in mouse splenocytes (4, 5, 25). This antibody inhibited ^{125}I-ASD-PT labeling of the 70-kDa PT receptor candidate in

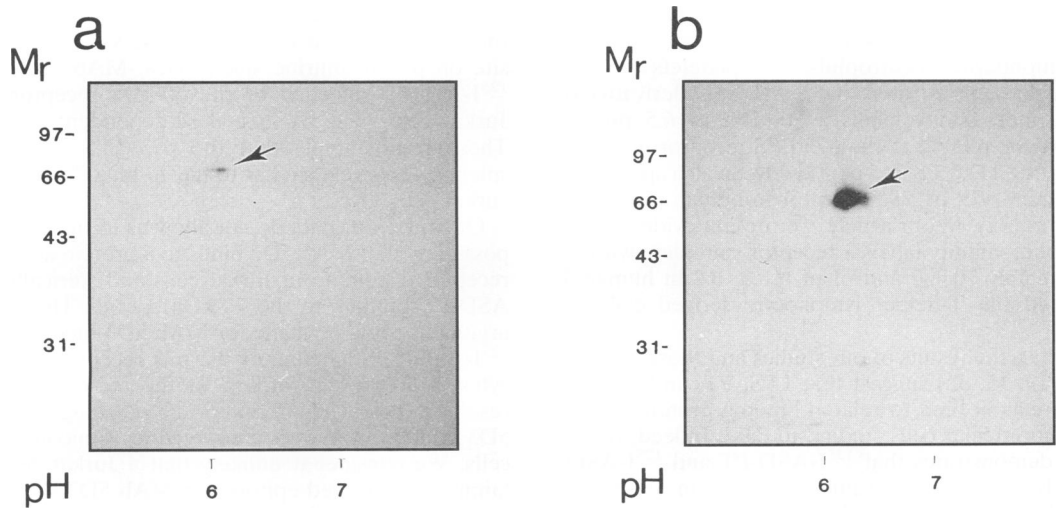

FIG. 2. Two-dimensional SDS-PAGE analysis of peripheral blood monocytic cells (a) and Jurkat cell (b) proteins (arrows) photoaffinity labeled with ^{125}I-ASD-PT. Samples were analyzed in the SDS-PAGE dimension in the presence of 50 mM dithiothreitol on 12.5% acrylamide separating gels. The autoradiogram used to produce the figure was prepared as described in the legend to Fig. 1. The pH gradient determined from duplicate isoelectric focusing gels is indicated at the bottom. The positions of protein standards are indicated (molecular weight in thousands) on the left of each panel.

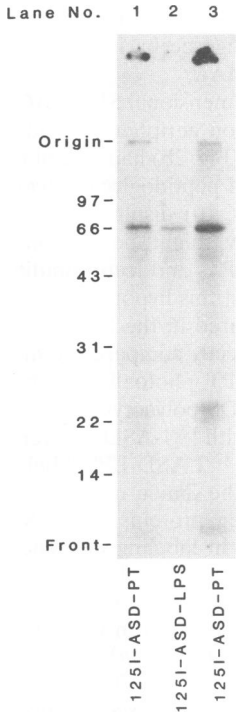

FIG. 3. Photoaffinity labeling of Jurkat cells with ^{125}I-ASD-PT (lanes 1 and 3) and ^{125}I-ASD-LPS from David Morrison (lane 2). Photoaffinity-labeled Jurkat cells were analyzed in the presence of 50 mM dithiothreitol on 12.5% polyacrylamide–SDS gels as described in Materials and Methods. The positions of protein standards are indicated (molecular weight in thousands) on the left.

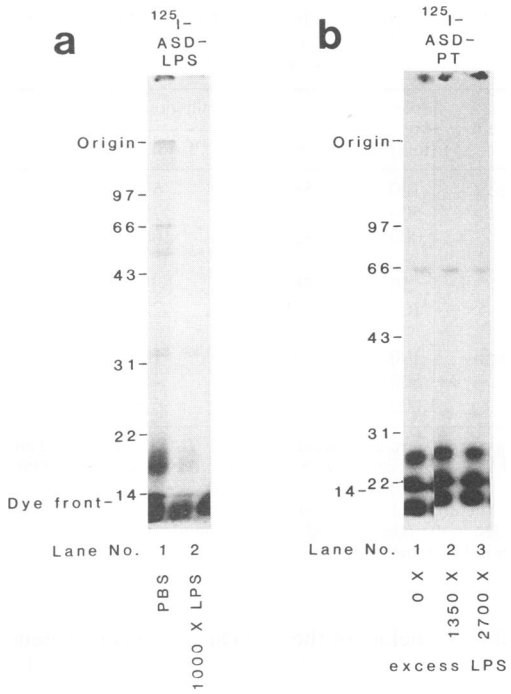

FIG. 4. SDS-PAGE analysis of Jurkat cells photoaffinity labeled with ^{125}I-ASD-LPS (a) and ^{125}I-ASD-PT (b). Jurkat cells were photoaffinity labeled in the presence and absence of underivatized LPS, as indicated at the bottom of the figure. The autoradiograms used to produce the figure were prepared as described in the legend to Fig. 1. The positions of protein standards are indicated (molecular weight in thousands) on the left.

Jurkat cells in a dose-dependent fashion (Fig. 5, lanes 5 and 8, and Fig. 6).

DISCUSSION

Morrison and colleagues previously demonstrated that ^{125}I-ASD-LPS photoaffinity labels a 73-kDa, pI 6.5 binding protein in mouse B lymphocytes, T lymphocytes, and macrophages (24) and human peripheral blood cell populations, including monocytes, lymphocytes, neutrophils, and platelets (15). In addition, Dziarski demonstrated that a ^{125}I-ASD derivative of peptidoglycan photoaffinity labels a 70-kDa, pI 6.5 protein species that may be related to the p73 LPS receptor in mouse B lymphocytes (10, 11). LPS and peptidoglycan also apparently bind to a common p73, pI 5.95 receptor in human peripheral blood monocytes (32). In our article, we present evidence that ^{125}I-ASD-PT photoaffinity labels a receptor candidate with an M_r of approximately 70,000 and pI of 6.1 ± 0.2 in human T lymphocytes and the T-helper lymphocyte-derived cell line Jurkat.

Taken together, the results of our studies and those of three other groups (10, 15, 32) suggest that LPS, PT, and perhaps peptidoglycan may all bind to related binding proteins in the different lymphoreticular cells studied to date. Indeed, recent evidence (20) demonstrates that ^{125}I-ASD-PT and ^{125}I-ASD-LPS apparently bind to the same p73 protein in mouse splenocytes. The results presented in our report suggest that the PT receptor candidate in human T lymphocytes and Jurkat cells is also very similar or identical to the LPS-binding protein in these cells. Furthermore, the 70-kDa protein appears to contain glycan sequences with which the plant lectins SNA and MAL, with specificities for sialyllactosamine, also interact. The p73 LPS receptor in mouse splenocytes also appears to contain carbohydrate sequences (4).

In addition to oligosaccharide sequences and LPS-binding domains, the p73 murine LPS receptor contains epitopes for MAb 5D3 (4). This antibody competes for ^{125}I-ASD-LPS photoaffinity labeling of the p73 receptor and protects mice from a lethal challenge with LPS (4, 25). Therefore, it appears that MAb 5D3 and LPS bind to the same or nearly the same site on p73 in murine splenocytes. MAb 5D3 also inhibited ^{125}I-ASD-PT labeling of the 70-kDa receptor candidate in Jurkat cells (Fig. 5) in a dose-dependent manner (Fig. 6). These results imply that the p73 LPS receptor in mouse splenocytes is very similar to the 70-kDa PT-binding protein in Jurkat cells.

Of course our data do not allow us to rigorously exclude the possibility that MAb 5D3 binds to a protein adjacent to the PT receptor candidate in Jurkat cells and sterically inhibits ^{125}I-ASD-PT binding to the 70-kDa species. However, the same argument could be made for MAb 5D3-mediated inhibition of ^{125}I-ASD-LPS labeling of the p73 receptor in mouse splenocytes. Moreover, regardless of the reason for the inhibition results shown in Fig. 5 and 6, the data suggest that the MAb 5D3 epitope in mice is conserved or semiconserved in Jurkat cells. We consider it unlikely that a Jurkat cell protein containing a conserved epitope for MAb 5D3 should, by chance, associate with another protein that appears to be similar to the p73 mouse LPS receptor. However, we are continuing to seek more conclusive evidence that MAb 5D3 and PT both physically interact with the 70-kDa PT receptor candidate in Jurkat cells.

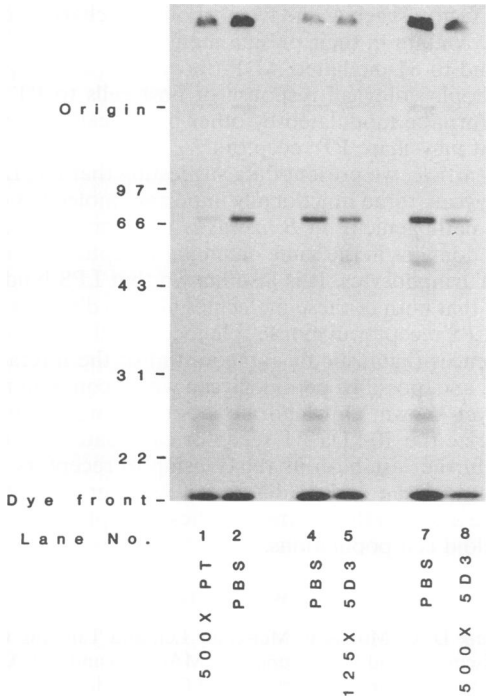

FIG. 5. Photoaffinity labeling of the 70-kDa PT-binding protein in Jurkat cells in the presence and absence of excess underivatized PT or MAb 5D3. Jurkat cells were photoaffinity labeled with ^{125}I-ASD-PT in the presence of a 500-fold molar excess of underivatized PT (lane 1) or a 125- or 500-fold molar excess of MAb 5D3 (lanes 5 and 8, respectively). The positions of protein standards are indicated (molecular weight in thousands) on the left. The 50-kDa protein that was occasionally photoaffinity labeled can be seen in lanes 5, 7, and 8.

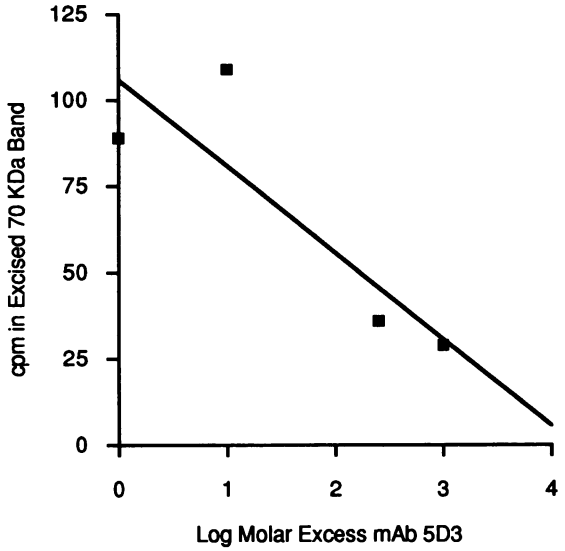

FIG. 6. Dose-dependent inhibition by MAb 5D3 of ^{125}I-ASD-PT labeling of the 70-kDa receptor candidate in Jurkat cells. Jurkat cells were photoaffinity labeled with ^{125}I-ASD-PT in the presence of increasing concentrations of MAb 5D3. The photoaffinity-labeled cells were then analyzed by SDS-PAGE and autoradiography. Counts in the region of the 70-kDa bands cut from the dried gels were recorded with a gamma counter and graphically plotted versus the molar excess of MAb 5D3. Values were corrected for background counts in gel pieces cut from the 70-kDa region of neighboring lanes containing the molecular weight standards. The line was fitted to the points by the least-squares method (r^2 = 0.747). Datum points are the averages of duplicate determinations.

In their recent article, Lei and Morrison (20) showed that PT and LPS probably bind to different sites on the same p73 receptor protein in mouse splenocytes. In this two-site model, it is possible that PT binding to sialyllactosamine glycan sequences on the murine p73 receptor may alter the conformation of the molecule so that its affinity for LPS is reduced. The receptor may contain more than one oligosaccharide chain capable of interacting with PT. The interaction of PT with multiple sugar chains on the p73 murine receptor could induce conformational changes in the polypeptide chain that negatively affect LPS binding. Alternatively, PT cross-linking of p73 receptors could result in the formation of receptor complexes in which the peptidyl-binding sites are no longer accessible to LPS. These possibilities could explain the ability of underivatized PT to inhibit ^{125}I-ASD-LPS labeling of the murine p73 receptor. In our photoaffinity-labeling investigations, we were unable to demonstrate the converse, i.e., to inhibit ^{125}I-ASD-PT photoaffinity labeling of the Jurkat cell 70-kDa protein with underivatized LPS. However, it is much less likely that underivatized LPS binding to peptide domains in the 70-kDa Jurkat cell protein would render the sialyllactosamine glycan sequences, which are probably totally exposed to the fluid phase, inaccessible to ^{125}I-ASD-PT.

The murine p73 LPS-binding protein appears to be a pharmacologically functional receptor by the following two criteria: (i) anti-p73 MAb 5D3 induces LPS-like responses in mouse macrophages in vitro and in vivo (5, 13, 25), and (ii) animals sensitive to endotoxin possess the p73 LPS receptor on their B lymphocytes, whereas endotoxin-resistant species such as frogs and chickens do not (33). Therefore, given that PT and LPS appear to bind to different sites on the same p73 receptor in mouse splenocytes, it is quite likely that the lectin-like interaction of PT with glycan sequences on this receptor may directly induce a biological response in these cells, albeit different from the response induced by LPS. Likewise, the 70-kDa glycoprotein in Jurkat cells may also represent a functional receptor for LPS and PT, but this remains to be confirmed in our laboratory.

Differences in the apparent molecular weights of the LPS-, peptidoglycan-, and PT-binding proteins in different cell types and species may be explained by structural heterogeneity in the glycan chains associated with these glycoproteins. Differences in the reported isoelectric points for these proteins (11, 24, 32) in mice and humans may also be due to species-specific differences in the amount of sialic acid present in the glycan sequences of these receptor candidates. In addition, the solubilized proteins may be sensitive to proteolytic processing. Indeed, we often observed differences in the mobility of the 70-kDa Jurkat cell receptor candidate on SDS-polyacrylamide gels. This can be clearly seen in the gel lanes in Fig. 4b.

Additional binding proteins of 50, 31, and 18 kDa have been observed when ^{125}I-ASD-LPS is used to photoaffinity label human lymphocytes and monocytes (15). We have also detected other photoaffinity-labeled binding proteins (i.e., 50 kDa, Fig. 5) in our experiments with ^{125}I-ASD-PT. It is possible that these lower-molecular-weight species represent important accessory molecules that are necessary for full expression of receptor function. The requirement for such accessory molecules for receptor function could explain why LPS-low-responder C3H/HeJ mice paradoxically express nor-

mal levels of the p73 protein (31). It is possible that the defect in these mice is at the level of accessory molecules rather than in the p73 receptor. Therefore, at present, we must be cautious in assuming that the 70-kDa protein in human T lymphocytes acts as a functional receptor for PT per se, because accessory proteins may also be involved in transmission of a biological response in these cells. The requirement for specific accessory proteins for the generation of appropriate biological signals might explain why T lymphocytes respond differently to LPS and PT.

SNA and MAL are lectins that bind to glycoconjugates having terminal sialic acid residues attached to galactose by α(2-6) and α(2-3) linkages, respectively (38, 49). These lectins photoaffinity labeled the same proteins as PT, competed for PT photoaffinity labeling of Jurkat cell proteins, and were competed for in the photoaffinity-labeling reaction by PT, indicating that some component of the PT, SNA, and MAL receptor contained oligosaccharide sequences having both α(2-3)- and α(2-6)-linked sialic acid residues.

In an earlier report, Rogers and colleagues (34) identified a 43-kDa PT receptor candidate in lymphocytes by the same photoaffinity-labeling procedure that we describe here. They also observed photoaffinity labeling of a PT-binding protein with a molecular mass (approximately 70 kDa) similar to that of the protein that we have characterized in the present study. They concluded, however, that photoaffinity labeling of the 70-kDa protein was nonspecific because the reaction could not be inhibited by adding a 100-fold excess of underivatized PT.

In our experiments, the larger amounts (500- to 1,000-fold) of underivatized PT required to achieve competitive inhibition of photoaffinity labeling may reflect a greater membrane concentration of the 70-kDa protein than is usually seen in high-affinity, low-capacity pharmacological receptors. Alternatively, aggregation of PT may result in the interaction of several toxin molecules with each receptor, leaving sufficient numbers of receptors available to bind both ^{125}I-ASD-PT and underivatized PT at lower competitor concentrations. We believe, however, that the ability of underivatized PT, lectins, glycoproteins, and MAb 5D3 to inhibit ^{125}I-ASD-PT labeling adequately demonstrates the specificity of the reaction for the 70-kDa receptor candidate.

PT may interact directly with plasma membrane calcium channel complexes in human T lymphocytes (41). Several surface antigens present on T lymphocytes, including CD2, CD3, CD4, CD5, CD6, CD7, CD8, and Tp44, are known to cause calcium mobilization after cross-linking with antibodies (18). The PT-binding protein that we have identified in T lymphocytes does not appear to be CD2, CD3, CD6, or CD7, since these antigens have different reported molecular weights. Moreover, CD8 should not be present on Jurkat cells, which are derived from T-helper lymphocytes. Preliminary results indicate that the 70-kDa protein is not CD5, since it is not precipitated by antiserum specific for this antigen (unpublished findings). The relationship of CD4 to the 70-kDa protein is currently under investigation, although reports that PT can deliver mitogenic signals to both $CD4^+$ and $CD8^+$ T lymphocytes (14) indicate that the 70-kDa receptor may not be the CD4 antigen.

The interaction of PT with host cells is complex. In addition to the toxin's ADP-ribosyltransferase activity, the B oligomer, by virtue of its lectin-like properties, may directly induce a pharmacological response in T lymphocytes (12, 40, 44, 52). Moreover, because of amino acid sequence differences between the S2 and S3 subunits, PT has the capacity to interact with more than one oligosaccharide receptor (1, 36, 46, 48, 51). Therefore, the effects of PT on host cells likely depend on multiple factors, such as the types of oligosaccharide receptors that they contain in their plasma membranes and their ability to respond to S1-mediated ADP-ribosylation of $G_{\alpha i}$ proteins. The pathophysiological response of host cells to PT binding may, in turn, be modulated by other biologically active molecules that may share PT receptors.

In this article, we present data suggesting that PT, LPS, and peptidoglycan, three functionally important molecules contributing to pathogenicity in *B. pertussis* (50), may interact with different domains in the same or similar receptor candidates in human T lymphocytes. It is also known that LPS binds to PT (21) and that both of these molecules bind to different sites on the p73 LPS receptor in mouse splenocytes (20). The biological consequences (agonistic or antagonistic) of the interaction of PT, LPS, and, possibly, peptidoglycan with a common receptor are not yet known. Our laboratory is therefore continuing to characterize the 70-kDa PT receptor candidate in T lymphocytes to further establish its relationship to receptors for LPS and peptidoglycan and to determine whether accessory proteins have a role in the function of these receptors in lymphoid and myeloid cell populations.

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