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Lymphocyte Receptors for Pertussis Toxin

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We have investigated human T-lymphocyte receptors for pertussis toxin by affinity isolation and photoaffinity labeling procedures. T lymphocytes were obtained from peripheral human blood, surface iodinated, and solubilized in Triton X-100. The iodinated mixture was then passed through pertussis toxin-agarose, and the fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Autoradiography of the fixed, dried gels revealed several bands in the pertussis toxin-bound fraction that were not observed in fractions obtained from histone or fetuin-agarose. Further investigations employed a photoaffinity labeling reagent, sulfosuccinimidyl 2-(*p*-azido-salicylamido)-1,3′-dithiopropionate, to identify pertussis toxin receptors in freshly isolated peripheral blood monocytic cells, T lymphocytes, and Jurkat cells. In all three cell systems, the pertussis toxin affinity probe specifically labeled a single protein species with an apparent molecular weight of 70,000 that was not observed when the procedure was performed in the presence of excess unmodified pertussis toxin. A protein comparable in molecular weight to the one detected by the photoaffinity labeling technique was also observed among the species that bound to pertussis toxin-agarose. The results suggest that pertussis toxin may bind to a 70,000-Da receptor in human T lymphocytes.

Pertussis toxin (PT) is a major virulence factor produced by *Bordetella pertussis*, the etiological agent of whooping cough (14). PT is also a protective antigen and has adjuvant-like activity (15) in the killed, whole-cell pertussis vaccine that is used to immunize children in North America (6). PT provides another example of an A-B class of bacterial toxin (25). It is composed of an enzymatic A (S1) subunit and a B oligomer which is required for toxin binding to receptors on host cell membranes, erythrocytes from several mammalian or avian species, and glycoproteins like fetuin (3, 20, 28, 30). The B oligomer also interacts with as yet unidentified receptors on T lymphocytes and induces generalized lymphocyte proliferation (24, 26) subsequent to the rapid intracellular accumulation of the second messengers calcium and diacylglycerol. Calcium is mobilized by PT from extracellular sources, presumably through the opening of membrane calcium channels (24). Apparently, this effect is not mediated through modification of intracellular GTP-binding proteins (G proteins) (13, 18), though signal transduction through the CD3–T-cell receptor complex may be involved (8, 27). Both intact PT and the isolated B oligomer are only weakly mitogenic in the absence of compounds which directly activate protein kinase C in vitro or of accessory cells in vivo (23), suggesting that PT may act in concert with other stimulatory ligands to transmit the mitogenic signal.

To obtain a better understanding of the molecular details of the PT B-oligomer activity in T lymphocytes, we have taken two approaches to identifying T-lymphocyte surface proteins to which PT binds. The first approach utilized the affinity isolation technique to identify PT-binding proteins in surface-iodinated, detergent-solubilized T lymphocytes. The second approach utilized an iodinatable photoaffinity reagent, sulfosuccinimidyl 2-(*p*-azido-salicylamido)-1,3′-dithiopropionate (SASD), to pass radioactive label from modified PT to T-lymphocyte receptor proteins in intact lymphocyte membranes. Our results suggested that PT binding to a 70,000-molecular-weight protein in T-lymphocyte membranes may represent the initial step in the process of PT-mediated T-lymphocyte activation.

MATERIALS AND METHODS

Preparation of lymphocytes. Peripheral blood monocytic cells (PBMC) were prepared by centrifuging buffy coats obtained from fresh human blood into Ficoll-Paque (Pharmacia). The PBMC were then washed three times with sterile sodium phosphate-buffered physiological saline (PBS; pH 7.2) and suspended in sterile PBS for the direct photoaffinity labeling experiments. Alternatively, to prepare T lymphocytes, the PBS-washed PBMC were suspended in fetal bovine serum (FBS)-supplemented RPMI 1640 at a concentration of approximately 10^8 cells per ml, and 1-ml volumes were applied to nylon wool (Fenwal Laboratories) columns in FBS-supplemented RPMI 1640 as described earlier (9). After incubation of the columns for 1 h at 37°C, nonadherent T lymphocytes were eluted with prewarmed RPMI and maintained at 4°C until use. T cells were enumerated by using anti-Leu-4 fluorescein isothiocyanate-conjugated antibodies (Becton Dickinson).

Surface iodination procedure. Jurkat cells were grown in RPMI 1640 growth medium (GIBCO) supplemented with 10% heat-inactivated FBS (GIBCO). Before iodination, T lymphocytes or Jurkat cells were washed three times with PBS to remove serum proteins and suspended at a final concentration of 10^8 cells per ml in PBS. A 100-μl sample of the resulting cell suspensions was then incubated with 4 MBq of $Na^{125}I$ (629 GBq/mg; Edmonton Radiopharmaceuticals) in the presence of 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril (IODO-GEN; Pierce) for 1 min. The iodinated cells were washed three times with PBS to remove unbound radioactive iodine and solubilized in Triton X-100 as reported previously (2). Next, the Triton-solubilized cells were added to PT-agarose, histone-agarose, or fetuin-agarose affinity columns and incubated for 15 min at room temperature. The procedure for preparing the agarose derivatives is described in our earlier report (29). Unbound proteins were removed by washing the columns with 5 ml of 50 mM sodium phosphate buffer, pH 7.4, containing 1 M NaCl (wash

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buffer), and the final 0.5-ml wash fraction was collected for analysis. Bound proteins were then eluted with 100 μl of 50 mM diethanolamine (pH 11.5) containing 0.15 M NaCl (7), followed by 400 μl of wash buffer. The fractions were dialyzed overnight at 4°C against 0.1% sodium dodecyl sulfate (SDS) in distilled water and lyophilized in a Savant model SVC100H Speed Vac concentrator. The dried samples were dissolved in 50 μl of laemmli sample buffer (10) containing 10% (vol/vol) 2-mercaptoethanol and analyzed by SDS-polyacrylamide gel electrophoresis (PAGE), using 7.5 to 15.0% linear acrylamide separating gels (2, 10). The gels were dried under vacuum in a Bio-Rad model 1125B slab gel drier and exposed at −96°C to Kodak X-Omat AR X-ray film, using Du Pont Cronex Lightning-Plus intensifying screens.

^{125}I labeling of SASD and preparation of ^{125}I-ASD-PT. ^{125}I-2-(*p*-azidosalicylamido)-1,3′-dithiopropionate (ASD)-PT was prepared by the method of Shephard et al. (21), with the following changes. All reactions were carried out in the dark or under dim lighting conditions in aluminum foil-wrapped disposable glass culture tubes (12 by 75 mm). SASD (Pierce) was dissolved in 0.33 M sodium phosphate (pH 7.5) containing 5% (vol/vol) dimethyl sulfoxide. The iodination reactions were carried out in IODO-GEN-coated culture tubes (12 by 75 mm) containing 10 μg of SASD in 100 μl of buffer and 20 MBq of Na[^{125}I] (629 GBq/mg). The reactions were allowed to proceed for 1 min, and the ^{125}I-SASD was removed from the IODO-GEN tube and incubated for 30 min with 10 μg of PT bound to 50 μl (bed volume) of fetuin-agarose (4). Next, the fetuin-agarose suspension was transferred to a glass wool-plugged Pasteur pipette and washed with 10 to 15 ml of PBS. ^{125}I-ASD-PT was eluted from the fetuin-agarose by using 200 μl of 50 mM diethanolamine (pH 11.5) containing 0.15 M NaCl (7). The ^{125}I-ASD-PT was collected in a culture tube containing 200 μl of 100 mM Tris hydrochloride (pH 4.4), and the pH of the resulting solution was adjusted to approximately 6.5 by using 1 N HCl. The amount of ^{125}I-ASD-PT recovered from the fetuin-agarose was determined by using the trypsinized goose erythrocyte agglutination assay (4) and the protein dye-binding assay described by Bradford (5). The specific activity of the preparations was determined by trichloroacetic acid precipitation (4). Radioactivity was quantified in an LKB model 1270 Rackgamma II gamma counter.

Photoaffinity labeling of lymphocytes. Cells for the photoaffinity labeling experiments were first washed four times with 15-ml volumes of PBS to remove unbound serum components. Approximately 0.1 μg (2.5 × 10^5 cpm) of freshly prepared ^{125}I-ASD-PT was added to 100 μl of PBS containing 3 × 10^7 T lymphocytes, 10^6 Jurkat cells, or 5 × 10^6 PBMC. Approximately 30 times more T lymphocytes were used in the experiments to compensate for the size difference between them and Jurkat cells. The ^{125}I-ASD-PT was allowed to react with the cells in the presence or absence of 1,000-fold-excess underivatized PT in the dark for 90 min on ice. The mixtures were then exposed to a UV light source (emission maximum, 302 nm) for 10 min at a fixed distance of 11 cm. Next, the cell suspensions were washed twice with 2.5 ml of PBS, dissolved in SDS sample buffer containing 10% (vol/vol) 2-mercaptoethanol, and analyzed by SDS-PAGE, using 12.5% acrylamide separating gels. In reactions receiving no cells, an equivalent amount of ^{125}I-ASD-PT in PBS was exposed to UV light and applied to the gels.

Immunoblotting procedure. Jurkat cell proteins were transferred from SDS-polyacrylamide gels to nitrocellulose membrane (Bio-Rad) in a Bio-Rad Transblot apparatus for 16 h at 27 V (constant). The transfer buffer was 25 mM sodium phosphate, pH 7.5. The nitrocellulose membranes were then incubated for 1 h at room temperature with 10 ml of 0.05% Tween 20 in PBS (blocking buffer). The PBS-Tween buffer was removed, and rabbit anti-bovine serum albumin (BSA) or anti-human serum albumin (HSA) antibodies (Nordic Immunology, Tilburg, The Netherlands) dissolved in fresh blocking buffer were added to duplicate blots. The blots were incubated for an additional 2 h at room temperature. After washing, bound antibodies were detected by using horseradish peroxidase-conjugated second antibody (Sigma).

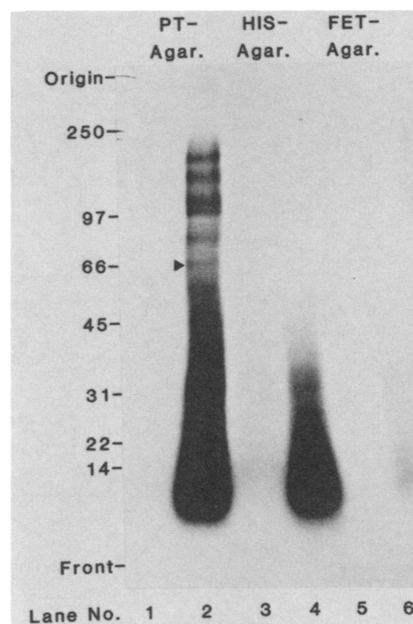

FIG. 1. SDS-PAGE analysis of PT receptors obtained from human T lymphocytes by affinity chromatography. The sample preparation, SDS-PAGE, and autoradiography techniques are described in Materials and Methods. Lanes: 1, 3, and 5, material derived from the final 0.5-ml wash fraction from each of the affinity columns; 2, 4, and 6, proteins from the diethanolamine fractions. The arrowhead indicates a protein with a relative mobility similar to that of the 70,000-Da species detected by the photoaffinity labeling technique. The positions to which the protein standards migrated are indicated by their molecular weights (in thousands) on the left.

RESULTS

Identification of PT receptors in T lymphocytes by affinity isolation. The results of experiments designed to identify PT receptors in T lymphocytes by affinity chromatography are displayed in Fig. 1. In these experiments at least six well-resolved bands, migrating in the 45,000- to 250,000-molecular-weight range, were found to bind to PT-agarose but not to agarose containing covalently bound histone or fetuin. Therefore, these six bands are potential lymphocyte receptors for PT. As discussed in our previous report (29), the diffuse material observed in the diethanolamine fractions obtained from both PT and histone, but not fetuin-agarose, probably represents anionic membrane components interacting nonspecifically with polycationic proteins such as histone or the B oligomer of PT but not interacting with anionic proteins such as fetuin (25).

Identification of Jurkat cell receptors by using ^{125}I-ASD-PT.

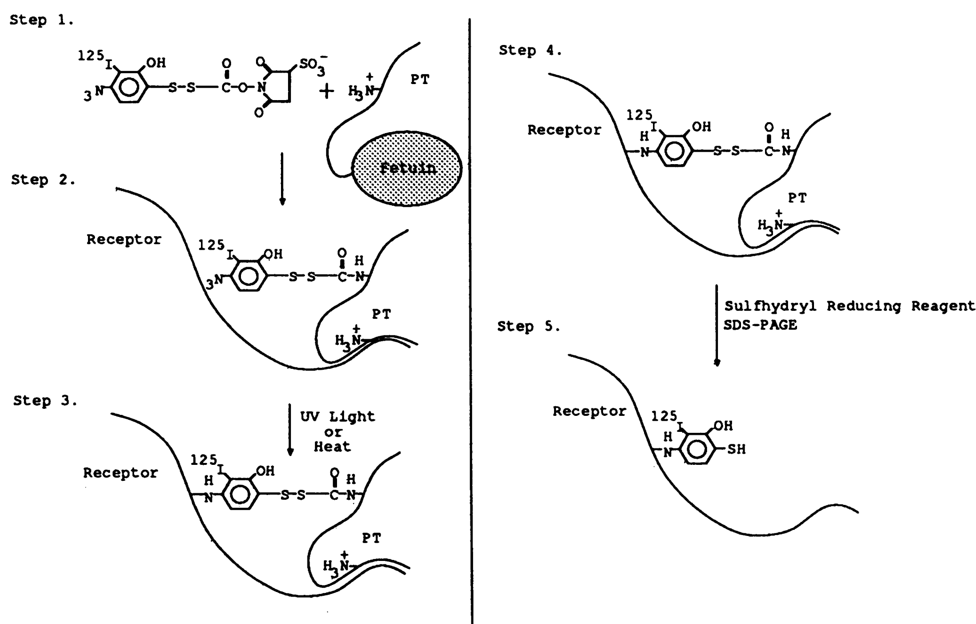

FIG. 2. Schematic illustration of the photoaffinity labeling technique. Step 1, Covalent attachment of ^{125}I-SASD to free amino groups of PT at alkaline pH; step 2, noncovalent interaction of ^{125}I-ASD-PT with receptors in the dark; step 3, covalent attachment of the photoactivated azido group of ^{125}I-ASD-PT to the PT receptor; steps 4 and 5, reduction of the disulfide bond of ^{125}I-ASD-PT and resolution of PT from the labeled receptor during SDS-PAGE analysis.

The next set of experiments used the ^{125}I-labeled photoaffinity labeling reagent, SASD, to investigate PT receptors in the Jurkat T-lymphocyte cell line. SASD has been used to identify lipopolysaccharide-specific binding proteins in lymphoid cells (11, 12, 31). The technique is most useful for investigating receptors for large or aggregated ligands because the resulting cross-linked complexes can be dissociated in the presence of sulfhydryl reducing reagents (Fig. 2). This leaves the ^{125}I-labeled probe attached to the receptor and facilitates its subsequent identification by SDS-PAGE.

When fetuin-agarose-bound PT was reacted with ^{125}I-SASD, the amount of protein recovered in the diethanolamine fractions ranged from 23 to 40% of the total PT (10 μg) applied. The average amount of ^{125}I-ASD-labeled PT obtained in 0.4 ml was 4.0 ± 3.3 μg (n = 8) as determined by the Bradford dye-binding assay (5) or 2.3 ± 1.5 μg (n = 8) as determined by agglutination of trypsinized goose erythrocytes (4). The difference between the amount of PT recovered as determined by the two assays was not significant (P > 0.2), and therefore it was apparent that all of the PT recovered from fetuin-agarose retained its ability to agglutinate goose erythrocytes. Otherwise, the goose hemagglutination assay would have indicated that significantly less PT was recovered in the diethanolamine fractions.

Incorporation of radioactive label into TCA-precipitable ^{125}I-ASD-PT averaged 84.4 ± 9.2% (n = 8) in the absence of 2-mercaptoethanol and 9.5 ± 6.9% in the presence of sulfhydryl reducing reagent. This observation was consistent with the incorporation of approximately 90% of the ^{125}I into the ASD portion of the derivatized PT. SDS-PAGE analysis of ^{125}I-ASD-PT in the absence of reducing reagent (Fig. 3) indicated that all of the PT subunits were labeled, though ^{125}I-ASD was incorporated preferentially into subunit S2. The low amount of label incorporated into the S1 subunit reflects the absence of lysine groups in this molecule. The mean specific activity of the eight ^{125}I-ASD-PT preparations was $3.3 \times 10^6 \pm 1.3 \times 10^6$ cpm/μg of PT and represented an average efficiency of labeling of roughly 15% (range 9 to 42%), assuming one molecule of radioactive iodine per ASD.

Noncovalently attached PT bound tightly enough to the surface of Jurkat cells that it was not removed by washing with PBS. Therefore, it was not possible to determine how much of the noncovalently bound ^{125}I-ASD-PT was converted to covalently bound ^{125}I-ASD-PT by exposure to UV

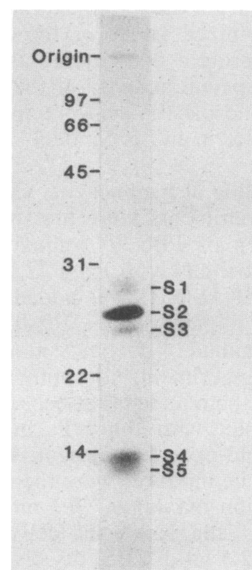

FIG. 3. PT subunits labeled with ^{125}I-ASD. ^{125}I-ASD-PT was analyzed by SDS-PAGE in the absence of sulfhydryl reducing reagent by using a 16% separating gel and prepared for autoradiography as described in Materials and Methods. PT subunits S1 to S5 derivatized with ^{125}I-ASD migrated to the positions indicated. The positions to which the protein standards migrated are indicated by their molecular weights (in thousands) on the left.

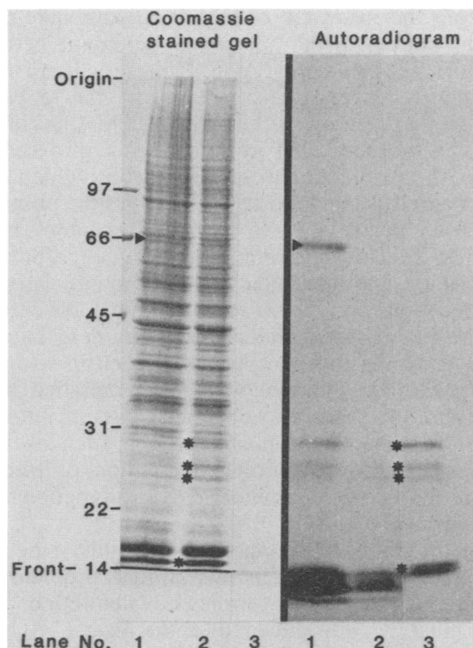

FIG. 4. SDS-PAGE analysis of photoaffinity-labeled Jurkat cells. Photoaffinity-labeled Jurkat cells were analyzed by SDS-PAGE in the presence of 2-mercaptoethanol, using 10% separating gels. (A) Coomassie-stained gel; (B) corresponding autoradiogram. ^{125}I-ASD-PT was used to photoaffinity label Jurkat cells in the absence (lanes 1) and presence (lanes 2) of excess unmodified PT. An amount of ^{125}I-ASD-PT equivalent to that in lanes 1 but exposed to UV light in the absence of Jurkat cells was applied to lanes 3. Proteins comigrating with the PT subunits are indicated (*).

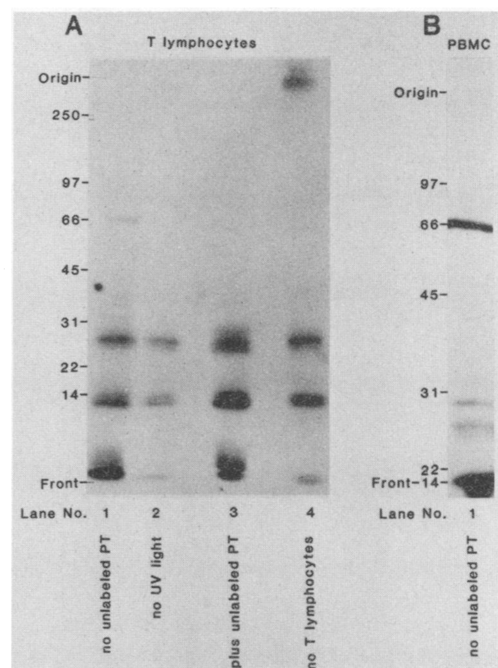

FIG. 5. SDS-PAGE analysis of photoaffinity-labeled T lymphocytes and PBMC. (A) Autoradiogram of 7.5 to 15% linear acrylamide separating gel. T lymphocytes were affinity labeled with ^{125}I-ASD-PT in the absence (lane 1) or presence (lane 3) of a 1,000-fold molar excess of unmodified PT. (B) Autoradiogram of a 10% acrylamide separating gel. PBMC were affinity labeled with ^{125}I-ASD-PT.

light because neither of them could be removed from the cells by nondestructive methods.

When ^{125}I-ASD-PT-labeled Jurkat cells were analyzed by SDS-PAGE in the presence of 2-mercaptoethanol (Fig. 4B, lane 1), a protein band that had a calculated M_r of 70,000 ± 2,400 (n = 4) was observed. This protein was not detected when ^{125}I-ASD-PT was irradiated with UV light in the absence of Jurkat cells (Fig. 4B, lane 3), nor was it detected in unirradiated ^{125}I-ASD-PT–Jurkat cell complexes (data not shown). Only lower-molecular-weight proteins were detected in the absence of Jurkat cells. These proteins comigrated with the five PT subunits (subunits S4 and S5 were not resolved in this SDS-PAGE system) observed in the Coomassie-stained gel (Fig. 4A, lane 2) and probably represent the products of endogenous subunit-subunit labeling or subunits that were directly iodinated because of residual Na^{125}I in the original ^{125}I-SASD preparation.

Some labeled material was detected at the dye front of the gels (Fig. 4B). This material was also observed when ^{125}I-ASD-PT-labeled Jurkat cells were analyzed on 16% gels (data not shown). The low-molecular-weight material probably represents free ^{125}I-ASD that was not covalently coupled to receptors. However, it is possible that the low-molecular-weight material represents small peptides or lipids which (as discussed below) may be part of a receptor complex.

The amount of ^{125}I-ASD that was actually incorporated into the 70,000-Da protein after UV irradiation of ^{125}I-ASD-PT-labeled Jurkat cells was less than 1% of the total label added to the reaction mixtures. This was determined by counting the radioactivity in the 70,000-Da receptor bands that were cut from the dried gels. Nonetheless, when the procedure was carried out in the presence of 1,000-fold-excess unmodified PT (Fig. 4B, lane 2), the amount of label transferred to the 70,000-Da receptor was reduced by 75%, demonstrating that unmodified PT was able to compete for receptor labeling with ^{125}I-ASD-modified PT and suggesting that the two ligands (ASD-PT and unmodified PT) were binding to the same receptor species. The reduction of incorporation of label into receptor was determined by comparing the radioactivity in receptor bands that were cut from dried gels and by scanning the autoradiograms in a densitometer. The two procedures gave the same results.

Identification of T-lymphocyte receptors for PT by photoaffinity labeling. A 70,000-Da band was also observed when enriched human peripheral blood T lymphocytes (about 92% T lymphocytes) were probed with ^{125}I-ASD-PT in the absence of unmodified PT (Fig. 5A, lane 1). In addition, although some labeling of the PT subunits was seen, the amount of label incorporated into the 70,000-molecular-weight T-lymphocyte band was greatly reduced (77%) in the presence of unmodified PT (Fig. 5A, lane 3) or (as in the Jurkat cell system discussed earlier) in T lymphocytes that were not exposed to UV light before analysis by SDS-PAGE (Fig. 5A, lane 2). Again, this observation is consistent with the photoinduced formation of covalent linkages between ^{125}I-ASD-PT and a 70,000-molecular-weight receptor in T lymphocytes.

It concerned us that the ^{125}I-ASD-PT-labeled lymphocyte band had approximately the same relative mobility on SDS-gels as fetuin and HSA or BSA. To address the possibility that the 70,000-molecular-weight protein was a contaminant derived from serum, we probed immunoblots to determine

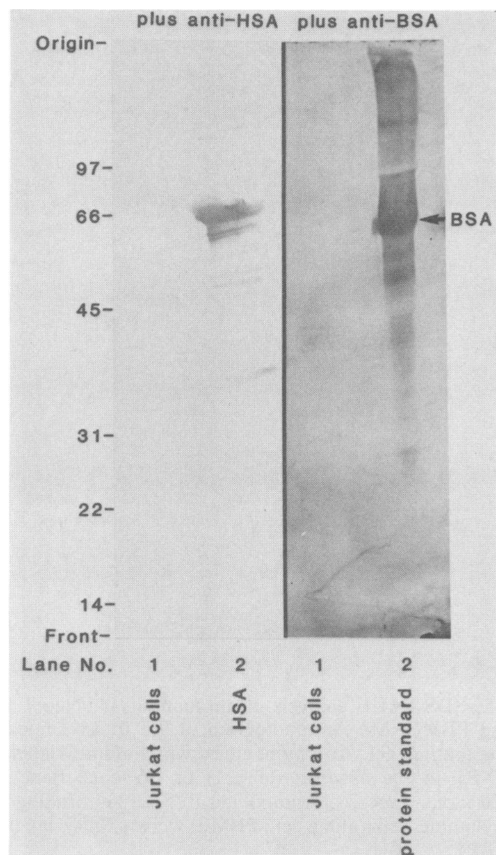

FIG. 6. Western immunoblot analysis of Jurkat cells, using anti-BSA and anti-HSA probes. Solubilized Jurkat cell proteins from 5 × 10^5 Jurkat cells, purified HSA, and protein standard containing BSA were analyzed by SDS-PAGE on 12.5% separating gels, electrophoretically transferred to nitrocellulose, and probed with anti-BSA or anti-HSA as described in Materials and Methods.

whether the PBS-washed Jurkat cells contained either BSA or HSA. Neither BSA nor HSA was detected in Jurkat cells that were probed with anti-BSA or anti-HSA (Fig. 6). Moreover, the 70,000-Da band was also apparent in human ^{125}I-ASD-PT-labeled PBMC (approximately 70% of which represented T lymphocytes) that had not been exposed to FBS during isolation (Fig. 5B). Labeling in the PBMC system was also specific, as determined by including unmodified PT in the reactions (which reduced labeling by 82%) or by not exposing the ^{125}I-ASD-PT-PBMC to UV light (data not shown). Therefore, it is also unlikely that the 70,000-Da protein detected by photoaffinity labeling was fetuin that would have been present in FBS used in the preparation of both Jurkat cells and T lymphocytes.

DISCUSSION

Although all of the lymphocyte components observed in the molecular weight range from 45,000 to 250,000 in Fig. 1 (lane 2) appeared to bind specifically to PT-agarose, the results of the affinity isolation experiments must be interpreted with caution. For example, the assumption that all T-lymphocyte proteins capable of binding to PT are efficiently labeled with iodine may not be correct; consequently, important receptor components that may not be iodinated could easily be overlooked. The procedure also suffers from the necessity of having to solubilize the iodinated lymphocyte membranes with detergent before performing affinity chromatography. This procedure may expose cryptic receptors that might never bind to PT in the native state. Alternatively, it is possible that important receptors fail to bind to PT in the presence of detergent.

Many of these potential problems may be avoided by using the photoaffinity labeling technique. With photoaffinity probes, it is possible to investigate receptors in intact membranes. This may prevent inappropriate (cryptic) receptors from being identified and avoids the potential problem of loss of receptor activity in the presence of detergents. Moreover, the lack of chemical specificity demonstrated by the azido group of the photoprobe (22) ensures maximum reactivity with receptors to which the modified ligand is bound. Therefore, the procedure also circumvents the requirement for specific chemical groups such as tyrosine residues in the case of surface iodination or free amino groups in the case of aminoreactive bifunctional cross-linking reagents.

Despite the many advantages, photoaffinity labeling procedures are not without their own unique problems. Since chemical groups capable of forming covalent bonds with the receptor must be introduced into the ligand, it becomes necessary to demonstrate that the chemically modified ligand still recognizes the same receptors as does the unmodified ligand. This is usually accomplished by demonstrating that the amount of affinity-labeled product is reduced when most of the available receptor-binding sites are occupied by the unmodified ligand (1, 17, 19).

Specific activity calculations indicated that the incorporation of ^{125}I-SASD into PT ranged from a low of 0.09 to a high of 0.42 ^{125}I-ASD molecule per toxin molecule. The average incorporation (0.15 ^{125}I-ASD-PT) was about three times lower than that reported for ^{125}I-ASD-lipopolysaccharide preparations (31). Nonetheless, although the efficiency of PT labeling was low, underivatized PT was able to compete for transfer of ^{125}I-ASD to the 70,000-molecular-weight receptor protein in Jurkat cells, T lymphocytes, and PBMC. The apparent differences between experiments in the efficiency of the photoaffinity labeling reaction are a reflection of the amount of ^{125}I-SASD incorporated into PT and hence the amount of ^{125}I-ASD that was available for transfer to the 70,000-molecular-weight receptor. This finding clearly demonstrates that ^{125}I-ASD-PT and underivatized PT bind to the same receptors of the surfaces of T-lymphocyte and Jurkat cells.

When ^{125}I-ASD-PT was used, only one PT receptor was identified in T lymphocytes that was not observed when the reaction was performed in the presence of unmodified PT. Nonetheless, it is possible that the 70,000-molecular-weight protein is not directly involved in delivering the mitogenic signal to the cell. The 70,000-molecular-weight receptor may, in fact, be only one part of a more complex multisubunit receptor system. Other signal transduction components may not have been labeled because, for one reason or another, they were not oriented correctly for attachment of the ^{125}I-ASD probe. Some of these receptor subunits might be represented by the additional proteins detected by the affinity isolation procedure (Fig. 1). The observation that one of the protein bands that bound specifically to PT-agarose in the affinity isolation experiment had a molecular weight of approximately 70,000 (arrowhead in Fig. 1) is consistent with the idea of an oligomeric PT receptor complex containing a 70,000-molecular-weight subunit, identified by affinity chromatography and photoaffinity labeling, and additional sub-

units that were detected only by affinity isolation. The inability, because of geometrical constraints, to identify all subunits in an oligomeric PT receptor system represents a potential limitation of the photoaffinity labeling procedure. The use of photoaffinity labeling reagents with variable spacing between the functional end groups could be useful in resolving this issue.

It was interesting that ^{125}I-SASD was incorporated preferentially into lysine groups of the S2 subunit of PT (Fig. 3) despite the fact that a computer program for predicting secondary structure of proteins indicated that the same number of lysine residues should be exposed on the surfaces of subunits S2, S3, and S4. Previously, Nogimori et al. (16) presented data suggesting that lysine groups in PT dimer 2 (composed of subunits S3 and S4) were important for the ability of PT to bind to receptors on mouse lymphocytes. More recent evidence (30) suggests that dimer 1 (composed of subunits S2 and S4) is more important than dimer 2 for PT binding to sialoglycoproteins such as fetuin and goose erythrocyte receptors. According to the data of Nogimori et al., the goose hemagglutination activity of PT was not altered by modification of lysine groups in dimer 1. Our data are consistent with those of Nogimori et al. because SASD modification of lysines in subunit S2 did not appear to affect the ability of ^{125}I-ASD-PT to agglutinate goose erythrocytes. However, it was apparent from the data in Fig. 3 that free amino groups in subunit S2 were extensively modified by ^{125}I-SASD even though the coupling reaction was performed while PT was bound to fetuin-agarose. This finding suggests that lysines on subunit S2 that were labeled by ^{125}I-SASD are not located close to the fetuin or goose erythrocyte receptor-binding site in dimer 1 because if they were, fetuin should have prevented them from becoming labeled. In light of the data of Nogimori et al. (16) suggesting that lysine groups in subunits S3 and S4 may be important for the interaction of PT with lymphocyte receptors, it is possible that the relative resistance of lysines in subunits S3 and S4 to modification by the SASD reagent was responsible for the sparing of the ability of ^{125}I-ASD-PT to bind to receptors in T lymphocytes.

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