Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 7552–7556, July 1996
Biochemistry

Binding of thalidomide to α_1-acid glycoprotein may be involved in its inhibition of tumor necrosis factor α production

(immunosuppression/photoaffinity label/monocytes)

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Communicated by Christopher T. Walsh, Harvard Medical School, Boston, MA, April 11, 1996 (received for review January 31, 1996)

ABSTRACT **In addition to its well known sedative and teratogenic effects, thalidomide also possesses potent immunomodulatory and antiinflammatory activities, being most effective against leprosy and chronic graft-versus-host disease. The immunomodulatory activity of thalidomide has been ascribed to the selective inhibition of tumor necrosis factor α from monocytes. The molecular mechanism for the immunomodulatory effect of thalidomide remains unknown. To elucidate this mechanism, we synthesized an active photoaffinity label of thalidomide as a probe to identify the molecular target of the drug. Using the probe, we specifically labeled a pair of proteins of 43–45 kDa with high acidity from bovine thymus extract. Purification of these proteins and partial peptide sequence determination revealed them to be α_1-acid glycoprotein (AGP). We show that the binding of thalidomide photoaffinity label to authentic human AGP is competed with both thalidomide and the nonradioactive photoaffinity label at concentrations comparable to those required for inhibition of production of tumor necrosis factor α from human monocytes, suggesting that AGP may be involved in the immunomodulatory activity of thalidomide.**

Thalidomide has a relatively simple chemical architecture (Fig. 1), but exhibits a multitude of physiological activities on mammals. In addition to its sedative and teratogenic effects (1, 2), thalidomide possesses significant and unique immunomodulatory and antiinflammatory activities. It has been found to be effective against several immune disorders, including chronic graft-versus-host disease (3), oral and orpharygal ulcers associated with HIV infection (4), erythema nodosum leprosum in leprosy (5), and several other inflammatory skin diseases (6). Most recently, thalidomide was found to have antiangiogenic activity (7), raising the possibility that it may be used to treat certain types of angiogenesis-dependent diseases such as diabetic retinopathy and cancer.

The immunomodulatory effect and the related antiinflammatory effect of thalidomide have attracted much attention in recent years. In searching for the cellular targets of thalidomide, several models of immune regulation have been examined. It was reported that the calcium-dependent lymphocyte proliferation in response to mitogen and alloantigens is inhibited by thalidomide (8). D. Neubert and coworkers (9) uncovered several cell surface molecules whose expression is either enhanced or inhibited by the drug *in vivo*. Kaplan and colleagues (10, 11) found that thalidomide selectively inhibits the production of tumor necrosis factor α (TNF-α) from stimulated human monocytes both *in vitro* and *in vivo*, providing a possible mechanism for both the immunomodulatory and the antiinflammatory activities of the drug. This TNF-α inhibitory effect has been ascribed to enhanced mRNA degradation by the drug (12). Blockade of TNF-α production seems to underlie the inhibition of replication of the type 1 HIV in human monocytes by thalidomide (13). Further delineation of the inhibitory mechanism has been hampered, in large part, by the relatively low potency of the drug with an IC_{50} between 10 and 100 μM for TNF-α inhibition (12, 13).

In an effort to elucidate the molecular mechanism of immunomodulation by thalidomide in general and the molecular target responsible for its inhibition of TNF-α in particular, we have synthesized an active photoaffinity label derived from the drug. Using this photoaffinity label, we detected a pair of proteins that specifically bind to thalidomide. These thalidomide-binding proteins have been identified as isoforms of α_1-acid glycoprotein (AGP), indicating a potential role for AGP as a mediator of the drug's immunomodulatory effect.

MATERIALS AND METHODS

Materials. Fresh calf thymi were obtained from Research 87 (Medford, MA). Human AGP and *Clostridium perfringens* neuraminidase were from Sigma. Mouse anti-human TNF-α monoclonal antibodies used for ELISA were from PharMingen. Carboxymethyl Sepharose was from Pharmacia. Cbz-glutamine was purchased from Bachem. ^{125}I-labeled and unlabeled azidoiodophenylpropionic acid succinimidyl ester were purchased from A. Ruoho (University of Wisconsin School of Medicine, Madison, WI). NMR solvents were from Cambridge Isotope Laboratories (Cambridge, MA). Propranolol and alprenolol were from Research Biochemicals International. All other chemicals were purchased from Aldrich.

Synthesis of Thalidomide Analogs. Thalidomide and most of its analogs were synthesized according to procedures reported previously (Fig. 1). All compounds except the photoaffinity label were characterized by ^{1}H NMR and mass spectrometry.

Synthesis of Thalidomide Photoaffinity Label [^{125}I]AIPPOT 9. All procedures were performed in the absence of direct light. [^{125}I]Azidoiodophenylpropionic acid succinimidyl ester (120 μCi in 120 μl of ethyl acetate) was concentrated under a stream of nitrogen to a volume of less than 5 μl. A suspension of 1 mg of 3-(aminoethoxy)thalidomide in 100 μl of ethyl acetate/0.5% triethylamine was added. The reaction mixture was left overnight at room temperature in a closed container. The product was purified by silica gel chromatography. Fractions were concentrated under a stream of nitrogen to <5 μl, and then the product was resuspended in 100 μl of methanol and stored at −80°C.

Monocyte Isolation and TNF-α Secretion Assay. Peripheral blood mononuclear cells were isolated from heparinized blood by centrifugation on Ficoll-Paque Plus (Pharmacia) step gradients. Cells were washed three times in cell culture medium (RPMI 1640 medium). A monocyte-enriched fraction was isolated by suspending the cells to 2.5×10^6/ml in complete

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Abbreviations: AGP, α_1-acid glycoprotein; AIPPOT, 3-(2-(3-azido-4-iodophenylpropionamido)ethoxy)thalidomide; DMSO, dimethyl sulfoxide; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor α.

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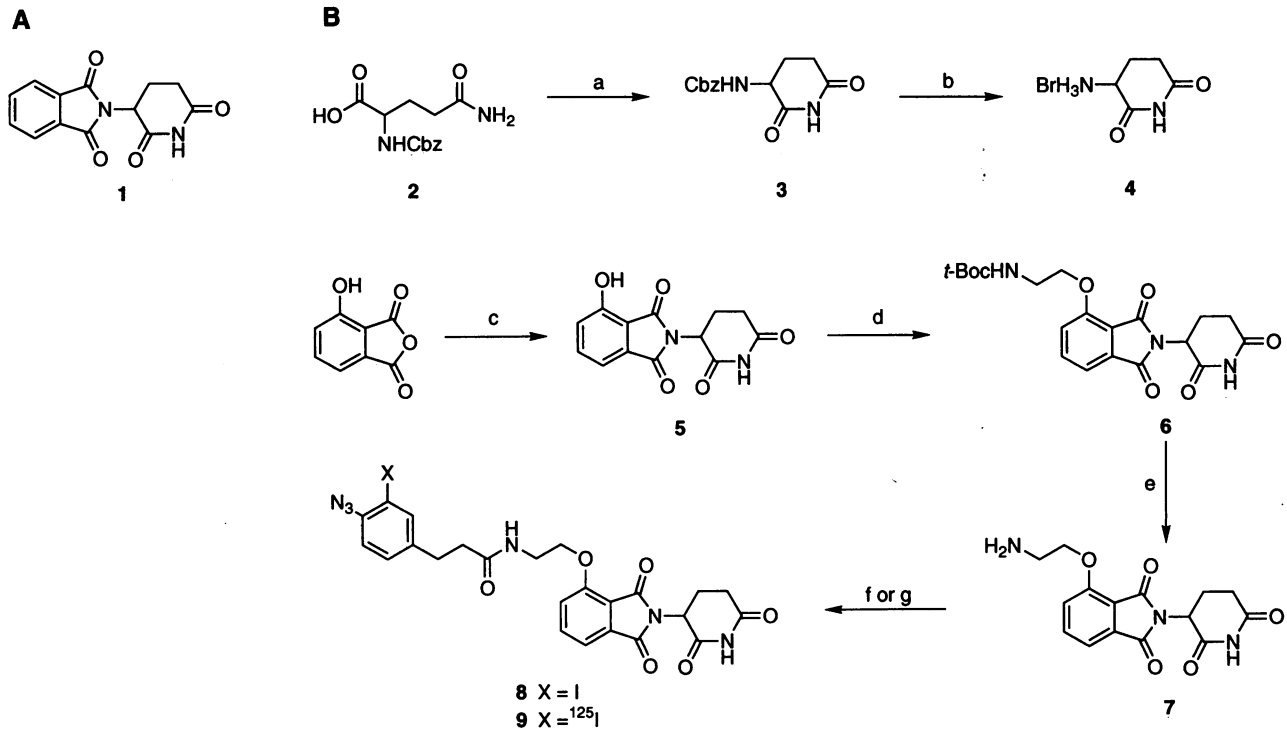

FIG. 1. (*A*) Structure of thalidomide (**1**). (*B*) Preparation of thalidomide photoaffinity labels 3-(2-(3-azido-4-iodophenylpropionamido)-ethoxy)thalidomide (AIPPOT) (**8**) and [^{125}I]AIPPOT (**9**). Reaction conditions: (a) $SOCl_2$, 1 equivalent in dimethylformamide, 2 hr, −5°C. (b) 30% HBr/HOAc, 80 min, room temperature (RT). (c) Compound **4**, 1 equivalent, and triethylamine, 1 equivalent, in dimethylformamide, 24 hr at 60°C, 1 equivalent dicyclohexylcarbodiimide and catalytic dimethylaminopyridine added, continued at 60°C for 72 hr. (d) *N*-(*t*-Butoxycarbonyl)-aminoethyl bromide, 5 equivalents, and tetraethylammonium fluoride, 1 equivalent, 72 hr, RT. (e) 25% trifluoroacetic acid/CH_2Cl_2, 30 min, RT. (f) Azidoiodophenylpropionic acid succinimidyl ester, 1 equivalent, triethylamine, 2 equivalents in EtOAc, 40 hr, RT. (g) [^{125}I]Azidoiodophenyl-propionic acid succinimidyl ester (carrier free) 100 μCi (1 Ci = 37 GBq) and 1 mg amine **7** in 0.5% Et_3N/EtOAc, 16 hr, RT.

medium (RPMI 1640 medium plus Hepes with 5% human AB serum, 50 units/ml penicillin, 50 μg/ml streptomycin, and 2.5 mM L-glutamine) and allowing cells to adhere to plastic tissue culture plates in a humidified atmosphere of 5% CO_2 at 37°C for 3 hr. Nonadherent cells were removed by washing with serum-free medium, and adherent cells were detached by scraping and washed twice with serum-free medium. To assay for TNF-α production, cells were resuspended in complete medium to 10^6 cells/0.9 ml and plated at 0.45 ml per well in 24-well plates. Cells were allowed to adhere for 2 hr at 37°C before drugs or analogs dissolved in dimethyl sulfoxide (DMSO) were added with a final concentration of 0.2% DMSO. This was followed by addition of lipopolysaccharide (LPS) (in 50 μl of complete medium) to a final concentration of 1 μg/ml. Plates were incubated for 16 hr in a humidified incubator (37°C/5% CO_2). Drug treatment did not significantly affect cell viability compared with DMSO controls, as judged by trypan blue exclusion. Culture supernatants were collected by centrifugation at 1300 × *g* for 6 min at 4°C. TNF-α was assayed by ELISA using mouse anti-human TNF-α monoclonal antibodies from PharMingen according to the supplier's instructions. The levels of TNF-α were derived from a second order polynomial standard curve ($r^2 > 0.995$ for each determination).

Preparation of Tissue Extracts. Freshly prepared bovine thymus tissue was shipped on ice and used the same day. After connective tissue was removed, the organs were cut into small pieces and frozen in liquid nitrogen. Frozen chunks were weighed, powdered, and then suspended in cytosol buffer (20 mM Tris·HCl, pH 6.9/100 mM NaCl/2 mM 2-mercaptoethanol/0.02% NaN_3/5% glycerol/1 mM phenylmethylsulfonyl fluoride), 1 ml/g of tissue, in a blender. Suspensions were Dounce homogenized with 10 up-and-down strokes and centrifuged at 9000 × *g* to remove most of the debris. The supernatant was centrifuged at 100,000 × *g*, and the S100 fraction was stored in aliquots at −80°C until needed.

Photoaffinity Labeling. Crude or partially purified tissue extract containing 5–20 μg of total protein or 0.1 μg of purified human AGP (Sigma) was diluted in labeling buffer (20 mM Tris·HCl, pH 7.0/50 mM NaCl/1 mM $MgCl_2$/1 mM $CaCl_2$/0.5 mM EDTA) to a final volume of 45 μl. Drug or DMSO (0.5% volume) was added followed by incubation at 37°C for 30 min. Photoaffinity label (≈1 μCi/μl) was diluted 5-fold in labeling buffer in bulk immediately before use, and 5 μl was added to each labeling tube in the absence of direct light. Samples were mixed and incubated at 37°C for 30 min in the dark and then irradiated in a Stratalinker (Stratagene) at 254 nm with a total energy output of 3.3 J/m^2. For one-dimensional SDS/PAGE, reactions were quenched by adding 3.5 μl of 2-mercaptoethanol followed by 18 μl of 4× SDS/PAGE sample buffer (250 mM Tris·HCl, pH 6.8/40% glycerol/5% SDS/0.1 mg/ml bromophenol blue). Samples were heated in a boiling water bath for 3 min before they were subjected to SDS/PAGE. For two-dimensional electrophoresis, reactions were quenched by adding 5 μl of 10% SDS/150 mM dithiothreitol. Samples were heated as above and cooled, and 5 μl of urea solution [65 mM dithiothreitol/4% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)/9 M urea/5% Bio-Rad Bio-Lyte 3–10 ampholyte] was added. Samples were loaded onto isoelectric focusing gels (4.5% acrylamide/0.4% bis-acrylamide/9.2 M urea/1% Bio-Lyte 5–7 ampholyte/4% Biolyte 3–10 ampholyte/1.5% CHAPS/0.5% Nonidet P-40, 14 cm × 2.5 mm) and run for 12 hr at 400 V followed by 2 hr at 1000 V in a Bio-Rad Protean II apparatus with 6 mM phosphoric acid in the lower chamber and 20 mM sodium hydroxide in the upper chamber. The SDS/PAGE dimension was 12% acrylamide/0.32% bis-acrylamide. Gels were stained with Coomassie blue, dried onto blotting paper, and exposed

to x-ray film (Kodak XAR 5) or to PhosphorImager cassettes (Molecular Dynamics). Labeling was quantified using IMAGEQUANT software.

Protein Purification. All procedures were carried out at 4°C. Ammonium sulfate (7.36 g, 61%) was added to 20 ml of bovine thymus S100 fraction (9.2 mg/ml protein) with mixing. The mixture was incubated on a rotator for 16 hr followed by centrifugation at 12,000 × *g* for 15 min. Ammonium sulfate (4.20 g, 92%) was added to the supernatant and rotated for 8 hr. The mixture was centrifuged as before. The pellet was resuspended in 2 ml of buffer containing 20 mM Tris·HCl (pH 6.8) and 100 mM NaCl, and dialyzed into 20 mM sodium acetate, pH 4.1/20 mM NaCl overnight. The resulting suspension was centrifuged at 16,000 × *g* for 10 min to remove the precipitate, and the supernatant was applied to a carboxymethyl Sepharose column. The column was washed with the dialysis buffer. The flow-through fractions were pooled and dialyzed into 50 mM ammonium carbonate for 24 hr with one change of buffer. The sample was lyophilized overnight and resuspended in 20 μl of H_2O.

Neuraminidase Digestion and Protein Sequencing. The purified thalidomide-binding proteins (10 μl), 17 μl 0.1 M sodium acetate (pH 5.5), and 3 μl of *C. perfringens* neuraminidase (Sigma Type V, 1 milliunits/μl) were mixed and incubated at 37°C for 5 hr. To the mixture were added 2.5 μl of 2-mercaptoethanol and 10 μl of 4× SDS sample buffer. The sample was heated in a boiling water bath for 3 min and subjected to SDS/PAGE as above. The proteins were then blotted onto poly(vinylidene difluoride) membrane. The blot was stained with Ponceau S and destained briefly in doubly distilled water before the band corresponding to the thalidomide-binding protein was sliced out. The membrane was submitted for proteolytic digestion with either Lys-C or chymotrypsin. Peptide fragments were separated by HPLC and sequenced by Edman degradation at the Harvard Microchemistry Facility and the Massachusetts Institute of Technology Biopolymer Laboratory.

RESULTS

The relatively low potency of thalidomide as an inhibitor of TNF-α production suggests that thalidomide may have a low affinity for its target, making it difficult to employ conventional affinity chromatography to identify and purify the potential target proteins. We therefore resorted to the more sensitive method of photoaffinity labeling to identify thalidomide-binding proteins. To make an active thalidomide photoaffinity label, we needed to define a position in thalidomide to which a radioactive photocrosslinking moiety could be attached without significantly abrogating its activity. A survey of the literature indicated that C-3 of the phthalimide group may be such a position as it has been shown that 3-hydroxythalidomide is active in animal models for both graft-versus-host disease (14) and embryopathy (15). We synthesized a nonradioactive photoaffinity label of thalidomide, AIPPOT, by attaching a 4-azido-3-iodophenylpropionyl group onto 3-hydroxythalidomide via an ethanolamine linker (Fig. 1). When the activity of the nonradioactive photoaffinity label was determined, it was found to be more active than thalidomide itself (Fig. 2). While thalidomide inhibits TNF-α production with an IC_{50} of over 200 μM, its solubility limit in aqueous medium, the attachment of the side chain onto the 3-hydroxy group in the photoaffinity label resulted in an increase in its potency, with an IC_{50} of less than 100 μM.

We subsequently synthesized the radioactive photoaffinity label containing ^{125}I (Fig. 1) and used it to identify potential thalidomide-binding proteins in membrane and soluble extracts prepared from fresh bovine thymus, an abundant source of proteins from lymphoid cells. Although no specific proteins were detected in the membrane fraction (data not shown),

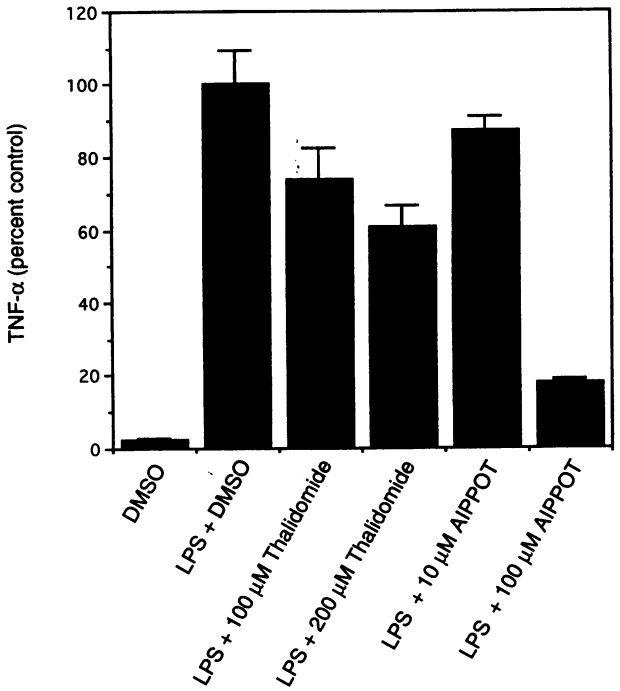

FIG. 2. Effect of thalidomide and AIPPOT on the secretion of TNF-α by LPS-stimulated human peripheral blood monocytes. Monocytes were treated with 1 μg/ml LPS for 16 hr in the presence of varying concentrations of drug or carrier alone (0.2% DMSO). The amount of TNF-α was determined in culture supernatants by ELISA. Results are expressed as the percentage of TNF-α produced compared with DMSO control and are the average of two experiments with separate donors each performed in triplicate.

treatment of the soluble extract with [^{125}I]AIPPOT followed by irradiation at 254 nm resulted in specific labeling of two proteins with apparent molecular mass of *ca.* 45 and 43 kDa (Fig. 3). This labeling can be competed with unmodified thalidomide at concentrations comparable to those required for TNF-α inhibition in the cellular assay (Figs. 2 and 3).

To find an optimal procedure for isolating the putative thalidomide-binding proteins, we determined, among other physical properties, their isoelectric points. Two-dimensional gel electrophoresis of labeled thymus extract revealed the putative thalidomide-binding proteins to be extremely acidic with isoelectric points of *ca.* 3.5 (Fig. 4). Moreover, the two labeled protein bands on one-dimensional SDS/PAGE were resolved into a series of spots in the isoelectric focusing dimension. The unusually high acidity of the putative thalidomide-binding proteins led us to search in the literature for proteins with similar isoelectric points. Indeed, we found that AGP, also called orosomucoid, was strikingly similar to the putative thalidomide-binding proteins on a two-dimensional gel electrophoresis map with few other proteins in its neighborhood (16). This finding helped to simplify the isolation and identification of the thalidomide-binding proteins.

Using the established purification procedures for AGP as a guide and the thalidomide photoaffinity labeling as an assay, we purified the thalidomide-binding proteins to near homogeneity in two steps. Starting with crude bovine thymus extract, the thalidomide-binding activity was concentrated in 61–92% ammonium sulfate precipitate (17). After dialysis into acidic sodium acetate buffer, the fraction containing thalidomide-binding protein was subjected to weak cation exchange carboxymethyl Sepharose chromatography (18). The thalidomide-binding proteins were present in the flow-through fraction at pH 4.1, which was virtually free of other proteins (Fig. 5, lane 1). The N-termini of the proteins thus purified were blocked, in agreement with the known N-terminal pyrogluta-

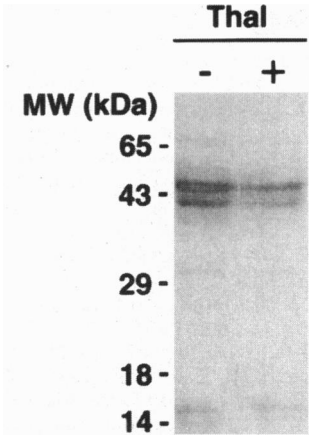

FIG. 3. Labeling of bovine thymus S100 fraction with [^{125}I]AIPPOT. Soluble bovine thymus extracts were preincubated with 200 μM cold competitor thalidomide (+) or DMSO (−) carrier (0.5%) for 30 min before the addition of 1 μCi [^{125}I]AIPPOT in the absence of direct light. After 30 min, samples were irradiated, quenched with 2-mercaptoethanol, and subjected to SDS/PAGE.

mate moiety of AGP (19). Since AGP is also known to have a high sialic acid content, we treated the purified thalidomide-binding proteins with neuraminidase. Upon neuraminidase treatment, the two thalidomide-binding protein bands were converted into a single protein band, suggesting that the two bands originated from the same protein with differential sialylation. Removal of the sialic acids also allowed for the subsequent proteolysis of the proteins and sequencing of the proteolytic fragments. The sequences of four proteolytic fragments were determined. All showed high homology with human AGP (Fig. 6). Although the bovine AGP has not been cloned, the four peptides sequences are 75% identical and 93% similar to human AGP (20). By comparison, rabbit AGP only bears 59% identity and 74% similarity to human AGP in the same regions, suggesting that the thalidomide-binding proteins in bovine thymus extract are isoforms of the bovine AGP.

To confirm that AGP binds to thalidomide, we labeled commercially available AGP from several species. The labeling of human AGP with thalidomide photoaffinity label is shown in Fig. 7A. Indeed, [^{125}I]AIPPOT labels human AGP and this labeling is competed by both thalidomide and the nonradioactive AIPPOT, with the latter being more potent. The potencies of thalidomide and AIPPOT are in general agreement with the relative potency of these two compounds in the TNF-α secretion assay (Fig. 2).

AGP in the plasma is known to bind to a large number of basic and occasionally neutral drugs and exert important

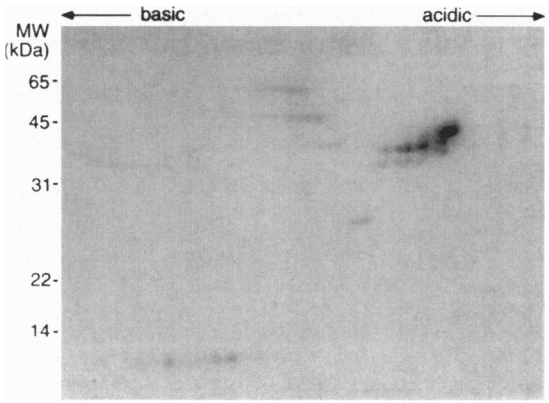

FIG. 4. Two-dimensional gel electrophoresis of [^{125}I]AIPPOT-labeled bovine thymus extract.

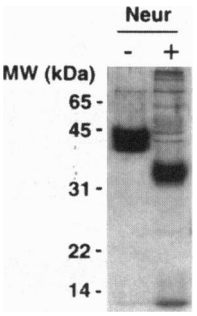

FIG. 5. Purified thalidomide-binding proteins. Proteins were purified as described in the *Results* section, and aliquots were incubated in 30 μl of digestion buffer in the presence or absence of 3 milliunits of *C. Perfringens* neuraminidase for 5 hr at 37°C. Portions of the samples were subjected to SDS/PAGE followed by silver staining.

effects on their pharmacokinetics (21). We compared the drug binding site for thalidomide and several other drugs using a competition assay in which the labeling of AGP by the thalidomide photoaffinity label was carried out in the presence of two known AGP-binding drugs, propranolol and alprenolol. As shown in Fig. 7*B*, both propranolol and alprenolol compete effectively against the thalidomide photoaffinity label for AGP, with alprenolol being more potent than propranolol. It is likely that thalidomide binds to AGP at the same or an overlapping site as propranolol and alprenolol.

DISCUSSION

Thalidomide is known to possess immunomodulatory and antiinflammatory effects. However, the cellular and molecular mechanisms of these activities are not understood. Among the various cellular effects of thalidomide, the inhibition of TNF-α seems to offer a plausible mechanism for several known immunomodulatory activities of the drug. Using LPS-induced TNF-α secretion in human monocytes as an assay and an active thalidomide photoaffinity label as a probe, we isolated and identified two thalidomide-binding proteins as isoforms of AGP.

AGP belongs to the class of acute phase proteins that are known to be induced in the liver in response to a wide variety of physiological stresses, including inflammation, infection, pregnancy, and malignancy (22, 23). It is distinct from other serum proteins by its high carbohydrate content (over 40% by weight) and high acidity. Although it has been known for more than a century (24), the precise biological function of AGP still remains unknown. Nevertheless, a number of studies have

Bovine Peptide-1	FYIGSAF
	• • • • • •
Human α_1-AGP	FYIASAF
Bovine Peptide-2	AIQAAF
	• • • • •
Human α_1-AGP	EIQAAF
Bovine Peptide-3	MLAASW
	• • • •
Human α_1-AGP	MLA-SY
Bovine Peptide-4	KXVGVXFYADK
	• • • • • • •
Human α_1-AGP	KQYGLSFYADK

FIG. 6. Comparison of peptide sequences derived from the purified thalidomide-binding protein and those of human AGP. X, unidentified residues.

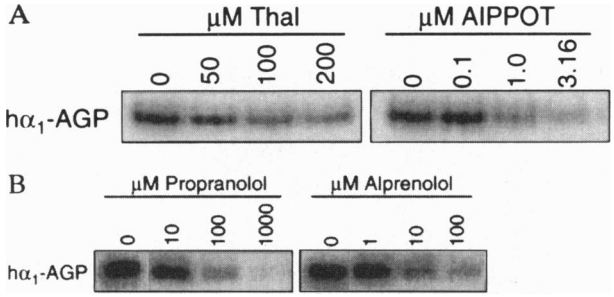

FIG. 7. Labeling of human AGP with increasing concentrations of cold competitors. A sample of 0.1 μg of human AGP per tube was preincubated with varying concentrations of competitor or with DMSO carrier alone (0.5%). [^{125}I]AIPPOT (1 μCi per tube) was added, and tubes were incubated for 30 min, irradiated, quenched with 2-mercaptoethanol, and subjected to SDS/PAGE followed by autoradiography. (*A*) Competition of labeling with thalidomide and AIPPOT. (*B*) Competition with the β-blockers propranolol and alprenolol.

pointed to potential roles of AGP in the regulation of the immune system and in inflammation. AGP was shown to have immunosuppressive activity in the mixed lymphocyte reaction (25), and an AGP-like immunosuppressive acidic protein was found from ascitic fluids of cancer patients (26). The potential role of AGP in inflammation has been somewhat controversial. On the one hand, AGP was shown to exert a protective effect against TNF-α and LPS-induced lethality when it is administered intraperitoneally in mice (27). It has also been shown to inhibit platelet aggregation (28) and thus may be capable of preventing secretion of other proinflammatory factors. On the other hand, AGP was shown to potentiate LPS-induced secretion of such inflammatory cytokines as TNF-α and interleukin 1 in human monocytes *in vitro* (29). Since thalidomide inhibits LPS-induced TNF-α secretion, it will be interesting to see whether thalidomide acts by inhibiting AGP's potentiating activity for LPS. It also remains to be determined whether thalidomide has any effect on other immunomodulatory activities of AGP.

As an abundant plasma protein, AGP binds to many drugs and can modulate their metabolism (21). Those drugs include known β-blockers such as propranolol and alprenolol. Competition experiments indicate that thalidomide binds to the same site in AGP as propranolol and alprenolol. None of the drugs, however, possess the same unique immunomodulatory and antiinflammatory activities of thalidomide. Whether binding of thalidomide to AGP confers a unique conformational change to the protein leading to a gain of function remains to be investigated.

The primary source of AGP is known to be hepatocytes (30). However, it has been shown that lymphocytes, granulocytes and monocytes also express a membrane-associated form of AGP (31). It has also been reported that monocytes can serve as a source of soluble AGP (32). In fact, we have found that upon LPS stimulation, the promonocytic cell line THP-1 differentiated by treatment with 1α, 25-dihydroxy vitamin D_3 produces large amount of AGP along with TNF-α (unpublished results). These observations are consistent with the aforementioned immunomodulatory activities of AGP and suggest that AGP may play an important role in immunoregulation. The fact that thalidomide binds to AGP with high specificity implies that AGP may mediate the immunomodulatory effect of the drug.

We thank William Lane (Harvard Microchemistry Facility) and Richard Cook (Center for Cancer Research, Massachusetts Institute of Technology) for peptide sequencing, Georgia Vogelsang (The Johns Hopkins University) for a sample of 3-hydroxythalidomide used in our initial studies, Arnold Ruoho for supplying us with the photoaffinity label precursor, and Edward Licitra for helpful discussions on synthesis of thalidomide analogs. We are grateful to Professors Richard Hynes and Phillip Sharp for their support. Financial support from Amgen Inc., Rita Allen Foundation, the Chicago Community Trust, March of Dimes, the Center for Cancer Research, and the Department of Biology of the Massachusetts Institute of Technology is gratefully acknowledged. J.L. is a Pfizer-Laubach Career Development Assistant Professor.

1. McBride, W. G. (1961) *Lancet* **ii,** 1358.
2. Lenz, W. (1962) *Lancet* **i,** 45.
3. Vogelsang, G. B., Taylor, S., Gordon, G. & Hess, A. D. (1986) *Transplant. Proc.* **18,** 904–906.
4. Gunzler, V. (1992) *Drug Safety* **7,** 116–134.
5. Sheskin, J. (1965) *Clin. Pharmacol. Ther.* **6,** 303–306.
6. Koch, H. P. (1985) in *Progress in Medicinal Chemistry*, eds. Ellis, G. P. & West, G. B. (Elsevier, Amsterdam), pp. 166–242.
7. D'Amato, R. J., Loughnan, M. S., Flynn, E. & Folkman, J. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 4082–4085.
8. Keenan, R. J., Eiras, G., Bruckart, G. J., Stuart, R. S., Hardesty, R. L., Vogelsang, G., Griffith, B. P. & Zeevi, A. (1991) *Transplantation* **52,** 908–910.
9. Nogueira, A. C., Neubert, R., Helge, H. & Neubert, D. (1994) *Life Sci.* **55,** 77–92.
10. Sampaio, E. P., Sarno, E. N., Galilly, R., Cohn, Z. A. & Kaplan, G. (1991) *J. Exp. Med.* **173,** 699–703.
11. Sampaio, E. P., Kaplan, G., Miranda, A., Nery, J. A. C., Miguel, C. P., Viana, S. M. & Sarno, E. N. (1993) *J. Infect. Dis.* **168,** 408–414.
12. Moreira, A. L., Sampaio, E. P., Zmuidzinas, A., Frindt, P., Smith, K. A. & Kaplan, G. (1993) *J. Exp. Med.* **177,** 1675–1680.
13. Makonkawkeyoon, S., Limson-Pobre, R. N. R., Moreira, A. L., Schauf, V. & Kaplan, G. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 5974–5978.
14. Vogelsang, G. B., Hess, A. D., Gordon, G., Brundrette, R. & Santos, G. W. (1987) *Transplant. Proc.* **19,** 2658–2661.
15. Boylen, J. B., Horne, H. H. & Johnson, W. J. (1963) *Proc. Can. Fed. Biol. Soc.* **6,** 12 (abstr.).
16. Golaz, O., Hughes, G. J., Frutiger, S., Paquet, N., Bairoch, A., Pasquali, C., Sanchez, J.-C., Tissot, J.-D., Appel, R. D., Walzer, C., Balant, L. & Hochstrasser, D. F. (1993) *Electrophoresis* **14,** 1223–1231.
17. Weimer, H. E., Mehl, J. W. & Winzler, R. J. (1950) *J. Biol. Chem.* **185,** 561–568.
18. Bezkorovainy, A. (1965) *Arch. Biochem. Biophys.* **110,** 558–567.
19. Ikenaka, T., Bammerlin, H., Kaufmann, H. & Schmid, K. (1966) *J. Biol. Chem.* **241,** 5560–5563.
20. Schmid, K., Kaufmann, H., Isemura, S., Bauer, F., Emura, J., Motoyama, T., Ishiguro, M. & Nanno, S. (1973) *Biochemistry* **12,** 2711–2724.
21. Kremer, J. M. H., Wilting, J. & Janssen, L. H. M. (1988) *Pharmacol. Rev.* **40,** 1–47.
22. Schmid, K. (1975) in *The Plasma Proteins: Structure, Function and Genetic Control*, ed. Putnam, F. W. (Academic, New York), pp. 184–228.
23. Arnaud, P., Miribel, L. & Roux, A. F. (1988) *Methods Enzymol.* **163,** 418–430.
24. Landwehr, H. A. (1882) *Z. Physiol. Chem.* **6,** 74–77.
25. Bennett, M. & Schmid, K. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 6109–6113.
26. Tamura, K., Shebata, Y., Matsuda, Y., & Ishida, N. (1981) *Cancer Res.* **41,** 3244–3252.
27. Libert, C., Brouckaert, P. & Fiers, W. (1994) *J. Exp. Med.* **180,** 1571–1575.
28. Costello, M., Fiedel, B. A. & Gweurz, H. (1979) *Nature (London)* **281,** 677–678.
29. Boutten, A., Dehoux, M., Deschenes, M., Rouzeau, J.-D., Bories, P. N. & Durand, G. (1992) *Eur. J. Immunol.* **22,** 2687–2695.
30. Jamieson, J. C. & Ashton, F. E. (1973) *Can. J. Biochem.* **51,** 1034–1045.
31. Gahmberg, C. G. & Andersson, L. C. (1978) *J. Exp. Med.* **148,** 507–521.
32. Nakamura, T., Board, P. G., Matsushita, K., Tanaka, H., Matsuyama, T. & Matsuda, T. (1993) *Inflammation* **17,** 33–45.