Characterization of a Human Lysophosphatidic Acid Acyltransferase That Is Encoded by a Gene Located in the Class III Region of the Human Major Histocompatibility Complex

(Received for publication, August 29, 1997, and in revised form, November 13, 1997)

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Sequence analysis of cDNA clones corresponding to a number of genes located in the class III region of the human major histocompatibility complex (MHC), in the chromosome band 6p21.3, has shown that the G15 gene encodes a 283-amino acid polypeptide with significant homology over the entire polypeptide with the enzyme lysophosphatidic acid acyltransferase (LPAAT) from different yeast, plant, and bacterial species. The amino acid sequence of the MHC-encoded human LPAAT (hLPAAT) is 48% identical to the recently described hLPAAT (Eberhardt, C., Gray, P. W., and Tjoelker, L. W. (1997) J. Biol. Chem. 272, 20299–20305), which is encoded by a gene located on chromosome 9p34.3. LPAAT is the enzyme that in lipid metabolism converts lysophosphatidic acid (LPA) into phosphatidic acid (PA). The expression of the hLPAAT polypeptide in the baculovirus system and in mammalian cells has shown that it is an intracellular protein that contains LPAAT activity. Cell extracts from insect cells overexpressing hLPAAT were analyzed in different LPAAT enzymatic assays using, as substrates, different acyl acceptors and acyl donors. These cell extracts were found to contain up to 5-fold more LPAAT activity compared with control cell extracts, indicating that the hLPAAT specifically converts LPA into PA, incorporating different acyl-CoAs with different affinities. The hLPAAT polypeptide expressed in the mammalian Chinese hamster ovary cell line was found, by confocal immunofluorescence, to be localized in the endoplasmic reticulum. Due to the known role of LPA and PA in intracellular signaling and inflammation, the hLPAAT gene represents a candidate gene for some MHC-associated diseases.

Lyosphosphatidic acid acyltransferase (LPAAT), also known as 1-acyl-sn-glycerol-3-phosphate acyltransferase (EC 2.3.1.51), is the enzyme that converts lysophosphatidic acid (LPA) into phosphatidic acid (PA) in the lipid metabolism. LPA or 1-acyl-sn-glycerol 3-phosphate consists of a glycerol backbone with a fatty acyl chain at the sn-1 position, a hydroxyl group at the sn-2 position, and a phosphate group at the sn-3 position. In the endoplasmic reticulum (ER) membrane, LPA is formed from glycerol 3-phosphate through the action of glycerol-3-phosphate acyltransferase. LPA is then further acylated in the ER by LPAAT to yield PA, the precursor of all glycerolipids.

The rate of acylation of LPA to PA is very high, and consequently, there is little accumulation of LPA at the site of biosynthesis. PA can either be hydrolyzed by phosphatidic acid phosphohydrolase to yield diacylglycerol (DAG) or, alternatively, can be converted to CDP-DAG for the synthesis of more complex phospholipids in the ER, from which they are transported to different subcellular compartments. PA can be produced by this de novo synthesis or, alternatively, by phospholipase D hydrolysis of phospholipids, such as phosphatidylcholine (PC) and phosphatidylethanolamine, or through phosphorylation of DAG by DAG kinase (for a general description, see Refs. 1 and 2).

Naturally occurring glycerolipids generally exhibit a nonrandom distribution of the acyl constituents; saturated fatty acids are esterified predominantly at the C-1 position and unsaturated fatty acids at the C-2 position (for a review see Refs. 3 and 4). Previous studies carried out using a semipurified LPAAT from rat liver microsomes indicated that LPAAT exhibited a significant acyl donor specificity for monoenoic and dienoic acyl-CoA thioesters (5). However, the activity of the enzyme was essentially not affected by the fatty acid constituent of the acyl acceptor (LPA), except that the 1-steaeryl and 1-arachidonoyl LPAs were somewhat less effective acyl acceptors (6). This acyl acceptor specificity was also found for lyso-PC acyltransferase when using different lysophosphatidylcholine (LPC) as substrates. The acyl acceptor specificity of LPAAT, however, does depend on the polar head group, and LPAAT is highly specific for LPA. In contrast, lyso-PC acyltransferase utilizes several acyl acceptors differing in the polar head group, except that LPA and lysophosphatidylethanol are ineffective substrates (6).

The human major histocompatibility complex (MHC) spans ~4 megabase pairs in the chromosome band 6p21.3 and is divided into three regions (7). The class I and II regions contain the classical MHC genes, which encode cell-surface glycoproteins involved in the presentation of antigenic peptides to T cells during an immune response. These are interspersed with a large number of other genes, some of which encode proteins involved in antigen processing. The class I and class II regions are separated by the central class III region that spans 1100 kilobase pairs of DNA (7, 8). Characterization of a 220-kilobase pair segment of DNA located between the class II region and

Vol. 273, No. 7, Issue of February 13, pp. 4096–4105, 1998
Printed in U.S.A.
the complement C4 genes in the class III region of the human MHC has revealed that the region contains at least nine genes (9–15), NOTCH-4, G18, PBX-2 (G17), RAGE, G16, G15, G14, G13 (Creb-rp), and TN-X (tenascin-X), of which only five (NOTCH-4, PBX-2, RAGE, Creb-rp, and TN-X), at present, encode proteins of known or putative function (for a review, see Ref. 8). We have found that one of the uncharacterized genes (G15) encodes a protein that has significant homology with LPAAT from bacteria, plant, and yeast species, suggesting that it could be the human homologue.

During the preparation of this manuscript, two other papers have been published describing the cloning and expression of human LPAAT (hLPAAT) (16, 17). In addition, another hLPAAT (hLPAAT) has been published describing the cloning and expression of it could be the human homologue.

This PCR copy was gel-isolated and ligated into the pBlsc), and several clones were sequenced.

cDNA Sequence Analysis—Screening of a U937 cDNA library, using two overlapping cosmids (D3A and E91) from the MHC class III region as probes, resulted in the isolation of 22 cDNA clones (9). Characterization of these clones by restriction enzyme mapping revealed that pG15–3B contained a full-length cDNA insert of 2.1 kilobase pairs. Both strands of this cDNA were sequenced by the dideoxy chain termination method after random sonicated fragments were cloned in the pBlsc). The spectrophotometric analysis for measurement of LPAAT activity was described as room temperature essentially as described by Yamashita et al. (3) with minor modifications. The enzyme activity was assayed by measuring the reaction of the thiol group of the released CoA with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) spectrophotometrically giving an increase in absorbance at 413 nm. A typical incubation mixture consisted of 100 mM Tris-HCl, pH 7.4, 1 mM DTNB, 50 μM LPA (oleoyl-sn-glycerol 3-phosphate) (Sigma), 10–45 μM acyl-CoA (Sigma), and 50–150 μg of cell homogenate in a total volume of 1 ml. DTNB was added as a 0.01 M solution in 0.1 M potassium phosphate buffer, pH 7.0. 0.02 μCi/ml [35S]methionine and 0.02 μCi/ml [1-14C]-oleoyl-CoA (Amersham). The reaction was terminated by directly spotting it onto a silica gel 60 TLC plate (Merck) and developed in chloroform/methanol/acetic acid/water (25:10:3:1). To determine the position of substrate and products, LPA and PA standards were labeled and visualized by exposure to iodine vapors. When 1H-labeled LPA was used, fluorography was done by immersion of the TLC plate in chloroform. Cellular labeling, and those groups of cells expressing the hLPAAT recombinant protein were diluted to obtain single clones expressing hLPAAT. Expression of the protein in the single clones was confirmed by Western blotting, and three of them were analyzed by immunofluorescence and for LPAAT activity. One of the clones, CHO-G15 (hLPAAT), was chosen for subsequent experiments. Single clones containing only the vector were created in parallel, and the presence of the vector was confirmed by PCR screening using specific pcDNA3 primers. Three of these clones were used in immunofluorescence and enzyme activity assays as negative controls for the hLPAAT transfectants, and one of these, CHOV, was chosen for further experiments.

Enzyme Assays—Si21 cells were infected with the wild type (A. californica nuclear polyhedrosis virus) or vG15Bac (hLPAAT) baculovirus at low multiplicity of infection (2 plaque-forming units/cell). Cells were infected for 72 h, postinfection (175 hpi) then Dounce homogenization. The homogenates were used as a 0.01 M solution in 0.1 M potassium phosphate buffer, pH 7.0. Absorbance of 13,600 M-1 cm-1 was used to calculate the activity.

To assay the enzyme activity by TLC, the assay procedure was essentially as described above except that 4 μg of enzyme homogenate in a total volume of 10 μl was used, and instead of adding DTNB, 0.02 μCi/ml [3H]-LPA (1-oleoyl) (NEN Life Science Products, Inc.) was used. The reaction was initiated by the addition of acyl-CoA after preincubation of the enzyme with all of the other components for 2 min. A molar absorbance of 13,600 M-1 cm-1 was used to calculate the activity.

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EXPERIMENTAL PROCEDURES

cDNA Sequence Analysis—Screening of a U937 cDNA library, using two overlapping cosmids (D3A and E91) from the MHC class III region as probes, resulted in the isolation of 22 cDNA clones (9). Characterization of these clones by restriction enzyme mapping revealed that pG15–3B contained a full-length cDNA insert of 2.1 kilobase pairs. Both strands of this cDNA were sequenced by the dideoxy chain termination method after random sonicated fragments were cloned in the pBlsc). The spectrophotometric analysis for measurement of LPAAT activity was described as room temperature essentially as described by Yamashita et al. (3) with minor modifications. The enzyme activity was assayed by measuring the reaction of the thiol group of the released CoA with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) spectrophotometrically giving an increase in absorbance at 413 nm. A typical incubation mixture consisted of 100 mM Tris-HCl, pH 7.4, 1 mM DTNB, 50 μM LPA (oleoyl-sn-glycerol 3-phosphate) (Sigma), 10–45 μM acyl-CoA (Sigma), and 50–150 μg of cell homogenate in a total volume of 1 ml. DTNB was added as a 0.01 M solution in 0.1 M potassium phosphate buffer, pH 7.0. The reaction was terminated by directly spotting it onto a silica gel 60 TLC plate (Merck) and developed in chloroform/methanol/acetic acid/water (25:10:3:1). To determine the position of substrate and products, LPA and PA standards were labeled and visualized by exposure to iodine vapors. When 1H-LPA was used, fluorography was done by immersion of the TLC plate in chloroform.
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RESULTS

The hLPAATα Gene Is Located in the MHC Class III Region.—A nearly full-length cDNA clone (pG15–3B) corresponding to the single copy gene G15, located in the MHC class III region, was isolated from a U937 cDNA library (9) and sequenced. The 2045-bp cDNA insert, with a poly(A) signal region, was isolated from a U937 cDNA library (9) and sequenced. The primary and secondary antibodies were added in 0.2% gelatin, 0.05% saponin in PBS for 45 min. The secondary antibody was fluorescein isothiocyanate-conjugated anti-mouse-IgG (Sigma Immunochromos), while the 1D3 primary monoclonal antibody (mAbs), was kindly donated by Dr D. Vaux (Sir William Dunn School of Pathology, Oxford). Non-permeabilized conditions were without detergent and involved incubation of the primary mAb at 4 °C to avoid permeabilization and internalization of the mAbs and membrane proteins. Immunofluorescence was observed using a Bio-Rad MRC 1024 confocal microscope.

Expression of hLPAATα in Insect Cells and in the Mammalian Cell Line CHO—To characterize the G15 gene product (hLPAATα), the protein was expressed in insect cells using the baculovirus system. Radiolabeling of Sf21 insect cells infected with vG15Bac (hLPAATα) showed, in cell extracts, a major band found for hLPAATα expressed in insect cells using baculovirus (Fig. 3). This suggests that the major cleavage site for the signal peptide is at amino acid 58 and that the cleavage at amino acid 22 observed in insect cells could be due to aberrant processing. Since it is also possible that anomalous migration of the protein (due to its hydrophobic nature) during SDS-polyacrylamide gel electrophoresis is taking place, further experiments will be required to characterize the amino terminus of the mature protein.

The Predict-protein program (EMBL, Heidelberg) (30), using the alignment of the different LPAATs and assuming cleavage of the hLPAATα after amino acid 58, predicts only two transmembrane domains, one from amino acid 130 to 147 and one from amino acid 195 to 211 (Fig. 1 and 2b). The predicted topology of the protein would be as illustrated in Fig. 4, with the amino terminus in the ER lumen, followed by a transmembrane domain, a cytoplasmic loop, another transmembrane domain, and the C terminus in the ER lumen. A highly conserved region, distinct from the transmembrane regions, between amino acids 176 and 196, would be located on the cytosolic side of the ER membrane, where phospholipid synthesis occurs (31), indicating a potential active center of the enzyme (Fig. 2b and 4). Comparative sequence analysis of the different LPAATs with the available glycerol-3-phosphate acyltransferase sequences revealed that they show a higher sequence similarity in this region, supporting the hypothesis of this being the active center (data not shown). The two conserved transmembrane regions could also be part of the active center and select for the length and degree of saturation of the acyl chains (Figs. 2b and 4). In this model, the potential glycosylation site would be located on the cytosolic side of the ER and for this reason would be unavailable for glycosylation. The model would still be valid for the localization of the active center and carboxyl terminus even if none of the signal peptide cleavage sites is used, or the cleavage site after amino acid 22 is used according to the Predict-protein program (data not shown). In these situations the predicted topology of the protein would only be different at the amino terminus, due to the addition of extra transmembrane domains.

1-Acylglycerol-3-Phosphate Acyltransferase Activity of hLPAATα—To demonstrate that the G15 gene product is the hLPAATα, the activity of the recombinant protein expressed using the baculovirus system was characterized in vitro by spectrophotometric and TLC analysis. To define the acyl-CoA (acyl donor) specificity of hLPAATα in relation to the length and degree of saturation of the fatty acid, we have used different saturated (stearoyl (C18:0), lignoceryl (C24:0), arachidoyl (C20:0), palmitoyl (C16:0), myristoyl (C14:0), and lauroyl (C12:0)) or unsaturated (palmitoleyl (C16:1), arachidonoyl (C20:4), linolenoyl (C18:3), linoleoyl (C18:2), and oleoyl (C18:1) acyl-
CoA. Cell extracts from vG15Bac (hLPAATa) baculovirus-infected insect cells, after 6 min of reaction using 10 μM of acyl-CoAs, showed that the highest activity was for palmitoleoyl-CoA (C16:1), which was 0.532 nmol of CoA released per mg of protein (nmols/mg prot) (Fig. 5a), and then the rank order was for the acyl-CoAs C16:0 > C14:0 > C12:0 > C18:2 > C18:3 > C18:0 > C20:4 > C24:0 (0.180 nmol/mg of protein). When using 45 μM acyl-CoAs, respectively (Fig. 5a). The background activity of the wild type cell extracts, under these conditions, was from 0.135 (C20:0) to 0.190 (C18:2) nmol/mg of protein.
Fig. 2. Percentage of amino acid identities (ID) or similarities (SIM) (a) and amino acid sequence alignment (b) of the complete amino acid sequence of the G15 gene product and LPAATs from different organisms. Human (G15 (hLPAATα)) and hLPAATβ, S. cerevisiae (S. cerevisiae), H. influenzae (H. influenzae), S. typhimurium (Salmonella), E. coli (E. coli), L. alba (L. alba), C. nucifera (C. nucifera), N. gonorrhoeae (N. gonorrhoeae), and N. meningitidis (N. meningitidis) are shown. a, the BestFit scores are shown. b, the conserved potential transmembrane sequences are underlined, and the potential active center is double underlined. c, BestFit analysis of hLPAATα (top line) and hLPAATβ (bottom line).
the enzyme kinetics showed that hLPAATα had maximal activity for the acyl-CoAs C16:0 ~ C14:0 ~ C16:1 ~ C18:2 ~ C18:3 ~ C12:0 (0.854–0.724 nmol/mg protein) (Fig. 5b) and intermediate activity for the acyl chains C20:4, C18:1, and C18:0 (0.512, 0.496, and 0.452 nmol/mg of protein, respectively) (Fig. 5b). Again it showed poor or no activity for long acyl chains (C20:0 and C24:0) (0.262 and 0.148 nmol/mg of protein, respectively) (Fig. 5b). The background activity of the wild type cell extracts when using 45 μM of acyl-CoAs was the same as that when using 10 μM of acyl-CoAs (Fig. 5a and data not shown). In these spectrophotometric assays, the recombinant hLPAATα enzyme activity was linear for at least 3–6 min when using 45 μM acyl-CoAs or 1–2 min when using 10 μM acyl-CoAs (Fig. 6a and data not shown). The wild type baculovirus background activity in these kinetic assays was nearly constant irrespective of the time and amount or type of acyl-CoA used (Fig. 6a and data not shown), suggesting that the wild type baculovirus contains very little endogenous LPAAT activity. Similar results of acyl donor specificity were obtained when the reactions were performed using 3H-LPA, and after 6 min the products were detected by TLC (Fig. 5c). When the TLC assay was performed with unlabeled LPA and 14C-oleoyl-CoA, specific labeled products (PA) were detected only when using vG15Bac (hLPAATα) cell extracts, which increased as the concentration of LPA was increased (Fig. 6b).

Miki et al. (6), using a semipurified rat liver LPAAT, found that the enzyme had no activity for lysophosphatidylethanolamine (LPE) or LPC. We have observed by spectrophotometric analysis, using wild type and vG15Bac (hLPAATα) baculovirus-infected insect cell extracts, with 50 μM LPE or LPC and with 20 μM C16:1, C12:1, C18:1, and C24:0 acyl-CoAs as substrates, that recombinant hLPAATα cannot incorporate those acyl-CoAs into LPE or LPC (data not shown), confirming these previous data.

The activity of the recombinant hLPAATα was also assayed in the pcDNA3G15 stable transfected mammalian cell line CHO, where it was found that cell extracts from CHOG15 (hLPAATα) showed a higher level of hLPAATα activity in both the spectrophotometric and TLC assays (Fig. 7). The enzyme activity was linear for 6 min of reaction (Fig. 7a) and increased in an extract dose-dependent manner (Fig. 7b). After 6 min of reaction and when using 45 μM oleoyl-CoA, CHOG15 (hLPAATα) cell extracts showed 1.4 times more activity than CHO or CHO cell extracts (Fig. 7a). Note the high activity of the endogenous LPAAT in CHO cells in comparison with the very low (or undetected) endogenous LPAAT activity found in wild type baculovirus-infected cells (compare Fig. 7a with Fig. 6a and Fig. 7b with Fig. 5c).

**Cellular Localization**—To localize hLPAATα in the cell, immunofluorescence on the different stable CHO cell lines was

### Table C

| Cells  | Medium  |
|--------|---------|
| vG15Bac | Ab15R   |
| WT     | 28A     |
| CHO    | 27P     |

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**Fig. 2.**—continued

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**Fig. 3.** Expression of hLPAATα. a, SF21 cells infected with vG15Bac (hLPAATα), AcB15R, or wild type (WT) baculovirus were pulse-labeled from 24 to 27 h postinfection, and proteins present in cells and in the medium were analyzed by SDS-polyacrylamide gel electrophoresis and visualized by autoradiography. The positions of the polyhedrin protein (P) and the vaccinia IL-1β receptor (*) are shown. b, Western blot analysis of cell extracts from the cell lines CHOG15 (hLPAATα), CHOV, or CHO using T7.TagMab. The proteins and the molecular size markers are indicated in kDa.
performed. In permeabilized cells expressing the tagged hLPAATα (CHOG15), an ER-staining pattern was observed using the T7.TagmAb (Fig. 8a) that was not present in the control cell lines (CHO, CHOV) using the same mAb (data not shown). A similar ER pattern was observed when the three cell lines were stained with the specific ER mAb 1D3 that labels protein-disulfide isomerase from the ER (Fig. 8, b, c, and d). The Predict-protein program predicts that, if hLPAATα is also located in the plasma membrane, the T7.Tag should be in the extracytoplasmic region, and thus, on nonpermeabilized cells, it would be accessible and recognized by the specific T7.TagmAb. However, immunostaining of unpermeabilized cells or fluorescence-activated cell sorting analysis did not show external protein labeling, indicating lack of plasma membrane localization (data not shown).

**DISCUSSION**

We have cloned and sequenced the cDNA of the G15 gene, located in the MHC class III region, and have found that the predicted polypeptide shares significant sequence identity with bacterial, yeast, and plant LPAATs. We have also expressed the protein product of that gene (hLPAATα) in insect cells and in the mammalian cell line CHO and have shown that it contains LPAAT activity. We have seen that baculovirus recombinant hLPAATα cannot incorporate acyl-CoAs into LPC or LPE. However, using 11 different acyl-CoAs, we have found that recombinant hLPAATα converts LPA into different PAs depending on the acyl-CoA incorporated, and it shows different specificities for different acyl-CoAs. Our results indicate that the hLPAATα has affinity for fatty acids of acyl chain lengths from 12 to 18 carbons with a slight dependence on the degree of saturation of the fatty acid. However, hLPAATα does not in-
corporate long chain fatty acids (C20:0, C24:0) unless they are saturated (C24:4). These hLPAAT substrate specificity results confirm previous reported data by Yamashita et al. (3, 5, 32) obtained using 10–20 μM acyl-CoA, where the rank order of specificities of the acyl donor was as follows: C18:1 > C18:2 > C16:1 > C16:0 > C14:0. However, C20:4, C18:0, and C12:0 were described as poor substrates for LPAAT, although in our assays the recombinant hLPAAT has significant activity for these substrates. In another study, Lands et al. (33), using rat liver microsomes and 20 μM acyl-CoA, described that the unsaturated acyl-CoAs C18:3, C18:2, and C18:1 were the best substrates for LPAAT followed by C16:0 and C18:0 and then, with low affinity, the unsaturated 20-carbon fatty acids. The discrepancy between these studies and our data may be due to the previous use of semipurified (32) or unpurified (33) enzyme preparations from rat liver microsomes, which could have been contaminated with other acyltransferases (3, 32), such as hLPAATβ, whereas in our assay specificity is only due to the recombinant hLPAATα.

Glycerophospholipids in animal tissues are known to contain large amounts of arachidonic acid (C20:4) at the sn-2 position. It has been proposed that PC would be deacylated at the sn-2 position and then reacylated, by the lyso-PC acyltransferase, with C20:4 (3). Here, we have demonstrated that hLPAATα is able to incorporate, with intermediate affinity, arachidonoyl-CoA (C20:4) into LPA to form PA. The incorporation of C20:4 by...
hLPAATα is of great relevance, since arachidonic acid, the most important prostaglandin precursor in humans, can be liberated from PA by the action of phospholipase A2. Prostaglandins mediate the inflammatory response, the production of pain and fever, the regulation of blood pressure, the induction of blood clotting, the control of several reproductive functions, and the regulation of the sleep/wake cycle (1).

Stamps et al. (17) have shown an increase in LPAAT activity in COS7 cells transiently transfected with hLPAATα, indicating an apparent preference of hLPAATα for C18:1 monounsaturated CoA as acyl donor. However, it is difficult to compare our data with theirs because, although using the same activity assay, they used an excess of substrates (10 times more LPA and 10 or 2.2 times more acyl-CoA) in their experiments. Comparison of our data on the acyl donor specificity of hLPAATα with that of Eberhardt et al. (18) on hLPAATβ could indicate that there is a difference in acyl donor specificity between the two hLPAATs. hLPAATβ was found to show more activity in the presence of the acyl donor C20:4, compared with the acyl donor C16:0 (18), and our data indicate the reverse is true for hLPAATα. However, we have to be careful in interpreting these data, since different LPAAT activity assays were used. Expression of the enzyme in the baculovirus system has been very successful, since the wild type baculovirus-infected cell extracts have shown very little endogenous activity, and in consequence the LPAAT activity observed is only due to the recombinant hLPAATα. This expression system could be used in the future for the characterization of other acyltransferases, including hLPAATβ, to elucidate their particular specificities. We have used homogenized cell extracts, instead of purified or detergent-treated enzyme, which maintain the hLPAATα in its natural hydrophobic environment and allow a better biochemical and functional characterization of the enzyme.

The initial steps in the biosynthesis of lipids have been described as occurring in the cytosolic part of the ER (for a review, see Ref. 31). We have shown that the hLPAATα is located in the ER using specific mAbs that only recognize the T7.Tag fused to the recombinant enzyme. This abolishes any possible cross-reaction with other endogenous acyltransferases that could lead to misinterpretation of the results when using Abs raised against the hLPAATα polypeptide. We propose a protein model in which hLPAATα is an ER transmembrane protein where the potential active center of the enzyme is facing the cytosolic part of the ER. This model supports previous data obtained from microsomal vesicles in which, by indirect biochemical methods (LPAAT inactivation by proteases and the ability of LPAAT to bind to the membrane-impermeable substrate palmitoyl-CoA), the active center of LPAAT has been suggested to be located on the external (cytosolic) part of the ER (31). Eberhardt et al. (18) propose that the hLPAATβ contains four putative transmembrane helices with the first and third oriented outside to inside and the second and fourth helices oriented inside to outside, within the membrane. This orientation, if we use it in relation to the ER membrane, is in agreement with the one that we propose for hLPAATα, since the protein region between the third and fourth helices (containing our proposed active center that is also conserved in hLPAATβ) would be in the cytosolic part of the ER.

While a major function of phospholipids is to form biological membranes, a subclass of phospholipids and their metabolites have been implicated as signaling molecules, acting either as intracellular second messengers or as extracellular agonists that modulate cell function (34–37). LPAAT is the enzyme that converts LPA into PA. LPA is an intracellular signaling molecule that is rapidly produced and released by activated cells, notably platelets, to influence target cells by acting on a specific cell surface receptor. LPA stimulates platelet aggregation and cell proliferation and can be involved in wound repair and blood clotting processes (34, 35). PA species can be potent growth factor molecules; stimulate phospholipase C, protein, and lipid kinases; mobilize Ca2+ flux; activate NADPH oxidase; induce hormone release, platelet aggregation, and gene transcription (37); and change cytoskeletal dynamics or cellular phenotype (see references in Ref. 38). Recent studies have shown that IL-1β (39), tumor necrosis factor-α (38), and platelet-activating factor (40), as well as bacterial cell wall products such as lipid A or lipopolysaccharide (41), may activate and signal, at least in part, through a common lipid intracellular signaling pathway, leading to rapid increases in intracellular levels of specific species of PA and DAG by the activation of the enzyme LPAAT. Recently, LPAAT inhibitors have been shown to block the inflammatory response produced by an increase in PA (42). West et al. (16) have found that overexpression of hLPAAT, in two different human cell lines, resulted in an increase in LPAAT activity that correlated with enhancement of transcription and synthesis of tumor necrosis factor-α and IL-6 from cells upon stimulation with IL-1β. The finding of which hLPAAT is involved in modulation of the inflammatory response will be crucial in relation to the designing of therapeutic agents.

Mobilization of intracellular stored Ca2+ by PA has been proposed (for a review see Ref. 37), which in this case could be from the ER, where hLPAATα is located, by the action of the induced PAs produced by this enzyme. Bursten et al. (39) have suggested that, in addition to the ER, LPAAT should be located on the plasma membrane, since they observed a 1.3-fold activation of LPAAT by IL-1β when using plasma membrane-enriched compared with crude-microsomal cell fractions. However, our data suggest that hLPAATα is only located in the ER but possibly very close to the plasma membrane (which could contaminate membrane-enriched fractions). LPAAT could be activated by direct contact with the plasma membrane or with receptors or receptor complexes associated with the plasma membrane. A mechanism has been described for the ryanodine receptor that, when located in the sarcoplasmic reticulum, is activated by direct contact with the dihydropyridine receptor located in the plasma membrane (43).

Human chromosomes 6 and 9 show gene family members present in both chromosomes such as NOTCH-4, PBX-2, and TN-X on chromosome 6p21.3 (class III region) and NOTCH-1, PBX-3, and TN-C (tenascin-C) on 9q34 (44). We have found that the hLPAATα (G15) gene is located on chromosome 6p21.3, while hLPAATβ is located on chromosome 9q34.3 (18), supporting the idea of duplication of an ancestral chromosomal segment giving rise to the present chromosome 6p21 and 9q34–33 segments. A large number of diseases are associated with the products of genes located in the MHC, many of which are not fully explained by the class I and class II antigens. Further characterization of hLPAATα at the genetic and molecular levels could extend the knowledge of the inflammatory response, lipid intracellular signaling pathways, MHC, and disease association and result in the rational design (based upon use of the recombinant protein) of novel inhibitors that could suppress intracellular signals used by several inflammatory mediators.

Acknowledgments—We are most grateful to Antonio Alcamí for helpful discussions and critical reading of the manuscript and for help with the baculovirus expression system. We also thank John Broxholme for help with computing; Ana Pombo, Alain Vanderplasschen, and Michael Hollisnhead for help with the confocal microscope; and Geoffrey Smith for access to specialized equipment.

REFERENCES

1. Voet, D., and Voet, J. G. (1995) Biochemistry, 2nd Ed., John Wiley & Sons, Inc., New York
