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A new monoclonal antibody, 4-1a, that binds to the amino terminus of human lipoprotein lipase

André Bensadoun [a,*], Charlene D. Mottler [a], Chris Pelletier [a], Daniel Wu [b], Jane J. Seo [b], Calvin S. Leung [b], Oludotun Adeyo [b], Chris N. Goulbourne [b], Peter Gin [b], Loren G. Fong [b], Stephen G. Young [b,c], Anne P. Beigneux [b,**]

[a] Division of Nutritional Science, Cornell University, Ithaca, NY 14853, USA
[b] Department of Medicine, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA 90095, USA
[c] Department of Human Genetics, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA 90095, USA

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ABSTRACT

Lipoprotein lipase (LPL) has been highly conserved through vertebrate evolution, making it challenging to generate useful antibodies. Some polyclonal antibodies against LPL have turned out to be nonspecific, and the available monoclonal antibodies (Mabs) against LPL, all of which bind to LPL's carboxyl terminus, have drawbacks for some purposes. We report a new LPL-specific monoclonal antibody, Mab 4-1a, which binds to the amino terminus of LPL (residues 5–25). Mab 4-1a binds human and bovine LPL avidly; it does not inhibit LPL catalytic activity nor does it interfere with the binding of LPL to heparin. Mab 4-1a does not bind to human hepatic lipase. Mab 4-1a binds to GPIHBP1-bound LPL and does not interfere with the ability of the LPL–GPIHBP1 complex to bind triglyceride-rich lipoproteins. Mab 4-1a will be a useful reagent for both biochemists and clinical laboratories.

1. Introduction

Lipoprotein lipase (LPL) is a crucial enzyme for the hydrolysis of triglycerides in plasma lipoproteins [1–3]. LPL is synthesized by adipocytes and myocytes and secreted into the interstitial spaces. The LPL is then picked up by GPIHBP1 (a glycosylphosphatidylinositol-anchored protein of capillary endothelial cells) and shuttled to the luminal face of capillaries. In the absence of GPIHBP1, LPL remains in the interstitial spaces around adipocytes and myocytes and never reaches its site of action within the capillary lumen [4]. A recent study by Gin and coworkers [5] suggested that the GPIHBP1–LPL complex may be crucial for the binding of triglyceride-rich lipoproteins (TRLs) to endothelial cells [5]. TRLs bound to the LPL–GPIHBP1 complex on the cell surface but not to GPIHBP1 alone [5]. GPIHBP1 and LPL are essential for the lipolytic processing of TRLs. A deficiency of either protein results in severe hypertriglyceridemia (chylomicronemia) [6,7] and impairs the delivery of lipid nutrients to parenchymal cells [8,9].

LPL is a key player in human plasma triglyceride metabolism, but studies of LPL biochemistry and function have been hampered by a paucity of antibody reagents. LPL is highly conserved in vertebrates, making it challenging to generate antibodies [10]. Widely used polyclonal antibodies against LPL have proven to be nonspecific [11]. Two mouse monoclonal antibodies (Mabs) against bovine LPL, 5D2 and 5F9 [12–14], have been widely used. Both bind to the carboxyl-terminal portion of bovine LPL and cross-react with human LPL (hLPL) [13]. Mab 5D2 has been useful for measurements of LPL mass [12,15], but it is not suitable for some studies because it blocks the catalytic activity of LPL [12,14]. Mab 5F9 binds to denatured human LPL but only weakly to native LPL [13].

Here, we report a new mouse monoclonal antibody against hLPL, 4-1a. Mab 4-1a binds to the amino terminus of LPL, does not inhibit catalytic activity, and binds avidly to GPIHBP1-bound LPL.

2. Material and methods

2.1. Lipase purification

Human lipoprotein lipase (hLPL) for the immunization of mice was purified from post-heparin human plasma [16]. The hLPL used to characterize Mab 4-1a was produced in suspension cultures of Chinese hamster ovary (CHO) cells and partially purified by heparin-Sepharose chromatography. The concentration of hLPL was measured with a sandwich

* Correspondence to: A. Bensadoun, Division of Nutritional Science, Cornell University, 321 Savage Hall, Ithaca, NY 14853, USA. Tel.: +1 607 592 9904; fax: +1 607 255 1033.
** Correspondence to: A.P. Beigneux, Department of Medicine, University of California at Los Angeles, 4506 Gonda, 695 Charles E. Young Dr. South, Los Angeles, CA 90095, USA. Tel.: +1 310 825 4422; fax: +1 310 206 0865.
E-mail addresses: ab55@cornell.edu (A. Bensadoun), abeigneux@mednet.ucla.edu (A.P. Beigneux).

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ELISA with Mabs 5F9 and 5D2 [13]. Mouse lipoprotein lipase (mLPL) was produced in suspension cultures of stably transfected CHO-Lec1 cells and purified by ceramic hydroxyapatite, heparin-Sepharose, and Superdex 200 chromatography. The concentration of mLPL was measured with an ELISA [17]. Chicken LPL (cLPL) was purified from chicken adipose tissue [18], and the concentration of cLPL was measured with an ELISA [19]. Bovine LPL (bLPL) was purified from fresh milk [20] by heparin-Sepharose, CHT hydroxyapatite, and Superdex 200 chromatography. LPL catalytic activity was determined with a [^{3}H]triolein substrate [21]. Human hepatic lipase (hHL) was prepared from CHO-K1 cells that had been transiently transfected with a hHL expression vector, pγk5-hHL, provided by Dr. Shau-Feng Chang (Heinrich-Pette-Institut, Hamburg, Germany). hHL was purified by heparin-Sepharose chromatography, and hHL mass was measured with an ELISA [22].

2.2. Monoclonal antibody production

Mice were immunized with hLPL, and hybridomas were selected after fusing splenocytes with myeloma cell line P3X [16,23]. The cells were plated on 96-well plates with mouse peritoneal macrophages. Ten days later, aliquots of the medium were tested for hLPL antibodies with an ELISA. 96-well plates were coated with hLPL (5 ng/well), and samples of the conditioned medium (100 μl) were added to the wells and incubated overnight. Mab binding was detected with an anti-mouse IgG coupled to horseradish peroxidase. One hybridoma, 4-1a, produced an antibody that bound hLPL; it was cloned twice by limiting dilution and grown in serum-free media (Gibco PFHM-II) in CELLine Two-Compartment Bioreactors (Wilsom Wolf). The isotype of Mab 4-1a was IgG_{2a} (Pierce Rapid Isotyping Kit). Mab 4-1a was purified on protein G-Sepharose columns (GE Healthcare); gel filtration revealed a single IgG peak.

2.3. Characterization of Mab 4-1a

Binding of Mab 4-1a to purified preparations of LPL and HL was assessed by western blotting. To localize the epitope for Mab 4-1a, CHO cells were transiently transfected with expression vectors for V5-tagged wild-type and mutant versions of hLPL and mLPL. Mutant LPLs were created by site-directed mutagenesis using the QuikChange Lightning Site-Directed Mutagenesis kit (Stratagene). After 48 h, western blots were performed on cell extracts (or conditioned medium samples) with Mab 4-1a and either Mab 5D2 or a V5 Mab. Antibody binding was detected with an Odyssey infrared scanner (Li-Cor).

Binding of Mab 4-1a to hLPL was also assessed with an ELISA. 96-well plates were coated with Mab 4-1a (1–3 μg/well), and the plates were blocked with Superblock (Pierce) for 2 h at room temperature.

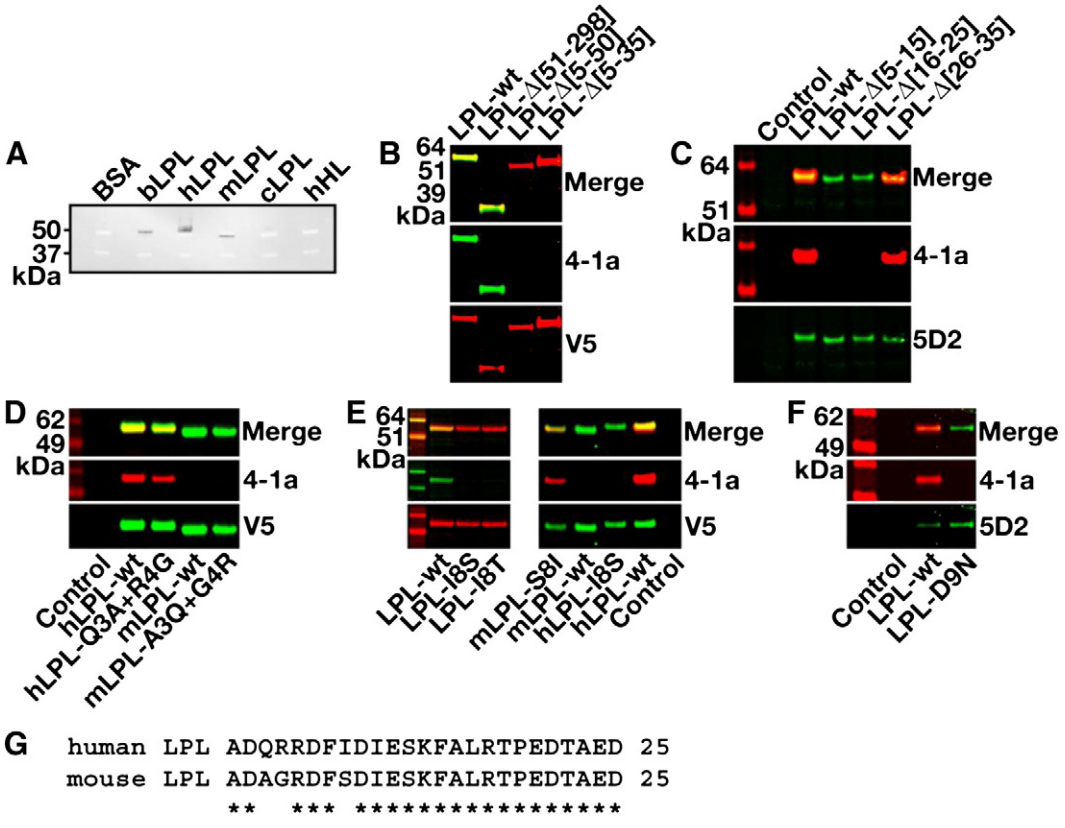

Fig. 1. Characterization of Mab 4-1a binding to LPL. A, Mab 4-1a binding to purified lipases in a western blot assay (350 ng/lane, as determined with an ELISA specific for each lipase). To each sample, we added Laemmli buffer that had been spiked with internal molecular weight standards (37 and 50 kDa) labeled with a fluorophore absorbing at 700 nm (white; serving as a loading control). The presence of the 4-1a antibody was detected with an IRDye800-labeled donkey anti-mouse IgG (black). Signals were detected with a Li-Cor infrared scanner. BSA, bovine serum albumin; bLPL, bovine LPL purified from milk; hLPL, human LPL purified from hLPL-transfected CHO cells; mLPL, mouse LPL purified from CHO-Lec1 cells; cLPL, chicken LPL from chicken adipose tissue; hHL, human hepatic lipase. B, Binding of Mab 4-1a to protein extracts prepared from CHO cells that had been transfected with a wild-type hLPL expression vector or a mutant hLPL vector containing deletions of amino acids 51–298, 5–35, or 5–50. Western blots were performed with Mab 4-1a (green) or with an anti-V5 antibody (red). C, Binding of Mab 4-1a (red) or 5D2 (green) to wild-type hLPL or mutant hLPLs containing deletions of amino acids 5–15, 16–25, or 26–35. D, Binding of Mab 4-1a (red) or a V5 Mab (green) to wild-type hLPL and mutant hLPLs containing Q3A and R4G mutations, and to wild-type mLPL and mutant mLPLs containing A3Q and G4R mutations. E, On the left, binding of Mab 4-1a (green) or the V5 Mab (red) to culture supernatants from CHO cells transfected with either a wild-type hLPL expression vector or mutant hLPL constructs containing I8S or I8T mutations. On the right, binding of Mab 4-1a (red) and the V5 Mab (green) to culture supernatants of cells that had been transfected with mLPL-S8I, wild-type mLPL, hLPL-I8S, and wild-type hLPL. The control lane shows nontransfected CHO cells. F, Binding of Mab 4-1a (red) and Mab 5D2 (green) to culture supernatants from cells that had been transfected with a wild-type hLPL vector or a mutant hLPL vector with a D9N mutation. G, Amino acid sequence alignment for the first 25 amino acids of mature human and mouse LPL. Conserved residues are indicated by an asterisk.