Genetically determined hypercholesterolemia in a rhesus monkey family due to a deficiency of the LDL receptor

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Abstract A family of rhesus monkeys comprising a sire, a dam, and four male offspring were fed a cholesterol-free Purina Chow diet for several months. The sire, 431-J, and two of the offspring, B-8204 and B-8806, had persistent plasma cholesterol levels in the range of 100–130 mg/dl, whereas the dam, 766-I, and the two other offspring, B-1000 and B-7643, exhibited a marked hypercholesterolemia in the 250–300 mg/dl range associated with an elevation of plasma LDL and apoB. When fed for 12 weeks a diet containing 12.5% lard and 0.25% cholesterol, sire, dam, B-1000 and B-7643 exhibited a marked hypercholesterolemia (500–800 mg/dl range), whereas B-8204 and B-8806 developed only a modest hypercholesterolemia (200–250 mg/dl). All animals were Lp[a]$^+$. Skin fibroblasts from each animal and from control cells were grown in 10% fetal calf serum, transferred to 10% lipoprotein-deficient serum for 48 hr, and then incubated at 4°C or 37°C with ^{125}I-labeled Lp[a]-free LDL. The fibroblasts from dam and offspring B-1000 and B-7643 bound and internalized ^{125}I-labeled LDL less efficiently than control cells. Mathematical analyses of the 4°C binding data indicated that there were no significant differences in LDL binding affinity between test and control cells suggesting that cells from the animals with a spontaneous hypercholesterolemia had a decreased number of LDL receptors. This conclusion was supported by the results of ligand and immunoblot analyses carried out on cell lysates separated by gradient gel electrophoresis. We conclude that a genetically determined LDL receptor deficiency was responsible, in part, for the spontaneous hypercholesterolemia observed in three out of the six family members and that this deficiency accounted for the hyperresponsiveness to a dietary fat and cholesterol challenge by the dam and the two offspring, B-1000 and B-7643. The hyperresponsiveness noted in the sire that had no evidence for LDL-receptor deficiency illustrates that factors other than the LDL receptor were responsible for the hypercholesterolemia attending the fat challenge. — **Scanu, A. M., A. Khalil, L. Neven, M. Tidore, G. Dawson, D. Pfaffinger, E. Jackson, K. D. Carey, H. C. McGill, and G. M. Fless.** Genetically determined hypercholesterolemia in a rhesus monkey family due to a deficiency of the LDL receptor. *J. Lipid Res.* 1988. **29:** 1671–1681.

Supplementary key words diet and plasma lipoproteins • rhesus monkey lipoproteins • spontaneous hypercholesterolemia • familial hypercholesterolemia • LDL receptor deficiency • genetics of plasma lipoprotein

In man, familial hypercholesterolemia (FH) is commonly secondary to a deficiency of the LDL receptor (1). A hypercholesterolemia with features similar to FH is present in the WHHL rabbit (2), the study of which has enlarged our understanding of the role of the LDL receptor in lipoprotein metabolism (3). In nonhuman primates a spontaneous hypercholesterolemia was reported in 1975 in two rhesus monkeys by Morris and Fitch (4) and Lee and Morris (5); however, its metabolic basis was not established. Studies of these animals by Guertler and St. Clair (6), using skin fibroblast cultures, were inconclusive regarding the nature of the molecular abnormality.

Through a preliminary screening program, we identified a six-member family of rhesus monkeys, three of which exhibited persistent elevation of plasma cholesterol, LDL cholesterol, and apoB while on a low-fat, cholesterol-free Purina Chow diet, and marked hypercholesterolemia following 12 weeks on a high-fat diet. Moreover, the skin fibroblasts of these animals showed a deficiency of the LDL receptor which appeared to account for part of their spontaneous hypercholesterolemia. A preliminary account of these studies has appeared (7).

Abbreviations: FH, familial hypercholesterolemia; LDL, low density lipoproteins; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline.

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MATERIALS AND METHODS

Animals

Screening was carried out on about 100 rhesus monkeys (*Macaca mulatta*) housed at Litton Bionetics (Kensington, MD) while this facility was functioning as a resource center for nonhuman primates with the support of the National Heart, Lung, and Blood Institute. From these monkeys we selected a five-member family in which both dam (766-I) and sire (431-J) were about 14 years of age at the start of the experiments, while the three male offspring (B-7643, B-8806, and B-8204) ranged between 5 and 7 years of age. When Litton Bionetics was no longer able to house the animals, they were transferred to the University of Chicago at which time a fourth male offspring, B-1000, was born from 431-J and 766-I. For the first 3 months, B-1000 was raised on his mother. Subsequently he was caged alone and, like the other animals, was fed monkey chow prepared by the Purina Company (Richmond, Indiana) twice daily and allowed to drink water ad libitum. During one 12-week period, the family was fed a diet containing 12.5% lard and 0.25% cholesterol. B-1000 consumed the challenge diet when he was 1 year old. During the fat challenge, blood samples were taken at 2-week intervals; otherwise the animals were bled, on the average, every 6 weeks. Blood (5–6 ml) was taken from the femoral vein after an overnight fast into tubes containing, at a final concentration, 0.12% Na_2 EDTA and 0.01% sodium azide in addition to gentamycin sulfate (0.8 mg/10 ml blood), chloramphenicol (0.8 mg/10 ml blood), and kallikrein inhibitor (80 units/10 ml blood). The blood was centrifuged at 4°C at 3,000 *g* and the plasma, free of blood cells, was stored at 4°C no longer than 48 hr before analysis. After these studies were completed and the need for further breeding was perceived, the animals were transferred to the Southwest Foundation for Biomedical Research in San Antonio, TX where they were continued on the Purina Chow diet. When needed, blood samples were collected under the same conditions as stated above, and sent to Chicago as plasma packed in wet ice in Styrofoam containers on the same day as the blood collection. The samples were received the following day and processed immediately. All studies of these animals were approved by the Animal Research Committee of the Foundation and were conducted in accord with the guidelines of the National Institutes of Health, the U.S. Department of Agriculture, and the American Association for Accreditation of Laboratory Animal Care.

Lipoprotein separation

Lipoprotein profiles on the plasma of each rhesus monkey were obtained on the average of every 6 weeks by the discontinuous density gradient ultracentrifugation technique of Nilsson et al. (8) as previously adapted to the study of rhesus monkeys (9). Following ultracentrifugation, 0.4-ml fractions were collected and those corresponding to the major lipoproteins, as assessed by continuous absorbance reading at 280 nm in an ISCO recorder, were pooled and dialyzed against saline, 0.05% EDTA, pH 7.0. The fractions so obtained were analyzed for protein and lipid content. The apolipoprotein distribution was also determined by electrophoretic methods (see below).

Electrophoretic analyses of lipoproteins

One percent agarose gel electrophoresis was carried out in 0.065 M barbital buffer, pH 8.6, using an agarose film cassette system from Corning (Palo Alto, CA). For lipoprotein analyses, polyacrylamide gradient gel electrophoresis (PAGE) was carried out under nondenaturing conditions as previously described (9) using polyacrylamide gel concentrations between 2 and 16%. After electrophoresis (150 V, 20 hr, 15°C), the gels were stained with 0.04% Coomassie blue R-250. When whole plasma was analyzed, the lipoprotein bands were identified with Fat Red 7B stain.

Electrophoretic analyses of apolipoproteins were carried out in 2–16% PAGE gels in the presence of 0.1% SDS according to the Laemmli method (10) in a V-16-2 model apparatus (Bethesda Research Laboratories, Inc., Gaithersburg, MD). Western blot analyses using antibodies raised in the rabbit against either LDL or Lp[a] were conducted after electrophoretic transfer to 0.45 μm nitrocellulose paper at 10°C, 30V for 18 hr in a buffer consisting of 20% methanol, 25 mM Tris, and 192 mM glycine.

Quantitation of apoB in whole plasma

The immunoquantification of apoB in the whole plasma was carried out by radial immunodiffusion (11) utilizing antisera raised in the rabbit against lipoprotein[a] or Lp[a]-free low density lipoproteins.

Preparation of LDL for binding studies

For binding studies, LDL was isolated by ultracentrifugal flotation from either normolipidemic human or rhesus monkey plasma having undetectable levels of Lp[a] in the density range between 1.025 and 1.045 g/ml using a Ti 50.2 rotor at 60,000 rpm for 24 hr at 10°C. The top fraction was refloated for an additional 24 hr in a d 1.063 g/ml solution. The final LDL preparations gave a single band by 1% agarose gel electrophoresis and 2–16% gradient PAGE (stain: Red Oil O). They also exhibited a single band corresponding to apoB-100 by SDS-GGE (2–16%) either in the presence or absence of β-mercaptoethanol. LDL samples free of Lp[a] were prepared about every fourth week and were kept in a d 1.063 g/ml solution at 4°C containing 0.02% EDTA and 20 μM vitamin E as an antioxidant until use, no longer than 1 week. Before each experiment, appropriate aliquots were dialyzed for 24 hr

against several changes of 150 mM NaCl at 4°C. LDL was labeled by the iodine monochloride method essentially according to Bilheimer, Eisenberg, and Levy (12) using 0.5 mCi of ^{125}I/mg LDL protein. The unincorporated radioiodine was separated from the labeled protein by passage through a G-25 Sephadex column equilibrated in 10 mM Tris, 0.9% NaCl, pH 7.5. The specific activities of the final product ranged between 50 and 300 cpm/ng protein. In a typical preparation, 97% of the radioactivity was precipitated by 10% trichloroacetic acid; only 2–5% of the total counts was extracted by chloroform–methanol 2:1 (v:v).

Preparation of lipoprotein-deficient serum (LPDS)

LPDS was prepared monthly from Gibco fetal calf serum kept frozen and then thawed for the purpose. All of the serum lipoproteins were floated at d 1.250 g/ml in a 50.2 Ti rotor at 49,000 rpm for 48 hr at 14°C. The d 1.250 g/ml bottom had no significant amount of cholesterol by a standard enzymatic cholesterol assay (see below). After an extensive dialysis against 150 mM NaCl and 0.02% EDTA, the LPDS was heat-inactivated for 30 min at 56°C, filtered through a 0.45-μm Millipore filter, and aliquots were stored in sterile tubes at − 80°C.

Skin fibroblast cultures

Skin biopsies, 15–20 mm in diameter, were obtained from the thigh region of each member of the rhesus monkey family as well as from nine normolipidemic controls and five control human subjects. Monkeys were immobilized with ketamine hydrochloride (10 mg/kg) and the biopsy site was prepared as for aseptic surgery. Intravenous sodium pentothal (15 mg/kg) was administered immediately before biopsy. Explants from each biopsy were placed in 25-cm^2 tissue culture flasks and allowed to grow in MEM medium supplemented with 10% heat-inactivated fetal calf serum, 20 mM/ml L-glutamine, 100 I.U. penicillin, and 100 μg streptomycin/ml medium in a humidified incubator at 37°C in a 10% CO_2 atmosphere. The growth of the cells was followed daily until they reached confluency. The cells were then trypsinized and transferred to 75-cm^2 flasks. Cell splitting was carried out at least four times, and once optimal growth conditions were achieved the cells were either used immediately or frozen at − 70°C in bovine serum containing 10% DMSO. Skin fibroblasts from patients with homozygous and heterozygous FH were purchased from the National Institute of General Medical Sciences, Human Mutant Cell Repository (Camden, NJ).

Binding of ^{125}I-labeled LDL

Fibroblasts were grown in six-well Costar plates until they reached 80% confluency in MEM containing 10% fetal bovine serum. In order to maximize the expression of the LDL receptor, the cells were incubated for 48 hr in MEM containing 10% bovine LPDS. Prior to the assay the medium was replaced with 2 ml ice-cold DMEM–10% LPDS containing ^{125}I-labeled LDL in the concentrations of 1, 2, 5, 10, and 25 μg/ml in the presence or absence of 50-fold excess unlabeled LDL protein. After 2 hr incubation at 4°C on a rotary shaker (60 rpm), the plates were washed three times with ice-cold 150 mM NaCl–50 mM Tris-HCl buffer, pH 7.4, containing 2 mg bovine serum albumin/ml and then twice with a Tris-NaCl buffer in order to remove the unbound ^{125}I-labeled LDL. To each well 1 ml of 0.1 M NaOH was added, the solubilized cells were collected in glass test tubes, and the bound radioactivity was counted in a scintillation counter. The protein content of each well was determined by the method of Lowry et al. (13) using bovine serum albumin as a standard. Specific binding was calculated as the difference between total counts and those observed in the presence of 50-fold excess of unlabeled LDL.

Internalization studies

The experimental conditions were the same as for the binding studies except that the incubation step was carried out for 2 hr at 37°C and the concentration of ^{125}I-labeled LDL varied between 1 and 50 μg/ml in the presence and absence of 50-fold excess unlabeled LDL protein. In each case the incubation reaction was stopped by placing the cells in ice, after which they were washed following the same protocol as for the 4°C binding. Thereafter, 2 ml of 50 mM NaCl–10 mM HEPES buffer, pH 7.4, containing 1000 U sodium heparin was added to each monolayer and the mixture was placed in a rotary shaker (60 rotations/min) for 60 min at 4°C. The heparinated buffer was collected and the number of counts was taken to represent the amount of ^{125}I-labeled LDL releasable from the cell surface. The cells were solubilized in 1 ml 0.1 M NaOH for 15 min at room temperature and aliquots of the cell suspension were counted to determine the amount of internalized ^{125}I-labeled LDL. The protein content of the cell suspension was determined by the method of Lowry et al. (13). The specific internalization was calculated by subtracting from the total radioactivity the nonspecific counts obtained in the presence of 50-fold excess unlabeled LDL protein and the background, i.e., radioactivity in the medium without cells. The results were expressed in terms of radioactivity/mg cell protein.

Ligand blot studies

Skin fibroblasts were incubated in MEM containing 10% LPDS for 48 hr before they were lysed. Each plate (100-mm Petri dish) of cells was solubilized in 0.5 ml of lysis buffer (10 mM HEPES, pH 7.4, 25 mM $MgCl_2$, 200 mM NaCl, 2 mM $CaCl_2$, 0.5 mM PMSF, 0.5 mg/ml leupeptin, 15 μg/ml soybean trypsin inhibitor, 1% Triton X-100 and 1000 KIU/ml kallikrein inactivator). Cells were scraped from the plate, placed in microfuge tubes, vor-

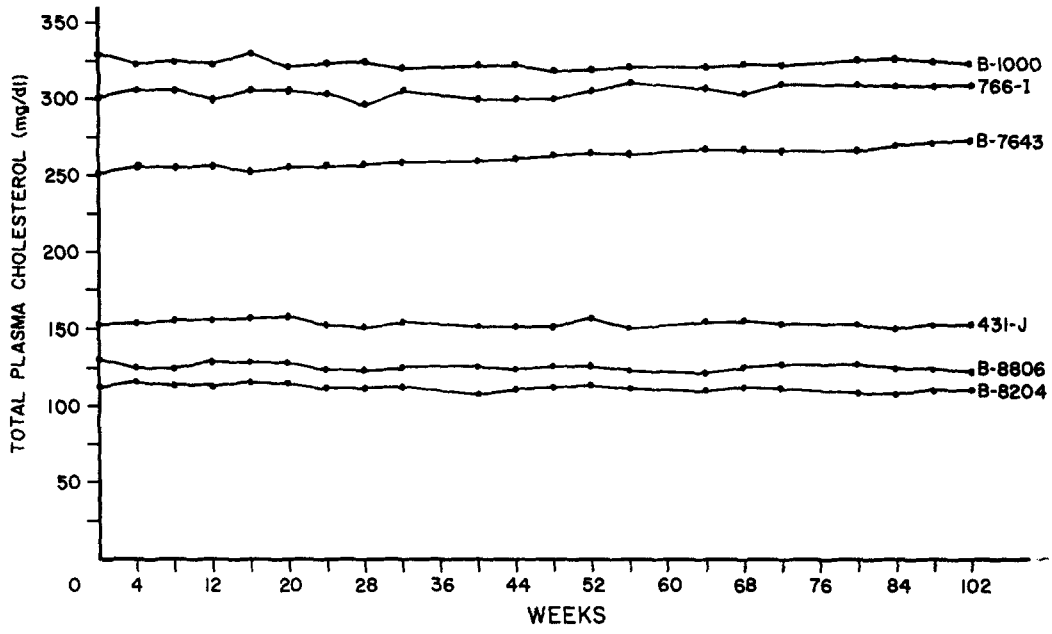

Fig. 1. Plasma cholesterol values of members of a rhesus monkey family fed a cholesterol-free Purina Chow diet.

texed for 30 sec, and incubated for 10 min on ice. The cell lysates were then centrifuged at 100,000 *g* for 20 min at 4°C using a TL-100.1 rotor in a Beckman TL-100 ultracentrifuge. The pellet containing nuclear material was discarded and the supernatant was saved. The protein content of supernatants of cell lysate precipitated by 10% TCA was determined following the method of Lowry et al. (13).

SDS-PAGE (6%) was carried out according to the method of Laemmli (9). Samples were not reduced or heated before electrophoresis. Equal concentrations, approximately 100 μg, of each cell lysate were added per well. Thereafter, the samples were electrophoretically transferred to 0.45 μm nitrocellulose paper at 10°C, 30V for 18 hr in a buffer of 20% methanol, 25 mM Tris, and 192 mM glycine. Transferred samples were blocked for 90 min at 37°C in blocking buffer (50 mM Tris, 2 mM $CaCl_2$, 90 mM NaCl, 50 mg/ml BSA, pH 8.0). The nitrocellulose paper was then incubated with 20 μg/ml ^{125}I-labeled LDL (~300 cpm/ng) in blocking buffer for 1 hr at room temperature. The nitrocellulose paper was washed with a buffer of 50 mM Tris, 2 mM $CaCl_2$, 90 mM NaCl, 5 mg/ml BSA, pH 8.0. The paper was then washed twice for 10 sec, twice for 20 min, then twice for 10 sec again. Thereafter the paper was dried and autoradiographed overnight using Kodak X-AR5 X-ray film.

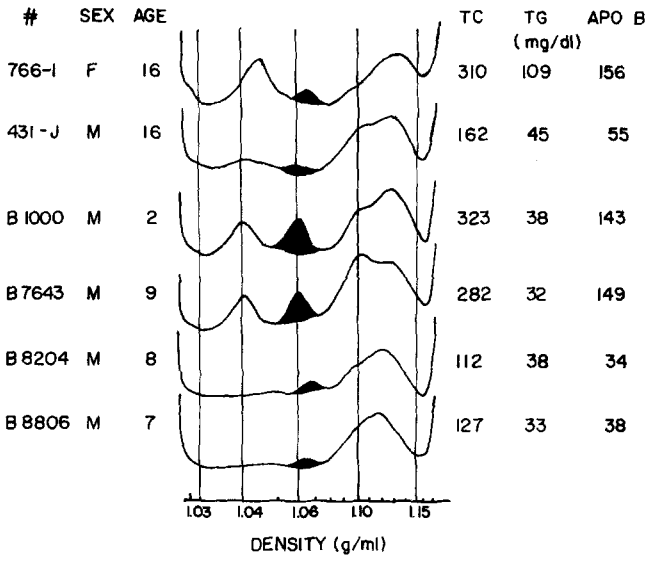

Fig. 2. Density gradient ultracentrifugal profiles of plasma samples from each members of the rhesus monkey family. The values of total cholesterol (TC), triglycerides (TG), and apoB were obtained on the same sample used for ultracentrifugal analysis. Lp[a] is indicated by the shaded area.

Immunoblot studies

The cell membrane extracts were prepared and separated on SDS-PAGE as for the ligand blot studies. The samples were always used fresh and were never frozen. Following electrophoretic transfer of the samples from SDS-PAGE, the nitrocellulose paper was blocked for 1 hr at room temperature in 10 mM Tris, pH 8.0, 50 mM NaCl, 2 mM $CaCl_2$, 1% milk (Carnation Instant Non-fat Dry Milk) and washed one time with PBS-milk buffer (10 mM NaH_2PO_4-Na_2HPO_4 buffer, 0.9% NaCl, 0.002% NaN_3, pH 8.0, 1% dry milk). This washing step was followed by a 90-min incubation at room temperature in the presence of PBS-milk buffer and 1 μl/ml of a polyclonal anti-LDL receptor serum raised in the rabbit against a partially purified preparation of bovine adrenal LDL

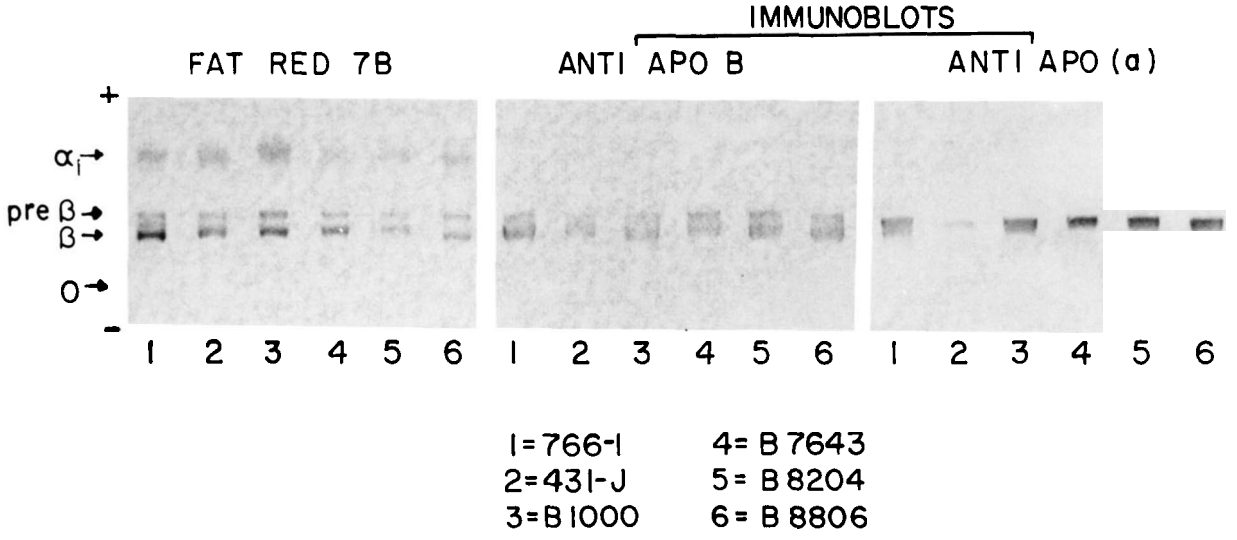

Fig. 3. One percent agarose gel electrophoresis of plasma samples from each family member. After electrophoretic separation, the pherograms were either stained by Fat Red 7B (left panel), Western blot against anti-apoB (center panel), or anti-apo[a] (right panel); O, origin.

receptor, a gift from Dr. Liang Ping of the Department of Pathology at the University of Chicago. The paper was subsequently washed three times in the PBS-milk buffer to remove unbound antibody and finally incubated with 0.4 μl ^{125}I-labeled Protein A (Dupont, 133.8 μCi/ml, 8.3 μCi/μg) per ml of PBS-milk buffer for 90 min at room temperature. The blot was washed five times in the PBS-milk buffer followed by two washes with PBS without milk. The blot was dried and autoradiographed for 48–52 hr using Kodak X-AR5 X-ray film.

Scanning laser densitometry

The relative areas of the autoradiographs obtained from the ligand and immunoblot studies were determined using an LKB Ultrascan XL Laser Densitometer with an Epson FX-86E line printer.

Chemical analyses

Total protein determinations were carried out according to the method of Lowry et al. (13). Total and unesterified

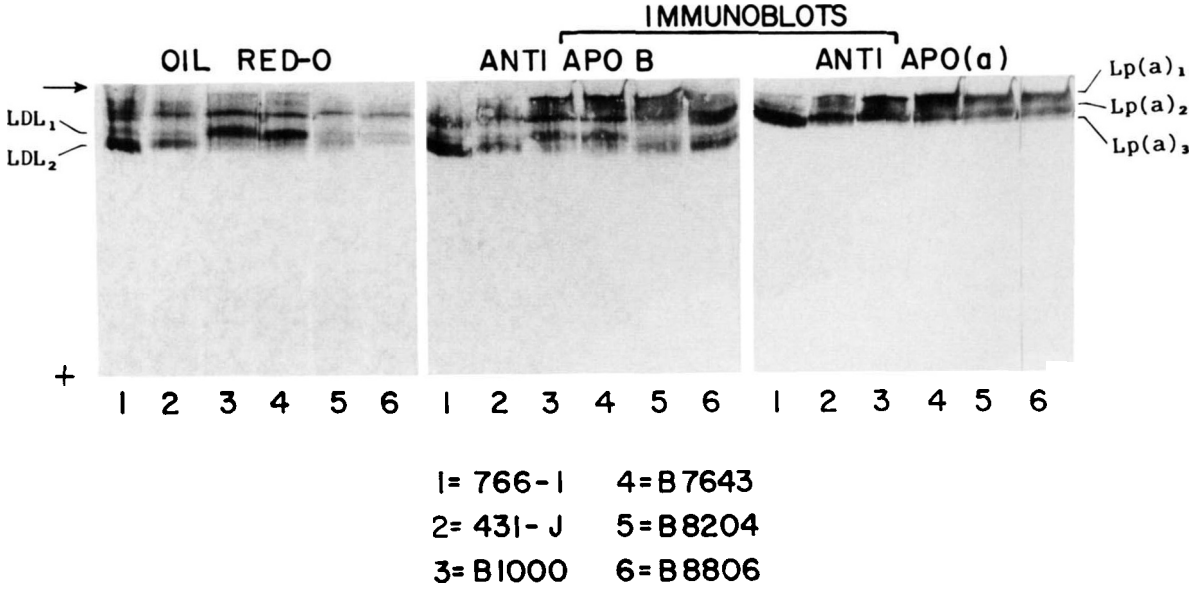

Fig. 4. Two to sixteen percent gradient gel electrophoresis of plasma samples from each family member. After electrophoretic separation, the pherograms were either stained by Fat Red 7B (left panel), Western blot against anti-apoB (center panel), or anti-apo[a] (right panel); arrow marks the origin.

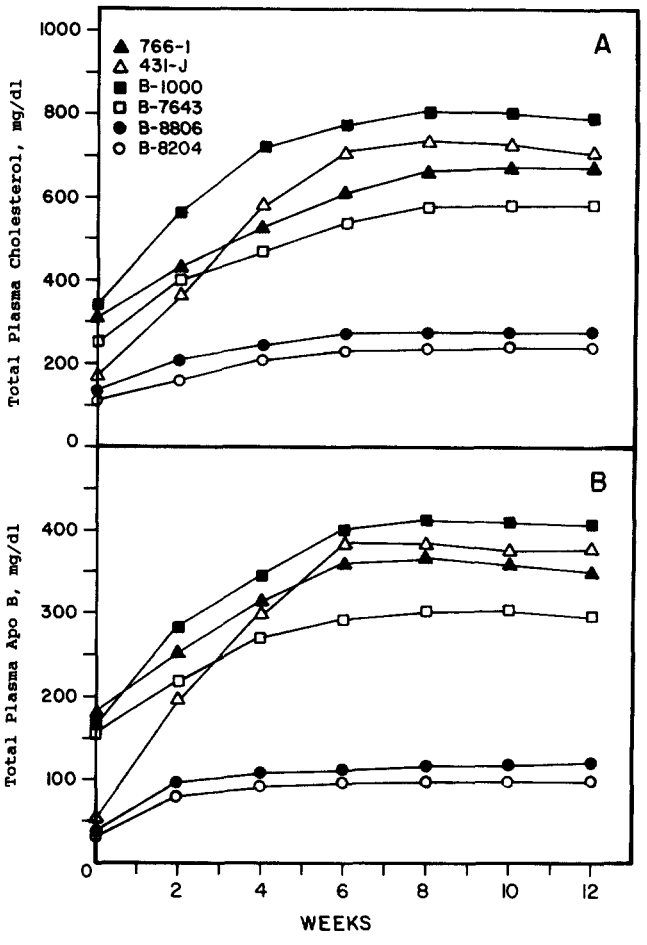

Fig. 5. Response of plasma total cholesterol (panel A) and apoB (panel B) of the rhesus monkeys fed a diet containing 25% lard, 0.25% cholesterol. Between 8 and 12 weeks after the animals were returned to a Purina Chow diet a normalization of these parameters was observed.

cholesterol, phospholipid, and triglyceride analyses were determined as previously described (14, 15).

RESULTS

Baseline results

The total plasma cholesterol analyses carried out as a function of time (**Fig. 1**) while the animals were fed a cholesterol-free Purina Chow diet showed that dam (766-I) and offspring B-1000 and B-7643 had a significant elevation of the total plasma cholesterol while the sire, 431-J, and offspring B-8204 and B-8806 were normocholesterolemic. All animals were normotriglyceridemic except that 766-I exhibited, although not persistently, triglyceride levels around 110 mg/dl (**Fig. 2**). Fractionation of the whole plasma by 1% agarose electrophoresis showed (by Fat Red 7B stain) that all of the animals exhibited β and pre-β bands identified as LDL and Lp[a] by the results of the Western blot analyses (**Fig. 3**). These results were corroborated by the 2-16% GGE data in nondenaturing gels showing a size heterogeneity of both LDL and Lp[a] particles (**Fig. 4**). As judged by density gradient ultracentrifugation, the animals and spontaneous hypercholesterolemia had a significant elevation of the LDL peak (Fig. 2). The assignment of the peak as LDL was based on *1*) position in the ultracentrifugal gradient; *2*) beta migration on 1% agarose gel electrophoresis with a band reacting against a polyclonal antibody specific for apoB by Western blot analyses; and *3*) molecular weight range of 500,000 in SDS-PAGE in the presence of 1% SDS (data not shown). All animals also had a band corresponding to Lp[a] (shadowed area in Fig. 2) as assessed by the following criteria: *1*) pre-β migration by 1% agarose gel electrophoresis; *2*) molecular weight higher than LDL in nondenaturing gradient PAGE and reactivity against both anti-LDL and anti-apo[a] antisera by Western blot analyses. There was no quantitative correlation between the LDL and Lp[a] band areas in each of the gradients. In all animals the LDL exhibited similar protein-to-lipid distributions that were in keeping with previous results by this laboratory (14, 15). Compositional data expressed per mole of LDL using the average molecular weight of $2.90 + 10^6$ from previous reports (14, 15) showed the following: protein, 580,000-610,000 g; unesterified cholesterol, 550-690 moles; cholesteryl esters, 1,500-1,900 moles; and triglycerides, 230-300 moles. We made no attempt to examine subfractions LDL-I and LDL-II that we previously reported (14, 15).

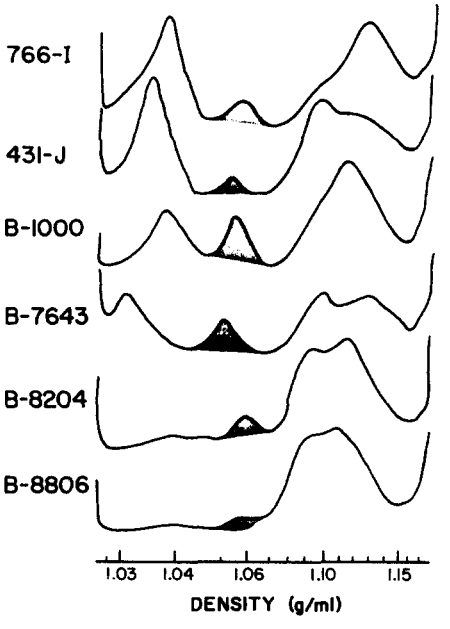

Fig. 6. Density gradient ultracentrifugal profiles of plasma samples from each member of the rhesus monkey family after 12 weeks on a diet containing 12.5% lard and 0.25% cholesterol.

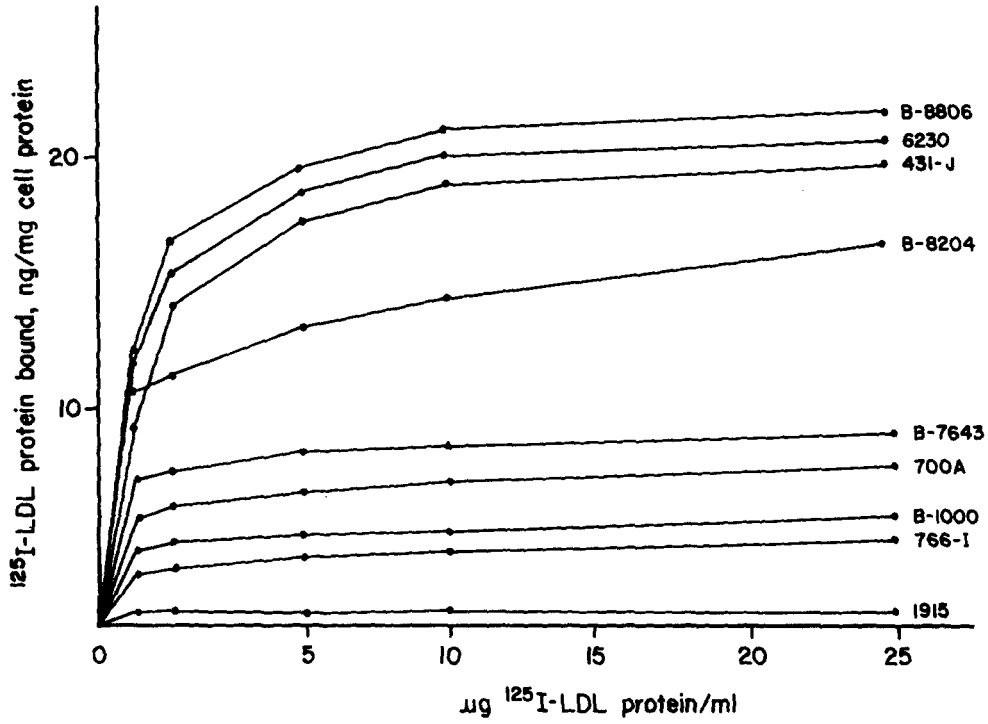

Fig. 7. Binding at 4°C of human ^{125}I-labeled LDL to skin fibroblasts of members of rhesus monkey family and controls. The results are the average of two experiments; 6230, normal rhesus monkey, non-member of the family; 700A, heterozygous FH obtained from NIGMS; and 1915, homozygous FH obtained from NIGMS.

Response of animals to a diet containing 12.5% lard and 0.25% cholesterol

Eating the high-fat, high-cholesterol diet for 12 weeks caused changes in plasma cholesterol LDL and apoB that differed markedly among animals. As shown in **Fig. 5A**, the plasma cholesterol levels reached a plateau after about 8 weeks, the highest responses (range 700–800 mg/dl) being exhibited by the sire 431-J and B-1000 followed by the dam 766-I and B-7643 (range 500–600 mg/dl). In contrast, both B-8204 and 8806 had a low response, below the 300 mg/dl range. The total plasma apoB (Fig. 5B) exhibited a similar response pattern. Based on the results of single-step ultracentrifugal analyses, fat feeding caused a marked elevation of the LDL peak with a shift to a lower density in 431-J and 766-I as compared to the baseline profiles in Fig. 2. The elevation of LDL was of a lesser degree in B-7643. In turn, no major changes were seen in B-8204 and B-8806, in keeping with their moderate plasma cholesterol response (**Fig. 6**). There were no significant changes of the Lp[a] area before and after dietary challenge, a finding in keeping with previous observations by this laboratory (14). Upon restoration of the Purina Chow diet, plasma cholesterol, apoB, and the single-spin profile returned to prechallenge values, but it took from 8 to 12 weeks for these variables to become normal. Hyperresponsiveness to the high fat diet was exhibited by the animals that had baseline hypercholesterolemia. An exceptional case was 431-J, which was normocholesterolemic on chow, but exhibited a marked hypercholesterolemia following the challenge diet.

TABLE 1. Binding at 4°C of ^{125}I-labeled LDL to skin fibroblasts from members of the rhesus monkey family and control cells

Origin of Cells	Constant of Dissociation K_d,[a] M
Family members	
766-I	$1.41 \pm 0.08 \times 10^{-9}$
431-J	$0.67 \pm 0.06 \times 10^{-9}$
B-1000	$0.74 \pm 0.20 \times 10^{-9}$
B-7643	$0.66 \pm 0.12 \times 10^{-9}$
B-8204	$0.48 \pm 0.07 \times 10^{-9}$
B-8806	$1.35 \pm 0.26 \times 10^{-9}$
Controls	
6230 (normal rhesus)	$1.28 \pm 0.19 \times 10^{-9}$
700A (human heterozygous FH)	$0.68 \pm 0.18 \times 10^{-9}$

[a] The data are the average of two experiments calculated according to Yamaoka et al. (28). By this program, which is based on the damping Gauss-Newton method, the data points were analyzed using a nonlinear least-squares fit computer program from which the K_d values were derived.

Cell studies

Binding and internalization results. In preliminary studies carried out at 4°C, we established that Lp[a]-free LDL

TABLE 2. Internalization at 37°C of ^{125}I-labeled LDL by skin fibroblasts from members of the rhesus monkey family and control cells

Origin of Cells	Internalization ng ^{125}I-labeled LDL/mg cell protein
Family members	
766-I	327 ± 10
431-J	772 ± 62
B-1000	357 ± 25
B-7643	294 ± 38
B-8204	1097 ± 80
B-8806	1528 ± 110
Controls	
6230 (normal rhesus)	898 ± 51
700A (human heterozygous FH)	352 ± 35
1915 (human homozygous FH)	89 ± 10

[a]The data are the average of two experiments.

isolated from normal human subjects had the same binding affinity to control rhesus monkey fibroblasts as the corresponding preparation isolated from the plasma of rhesus monkey fed a Purina Chow diet. Thus, in subsequent studies we found it practical to use only human LDL. As shown in **Fig. 7**, the skin fibroblasts from B-7643, B-1000, and 766-I bound less ^{125}I-labeled LDL than the cells from B-8806, B-8204, and 431-J. Saturation was reached between 5 and 10 μg LDL protein/ml. The GM1915B cell line obtained from a homozygous FH patient exhibited negligible binding of ^{125}I-labeled LDL; in turn, the binding by 700A cells obtained from a heterozygous FH human subject was between those by B-7643 and B-1000. The dissociation constants (K_d), calculated from these results by computer-assisted nonlinear regression analyses (16), were of the same order in all animals studied (see **Table 1** for details) indicating that binding differences were due not to affinity but to LDL receptor number. The internalization studies shown in **Table 2** were in keeping with the binding experiments; that is, there was a correlation between 37°C internalization and maximum binding at 4°C of radiolabeled LDL.

Ligand blot studies. The ligand blot analyses revealed important differences among the animals studied (**Fig. 8** and **Table 3**). From densitometric scans, the two offspring, B-8806 and B-8204, had LDL receptor levels in the range of that of normal control monkey 6277, whereas sire 431-J had levels about three times above normal. On the other hand, in the dam 766-I and offspring, B-1000 and B-7643, the receptor levels were significantly lower than control. The dam gave scanning data that were similar to those of the homozygous FH cells GM-0486. Cells from all animals had a receptor with a molecular weight of about 160,000.

Immunblot studies. The autoradiographs of the immunoblots and relative densitometric scannings are shown in **Fig. 9** and Table 3, respectively. The sire, 431-J, had levels of immunodetectable receptor that were about 40% higher than that of the normal control; in turn, offspring B-8204 and B-8806 had levels that were much closer to those of normal cells. The densitometric readings of 431-J, B-8204, and B-8806 did not take into account the presence of a high molecular weight band probably representing a dimer as suggested by the recent work by Van Driel et al. (17). This high molecular weight component was not seen by the ligand blot, a finding that indicated its difference in affinity for ligand LDL and the LDL receptor antibody. The densitometric readings of immunoblots of 766-I were only about 50% of normal, whereas B-1000 and B-7643 exhibited levels comparable to that of the FH cells GM-0486. Overall, the immunoblot analyses were in keeping with the results of the ligand blot data except for the magnitude of the changes, probably because the two techniques are probing two different LDL-receptor functions (see Discussion).

DISCUSSION

As summarized in **Fig. 10**, the present studies have identified a family of six rhesus monkeys in which the dam and two offspring, B-1000 and B-7643, exhibited persistent hypercholesterolemia on a chow diet. The hypercholesterolemia was markedly enhanced by a dietary fat

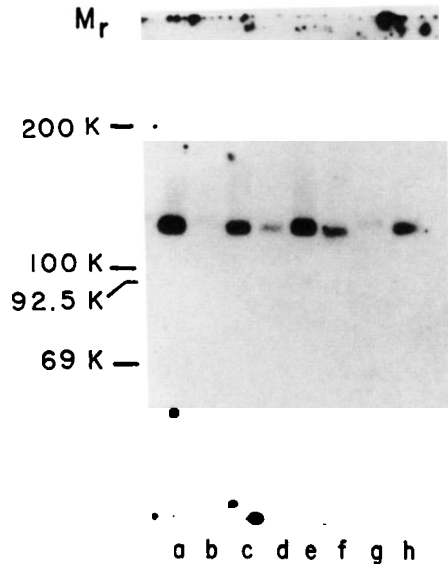

Fig. 8. Ligand blot of a 6% SDS-polyacrylamide gel electrophoresis of rhesus and human skin fibroblast lysates. Lanes: a, 431-J; b, 766-I; c, B-8204; d, B-1000; e, B-8806; f, B-7643; g, GM-0486 (a receptor-negative human skin fibroblast cell line; h, 6277 (normal rhesus skin fibroblast cell line). One hundred μg total lysate protein was added per well. Nitrocellulose blot was developed with ^{125}I-labeled LDL (3×10^5 cpm/μg). Autoradiograph was developed 24 hr at − 80°C; M_r, molecular weight. The ^{14}C standards used were myosin (200,000), phospholipase B (100,000 and 92,500) and BSA (69,000). They were purchased from Amersham Co., Arlington Heights, IL.

TABLE 3. Summary of ligand and immunoblot analyses

Fibroblast Line	Ligand Blot			Immunoblot		
	Area[a]	% Normal[b]	± % Error	Area[a]	% Normal[b]	± % Error
766-I	0.26	12.9	3.1	0.83	49.0	5.7
431-J	6.17	301.0	19.1	2.35	139.5	26.1
B-1000	0.77	37.5	10.8	0.21	12.7	0.4
B-7643	1.27	61.8	4.6	0.44	25.8	1.4
B-8204	2.80	137.0	2.8	1.40	83.0	7.6
B-8806	4.47	218.5	16.2	1.07	63.5	2.0
GM-0486	0.16	7.7	2.3	0.57	33.8	1.8
6277	2.05	100.0		1.69	100.0	

The data are the average of three separate experiments.
[a]Calculated from laser densitometry readings.
[b]Data expressed using fibroblasts from control #6277 = 100.

and cholesterol challenge. An LDL receptor deficiency was detected in cultures of skin fibroblasts by binding, internalization, ligand, and immunoblot analyses. Two additional phenotypes were observed: one was represented by baseline normocholesterolemia, high response to dietary fat and cholesterol challenge, normal LDL receptor function (sire); and the other by baseline normocholesterolemia, low response to the dietary fat and cholesterol challenge, and normal LDL receptor function (B-8204 and B-8806).

The occurrence of spontaneous hypercholesterolemia in nonhuman primates is rare. Eggen et al. (18), in a survey of 811 wild-caught rhesus monkeys, found no animals with this disorder. Thus far, a similar abnormality was observed in only two rhesus monkeys by Morris and Fitch (4) and Lee and Morris (5). At the time of the first report in 1968, these animals, while on a cholesterol-free diet, had average total plasma cholesterol concentrations of 436 and 526 mg/dl. About 6 years later these levels had increased to 705 and 725 mg/dl, respectively. Like our rhesus monkeys 766-I, B-1000, and B-7643, the increased plasma cholesterol in these animals (4, 5) was largely carried by the LDL fraction. However, when Guertler and St. Clair (6) examined the activity of the LDL receptor in skin fibroblast cultures, the animals had no significant defect in the LDL receptor or in regulation of HMG-CoA-reductase activity. Moreover, Guertler and St. Clair (19), when examining skin fibroblast cultures of low and high responder squirrel monkeys, found no differences in their LDL receptor activity as compared to control animals. In contrast to these early reports, the monkeys that in the current studies had a spontaneous hypercholesterolemia were also LDL receptor-deficient, thus documenting for the first time the occurrence in nonhuman primates of a familial hypercholesterolemia with features similar to those of human FH. It is interesting to note that at the time of the study none of the affected animals had evidence of corneal arcus, xanthelasmas or xanthomas, manifestations often seen in human FH.

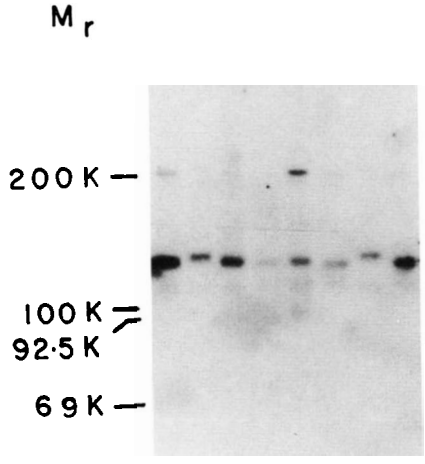

Fig. 9. Immunoblot of a 6% SDS-polyacrylamide gel electrophoresis of rhesus and human skin fibroblast lysates. Lanes: a, 431-J; b, 766-I; c, B-8204; d, B-1000; e, B-8806; f, B-7643; g, GM-0486 (a receptor-negative human skin fibroblast cell line; h, 6277 (normal rhesus skin fibroblast cell line). One hundred μg total lysate protein was added per well. Nitrocellulose blot was developed with rabbit polyclonal antibody to partially purified bovine adrenal LDL-receptor (1 μl/ml) and ^{125}I-labeled Protein A (0.053 μCi/ml). Autoradiograph was developed 48 hrs at − 80°C with one intensifying screen. The molecular weight markers were the same as in Fig. 8.

Although an LDL receptor deficiency was well documented by the skin fibroblast data, the results of the ligand and immunoblot analyses deserve comment from the quantitative standpoint. In the case of the dam, the ligand blot technique revealed a degree of LDL receptor deficiency much higher than that determined by the immunoblot analyses and this was also true of the GM-0486 cells derived from a patient with established homozygous FH. Based on these results it would be difficult to determine the actual degree of LDL receptor deficiency in those animals since ligand LDL and the LDL receptor an-

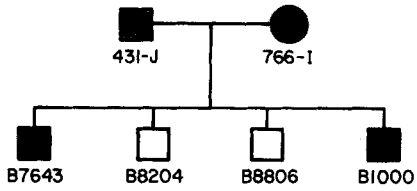

Fig. 10. Summary of the results in the six-member rhesus monkey family: (□), baseline normocholesterolemia, low response to fat challenge, normal LDL receptor activity; (▨), baseline normocholesterolemia, high response to fat challenge, normal LDL receptor activity; (■), baseline hypercholesterolemia, high response to fat challenge, LDL-receptor deficiency.

tibody used in this study likely probed different domains of the LDL receptor. Thus, additional data are required and useful information should be obtained from pulse-chase studies as well as from DNA and RNA analyses now in progress. The results with the two offspring, B-1000 and B-7643, invite similar consideration.

The results of the current studies also suggest that the LDL receptor deficiency was responsible for the hyperresponsiveness to a high-fat dietary challenge of the animals with baseline hypercholesterolemia. On the other had the sire, which was a hyperresponder with no LDL receptor deficiency, represents a different metabolic phenotype not explained by an abnormality in the LDL receptor. Thus far no unifying molecular mechanism has been found to explain the divergent response of animals to a dietary fat and cholesterol challenge (20–26). Also unexplained is the molecular basis for hyporesponsiveness exemplified in our animals B-8204 and B-8806.

Lp[a] particles were present in all family members. Our ultracentrifugal assays indicate that the levels of the particles were unrelated to the overall plasma apoB and to the fat challenge. Little is yet known about the metabolism and site(s) of degradation of Lp[a] (27, 28). Our family, comprising both normal and LDL receptor-deficient animals, should be valuable in comparing the degradation of LDL and Lp[a] particles.

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