AIBP protects against metabolic abnormalities and atherosclerosis

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Abstract Apolipoprotein A-I binding protein (AIBP) has been shown to augment cholesterol efflux from endothelial cells and macrophages. In zebrafish and mice, AIBP-mediated regulation of cholesterol levels in the plasma membrane of endothelial cells controls angiogenesis. The goal of this work was to evaluate metabolic changes and atherosclerosis in AIBP loss-of-function and gain-of-function animal studies. Here, we show that Apoa1bp<sup>−/−</sup>Ldlr<sup>−/−</sup> mice fed a high-cholesterol, high-fat diet had exacerbated weight gain, liver steatosis, glucose intolerance, hypercholesterolemia, hypertriglyceridemia, and larger atherosclerotic lesions compared with Ldlr<sup>−/−</sup> mice. Feeding Apoa1bp<sup>−/−</sup>Ldlr<sup>−/−</sup> mice a high-cholesterol, normal-fat diet did not result in significant differences in lipid levels or size of atherosclerotic lesions from Ldlr<sup>−/−</sup> mice. Conversely, adeno-associated virus-mediated overexpression of AIBP reduced hyperlipidemia and atherosclerosis in high-cholesterol, high-fat diet-fed Ldlr<sup>−/−</sup> mice. Injections of recombinant AIBP reduced aortic inflammation in Ldlr<sup>−/−</sup> mice fed a short high-cholesterol, high-fat diet. Conditional overexpression of AIBP in zebrafish also reduced diet-induced vascular lipid accumulation. In experiments with isolated macrophages, AIBP facilitated cholesterol efflux to HDL, reduced lipid rafts content, and inhibited inflammatory responses to lipopolysaccharide.

Our recent work and the work of others have highlighted apolipoprotein A-I binding protein (AIBP), which augments cholesterol efflux from endothelial cells (9, 10) and macrophages (11) to HDL. AIBP is a secreted protein disulfide isomerase, with apoA-I (12). Human APOA1BP mRNA encoding AIBP is ubiquitously expressed, with the highest expression in kidney, heart, liver, thyroid gland, adrenal gland, and testis (12). AIBP is found in cerebrospinal fluid and urine and in plasma of patients with sepsis (12). The human APOA1BP gene is located at 1q21.2-1q22 on chromosome 1, which corresponds to the 1q21-q23 locus for familial combined hypercholesterolemia (FCH). The human APOA1BP gene contains a supplement.

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality in the United States, accounting for approximately one-third of all US deaths (1). Therapy to reduce LDL cholesterol (LDL-C) has led to remarkable improvement in clinical outcomes, decreasing the incidence of acute cardiovascular events by 25–35% (2, 3). Statins, ezetimibe, and recently apoB antisense and PCSK9 monoclonal antibodies all target plasma LDL-C as a major atherogenic factor. However, improving the other arc of cholesterol homeostasis—reverse cholesterol transport and atheroprotective HDL—has proven to be a challenging task. Results of clinical trials of cholesteryl ester transfer protein inhibitors put in doubt the efficacy of simply raising HDL cholesterol (HDL-C) levels as an ultimate therapeutic goal (4). Although HDL-C clearly correlates with atheroprotection in epidemiology studies, the current consensus in the cardiovascular field underscores the added importance of improving the functionality of HDL (5–8). However, in vivo mechanisms regulating HDL function are not sufficiently understood.

Our recent work and the work of others have highlighted apolipoprotein A-I binding protein (AIBP), which augments cholesterol efflux from endothelial cells (9, 10) and macrophages (11) to HDL. AIBP is a secreted protein discovered in a screen of proteins that physically associate with apoA-I (12). Human APOA1BP mRNA encoding AIBP is ubiquitously expressed, with the highest expression in kidney, heart, liver, thyroid gland, adrenal gland, and testis (12). AIBP is found in cerebrospinal fluid and urine and in plasma of patients with sepsis (12). The human APOA1BP gene is located at 1q21.2-1q22 on chromosome 1, which corresponds to the 1q21-q23 locus for familial combined hypercholesterolemia.

Supplementary key words hypercholesterolemia • hypertriglyceridemia • lipid rafts • hepatosteatosis

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Abbreviations: AAV, adeno-associated virus; AIBP, apolipoprotein A-I binding protein; CTB, cholera toxin B; dpf, days postfertilization; FIB, fibronectin secretion sequence; HDL-C, HDL cholesterol; HFD, high-fat, normal-cholesterol diet; LDL-C, LDL cholesterol; LPS, lipopolysaccharide; qPCR, quantitative PCR; WAT, white adipose tissue.

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hyperlipidemia, a common multifactorial and heterogeneous dyslipidemia predisposing to premature coronary artery disease (13). Yet, there are no studies directly linking AIBP polymorphism with dyslipidemia or risk of CVD.

AIBP does not bind cholesterol or induce cholesterol efflux in the absence of HDL or apoA-I, but it does increase the turnover of HDL and thus accelerates cholesterol efflux (9). The goal of this work was to evaluate metabolic changes and atherosclerosis in AIBP loss-of-function and gain-of-function animal studies. We report that AIBP deficiency exacerbates weight gain, hyperlipidemia, and atherosclerosis, while over-expression of AIBP is protective against vascular lipid accumulation, atherosclerosis, and metabolic abnormalities.

MATERIALS AND METHODS

Animals and diets

All animal experiments were conducted according to protocols approved by the Institutional Animal Care and Use Committee of the University of California at San Diego. Mice were housed up to five per standard cage at room temperature and maintained on a 12:12 h light:dark cycle, with lights on at 07:00. Both food and water were available ad libitum. Wild-type C57BL/6 and Ldlr−/− mice were initially purchased from the Jackson Laboratory (Bar Harbor, ME) and bred in-house for experiments. Apoa1bp−/− mice on a C57BL/6 background were generated in our group as previously described (10) and cross-bred with Ldlr−/− mice. Apoa1bp−/− Ldlr−/− mice were initially purchased from the Jackson Laboratory and bred in-house for experiments. Additional mice were available ad libitum. Wild-type C57BL/6 and Ldlr−/− mice develop and breed normally. For metabolic studies, mice on a C57BL/6 background were fed a high-fat diet (HFD; Research Diets D12492; supplemental Table S2) containing 45% kcal from fat, starting at age 10 weeks. For metabolic and atherosclerosis studies, mice on an Ldlr−/− background were fed either a Western diet (Teklad TD.96121; supplemental Table S2) containing 42% kcal from fat (21% milkfat) and 1.25% cholesterol or a high-cholesterol, normal-fat diet (Teklad TD.97131; supplemental Table S3) containing 1% cholesterol, starting at age 8 weeks.

Adult zebrafish were maintained at 28°C on a 14 h light/10 h dark cycle as previously described (14) and fed brine shrimp twice a day. Zebrafish larvae were fed Golden Pearls (Brine Shrimp Direct, Ogden, UT) twice a day, starting from 5 days postfertilization (dpf). The transgenic hsp70:apoa1bp-2A-mRFP zebrafish was generated by using constructs and methods previously described (9, 15, 16). The self-cleavage 2A peptide allows for expression of two separate proteins in the same tissue, with mRFP serving as an indicator of successful protein expression. Transgene expression was initiated by single or repeated heat shocks (transferring zebrafish for 1 h into water warmed to 37°C) and detected via mRFP fluorescence. Expression of the zebrafish Apoa1bp protein was confirmed in Western blot with a guinea pig polyclonal antibody (9). For vascular lipid deposit experiments, Golden Pearls supplemented with 4% (wt/wt) cholesterol and 1 μg/g TopFluor Cholesterol (Avanti Polar Lipids) were fed to zebrafish from 5 to 13 dpf as described (15). Zebrafish were imaged by using a Nikon A1 confocal microscope, and images were analyzed as described (15).

Cohort sizes and blinding

Cohort sizes for experiments involving Apoa1bp−/− mice, which were considered the major goal of the study, were calculated based on an assumption that the difference between means would be 1.3- to 2.0-fold, with SDs of 10-50% of mean, depending on experiment; 80% power; and P < 0.05. Animals were assigned to respective groups randomly within genotype. Individuals performing atherosclerosis analysis were not informed of genetic background or hypothesis.

Glucose, insulin, and glucose tolerance test

Glucose tolerance test was performed at 8 weeks of diet feeding and 18 weeks of age. Mice on the C57BL/6 background were fasted 4 h prior to testing. Glucose was administered to mice at a dose of 1 g per kg of body weight via ip injection of 25% dextrose in sterile saline (VetOne). Blood was collected from tail at times 0, 10, 30, 60, 90, and 120 min, and blood glucose was measured on a OneTouch Ultra glucose monitor. Additional blood was collected into heparinized capillary tubes at times 0 and 10 min for measurement of plasma insulin via ELISA (Alpco; catalog no. 80-INSMSH-E01). In cohorts of mice on the Ldlr−/− background, plasma was isolated from terminal blood, and plasma glucose levels were measured by using a kit from Crystal Chem (catalog no. 81692) and insulin levels by using an ultrasensitive insulin ELISA (Alpco; catalog no. 80-INSMSU-E01).

Lipids and lipoprotein profile

Blood was collected from mice into EDTA tubes upon euthanization, and plasma was collected following centrifugation. Lipoprotein profiles were determined by pooling plasma from each genotype and quantifying cholesterol content of each fraction via fast protein liquid chromatography with a Superose 6 column.

Atherosclerosis studies

Atherosclerosis was assessed as previously described (17). Briefly, en face atherosclerosis was quantified via computer-assisted image analysis (ImagePro) of Sudan-stained whole aortas. Aortic root atherosclerosis was quantified by cutting cross-sections starting from the aortic origin until the last leaflet. Sections were stained with a modified Van Gieson stain, and lesion area was quantified via computer-assisted image analysis (ImagePro).

Cells

RAW264.7 cells were cultured in DMEM (Cellgro) supplemented with 10% heat-inactivated FBS (Omega Scientific) and 50 μg/ml gentamicin (Omega Scientific).

Western blot

Cell and tissue lysates were subjected to gel electrophoresis and immunoblot as described (18). Antibodies were purchased from Cell Signaling Technology: p65 (catalog no. 4767), phospho-p65 (catalog no. 3033), ERK1/2 (catalog no. 9101), and GAPDH (catalog no. 2118).

Quantitative PCR

Total RNA was isolated by using Nucleospin RNA columns (Clontech). Isolated RNA was reverse-transcribed by using RNA to cDNA EcoDry (Clontech) following the manufacturer’s protocol. Quantitative PCR (qPCR) was performed by using a KAPA SYBR FAST Universal qPCR kit (KAPA Biosystems, KK4602), with primers ordered from Integrated DNA Technologies and aRotor Gene Q thermocycler (Qiagen). Primer sequences are listed in supplemental Table S4.

Recombinant AIBP

AIBP was produced in a baculovirus/insect cell system to allow for posttranslational modification and to ensure endotoxin-free preparation. Human AIBP was cloned into a pAcHLT-C vector.
behind the polyhedrin promoter. The vector contained an N-terminal His-tag to enable purification and detection. Insect Sf9 cells were transfected with BD BaculoGold Baculovirus DNA and the AIBP vector. After 4–5 days, the supernatant was collected to afford a baculovirus stock. Fresh Sf9 cells were infected with the AIBP-producing baculovirus; cell pellets were collected after 3 days, lysed, sonicated, and cleared by centrifugation; and the supernatants were loaded onto a Ni-NTA agarose column and eluted with imidazole. Protein was dialyzed against PBS, and the concentration was measured. Aliquots were stored at −80°C.

**LPS/Kdo2-LipidA**

In vitro experiments were conducted with Kdo2-LipidA (Avanti Polar Lipids), a well-characterized active component of lipopoly saccharide (LPS) and a highly specific Toll-like receptor 4 (TLR4) agonist (19). In text and figure legends, we refer to Kdo2-LipidA as LPS.

**Adeno-associated virus 2-AIBP**

Mouse AIBP (25–283 aa) was fused with fibronectin secretion sequence (FIB) at the N terminus and 6XHis at the C terminus (FIB-AIBP-His). FIB-AIBP-His was cloned into the pAAV-MCS vector (Agilent Technologies). All clones were sequenced to confirm the presence of the insert. Adeno-associated virus (AAV)-293 cells (Agilent Technologies) were transfected with 20 µg each of pAAV-FIB-AIBP-His, pAAV2 (Agilent Technologies), and pHelper DNA (Agilent Technologies) following the routine calcium phosphate-based protocol (Agilent Technologies). Subsequent steps of virus harvest, purification, and storage were according to published protocols (20). Viral DNA was extracted from purified virus, and the number of gene copies (gc) was determined by using qPCR (21). Viral DNA was extracted from purified virus, and the number of gene copies (gc) was determined by using qPCR with primers for the inverted terminal repeats (Takara Bio number of gene copies (gc) was determined by using qPCR (20). Viral DNA was extracted from purified virus, and the number of gene copies (gc) was determined by using qPCR with primers for the inverted terminal repeats (Takara Bio Inc.). The testing the FIB-AIBP-His plasmid and the AA2V-FIB-AIBP-His, we found that infected HEK 293 cells expressed AIBP of the correct molecular mass, recognized by anti-His (ThermoFisher, catalog no. MA1-21315). For AAV2-mediated AIBP expression, Ldlr−/− mice were iv injected with empty virus or AAV2-AIBP at 1 × 10^{12} gc per mouse. At 3 weeks postinjection, mice were fed a Western diet (Taklad TD.96121) for an additional 16 weeks. Testing for the expression of FIB-AIBP-His, liver was homogenized in RIPA buffer, and lysates were analyzed in Western blot.

**White adipose tissue FACS**

Flow cytometry of white adipose tissue (WAT) was performed as previously described (21). In brief, mice were euthanized, and tissue was perfused with PBS. WAT was minced in 5% BSA Dulbecco’s PBS and centrifuged to remove erythrocytes and free leukocytes. Tissue was dissociated by using collagenase (Sigma C6885), filtered, and separated from adipocytes via centrifugation. The stromal vascular fraction was used for FACS analysis. Antibodies used were CD11b-FITC (BD 553310), F4/80-APC (Ab Serotec MCA497APC), and CD11c-PE (BD 553802).

**Cholesterol efflux and lipid raft assays**

A cholesterol efflux assay was performed as described (22, 23), with modifications. In brief, RAW264.7 cells were loaded with 2 µCi/ml [3H]cholesterol (American Radiolabeled Chemicals), and cholesterol efflux was initiated by the addition of efflux medium containing 25 µg/ml HDL, in the presence or absence of 0.2 µg/ml recombinant AIBP, for 4 h. Background, nonspecific release of [3H]cholesterol was measured in the absence of HDL. After incubation, the medium was collected and counted in a liquid scintillation counter LS 6500 (Beckman Coulter). The cells were extracted with 2-propanol, and the lipid extract was added to ScintiVerse BD Cocktail (Fisher) and counted. Cholesterol efflux was expressed as a percentage of [3H] counts in the medium compared with combined [3H] counts in the cells and the medium. In these experiments, to replicate in vivo conditions, cells were not treated with a liver X receptor agonist or acyl-CoA cholesterol acyltransferase inhibitor.

For lipid raft measurements, RAW264.7 macrophages were incubated with FITC-conjugated cholera toxin (CTB; Sigma) for 1 h on ice. Cells were washed two times with a FACS buffer, fixed with 3.7% formaldehyde for 15 min on ice, washed three times with a FACS buffer, and analyzed by using a FACS Canto II (BD Biosciences) flow cytometer.

**Statistical analyses**

Results were analyzed by using Student’s t test (for differences between two groups), one-way ANOVA (for multiple groups), or two-way ANOVA with the Bonferroni post hoc test (for multiple groups time course experiments), using GraphPad Prism. Differences between groups with P < 0.05 were considered statistically significant. Values were excluded if determined to be a significant outlier via the extreme studentized deviate test.

**RESULTS**

**Metabolic abnormalities in Apoa1bp−/− mice**

Systemic Apoa1bp knockout mice have been developed in our laboratory as described (10). C57BL/6 and Apoa1bp−/− mice were fed a high-fat, normal-cholesterol diet (HFD) for 10 weeks starting from the age of 10 weeks. Despite having similar weights prior to diet feeding, Apoa1bp−/− mice were significantly heavier than their wild-type counterparts following the diet, despite consuming equivalent amounts of food (Fig. 1A, B). Apoa1bp−/− mice also exhibited impaired glucose clearance and increased circulating plasma insulin levels when subjected to a glucose tolerance test (Fig. 1C–E). Flow cytometry analysis of the WAT stromal vascular fraction revealed that epididymal WAT from Apoa1bp−/− mice contained a greater proportion of F4/80+CD11b+CD11c+ proinflammatory macrophages (Fig. 1F). Overall, Apoa1bp−/− mice exhibited more hallmarks of metabolic disease than their wild-type counterparts when fed a HFD.

Similar to HFD-fed Apoa1bp−/− mice, Apoa1bp−/−Ldlr−/− mice fed a Western diet (high-fat, high-cholesterol) for 12 weeks became significantly heavier than Ldlr−/− mice (Fig. 2A). The livers of Apoa1bp−/−Ldlr−/− mice had increased cholesterol and triglyceride content (Fig. 2B, C). Liver expression of TNFXa mRNA was increased nearly 3-fold in Apoa1bp−/−Ldlr−/− mice, suggesting increased liver inflammation (Fig. 2D). Plasma levels of glucose and insulin trended higher in terminal blood of nonfasted Apoa1bp−/−Ldlr−/− mice (supplemental Fig. S1A, B).

**AIBP deficiency results in exacerbated hyperlipidemia and atherosclerosis**

Apoa1bp−/−Ldlr−/− mice fed a Western diet had higher plasma cholesterol and triglyceride levels, primarily due
to elevated VLDL and LDL, as compared with Ldlr−/− mice (Fig. 3A–D). Importantly, atherosclerotic lesions in the aortic root were significantly larger in Apoa1bp−/−Ldlr−/− mice compared with Ldlr−/− mice (Fig. 3E–G). Overall, mice lacking AIBP had significantly increased weight gain, lipid levels in both plasma and liver tissue, and atherosclerosis when fed a high-fat, high-cholesterol diet. However, feeding Apoa1bp−/−Ldlr−/− mice a diet enriched in cholesterol (1%) but with normal fat content did not result in significant differences in weight, plasma cholesterol, triglycerides, glucose levels, or atherosclerosis when compared with Ldlr−/− mice (Fig. 4 and supplemental Fig. S1C). Plasma insulin levels in Apoa1bp−/−Ldlr−/− mice were higher than in Ldlr−/−
mice, but absolute numbers remained low (supplemental Fig. S1D). These results suggest the importance of AIBP-mediated metabolic changes in the development of atherosclerosis in the context of high-fat, high-cholesterol diets.

AAV-mediated expression of AIBP reduces weight gain, hyperlipidemia, and atherosclerosis

Next, we tested if sustained overexpression of mouse AIBP can reduce atherosclerosis. We generated an AAV2 with the FIB-AIBP-His construct. The fibronectin signal...
peptide (FIB) was inserted to ensure secretion of His-tagged mouse AIBP. Five-week-old Ldlr−/− mice were infected with AAV2-AIBP or the empty AAV2 (control) and, starting at 8 weeks of age, were fed a Western diet for 16 weeks. At the time of euthanization, livers were collected to confirm AIBP-His protein expression (supplemental Fig. S2); however, AIBP-His was not detectable in plasma. Both the control and AAV2-AIBP cohorts had similar weights prior to the start of the diet, but the mice overexpressing AIBP were significantly protected against diet-induced weight gain (Fig. 5A). Liver triglyceride levels were significantly lower and cholesterol levels trended lower in mice infected with AAV2-AIBP compared with the mice infected with the empty AAV2 (Fig. 5B, C). Plasma cholesterol levels had a trend toward reduction (Fig. 5D, E), and plasma triglycerides were significantly decreased (Fig. 5F, G). Importantly, there was a significant decrease in en face lesions (Fig. 5H), as well as a trend of reduced aortic root atherosclerosis in the AAV2-AIBP mice (Fig. 5I, J). Together, these data show protection against weight gain, plasma lipid increases, and atherosclerosis by AAV-delivered AIBP.

Transgenic AIBP expression in zebrafish reduces diet-induced vascular lipid accumulation

We have previously described a hyperlipidemic zebrafish model in which vascular accumulation of lipid deposits was observed in live animals 10–14 days after initiation of high-cholesterol feeding (15, 24). To investigate whether AIBP can reduce lipid accumulation in the vasculature, we generated a transgenic zebrafish line in which expression of the apoalbp-2A-mRFP construct is controlled by the hsp70 heat shock promoter (Fig. 6A). Following protein expression, self-cleavage of the translated 2A peptide (16) released untagged zebrafish Apoa1bp and mRFP, the latter serving as a reporter for Apoa1bp expression. Heat shock-induced expression of the Apoa1bp protein was confirmed in Western blot (Fig. 6B). When zebrafish were fed a high-cholesterol diet (HCD) supplemented with fluorescently labeled cholesterol for 10 days, vascular lipid deposits...
Fig. 6. Induced AIBP expression in zebrafish reduces diet-induced vascular lipid accumulation. A: Construct used to make transgenic hsp70:apoalbp-2A-mRFP zebrafish. B: Heat shock-induced Apoa1bp protein expression. Embryos were subjected for 1 h to heat shock (37°C) at 1 dpf and again at 2 dpf. Three mRFP-negative and three mRFP-positive embryos were selected and homogenized in SDS-PAGE loading buffer. Lysates were run on SDS-PAGE, blotted, and probed with anti-zebrafish Apoa1bp and tubulin antibodies. C: Vascular lipid accumulation. Transgenic hsp70:apoalbp-2A-mRFP zebrafish were fed control or 4% cholesterol diet supplemented with TopFluor-cholesterol for 10 days, and one group was subjected to three heat shock sessions during the 10-day feeding period. Left demonstrates heat shock-induced expression of mRFP (red) in zebrafish. Right shows vascular cholesterol deposits (green). White dashed lines trace the caudal vein in zebrafish. D: Quantification of vascular lipid deposits. Mean ± SEM; n = 4–11; * P < 0.05 (one-way ANOVA with Tukey’s multiple comparison test).

increased significantly (Fig. 6C, D). Our previous work has shown that heat shock itself does not affect diet-induced vascular lipid accumulation (15). However, heat shock-induced overexpression of Apoa1bp prevented the HCD-induced increase in vascular lipid deposition (Fig. 6C, D), independently supporting the hypothesis that AIBP overexpression is atheroprotective.

Recombinant AIBP inhibits inflammatory responses by macrophages

Because AIBP was shown to augment HDL function (9, 10) and AIBP deficiency resulted in a higher liver expression of Tnfa (Fig. 2D), we hypothesized that AIBP reduces inflammatory responses by macrophages. In cell culture experiments, recombinant AIBP significantly increased cholesterol efflux from RAW264.7 macrophages (Fig. 7A) and reduced lipid raft content as assessed by FACS analysis of CTB binding to cells (Fig. 7B). To test whether AIBP inhibits inflammatory signaling, RAW264.7 macrophages were stimulated with LPS, in the presence of AIBP or BSA control. LPS-induced phosphorylation of p65 and ERK1/2 in macrophages was significantly reduced in AIBP-treated cells (Fig. 7C–E). In addition, recombinant AIBP significantly reduced gene expression of inflammatory cytokines in macrophages that were stimulated with LPS+IFNγ, a common inflammatory, M1-like polarization signal (Fig. 8).

We next tested if injections of recombinant AIBP can reduce vascular inflammation in mice. Hyperlipidemic Ldlr−/− mice that had been fed a Western diet for 4 weeks were injected three times during the final week of feeding
Atheroprotective role of AIBP

with PBS control, AIBP, or heat-inactivated AIBP. Injections did not affect mouse weight or plasma lipids (Fig. 9A–C). Aortas of these mice were used for total RNA isolation and gene expression analysis via qPCR. While heat-inactivated AIBP had no effect, nondenatured AIBP injection significantly reduced transcript levels of the inflammatory cytokines IL-6, IL-1β, and CCL2 compared with PBS-treated mice (Fig. 9D).

DISCUSSION

In this study, using loss-of-function and gain-of-function animal models, we explored the role of AIBP in metabolism and atherosclerosis. We found that Apoa1bp−/−Ldlr−/− mice fed a Western diet had exacerbated liver steatosis, hyperlipidemia, and atherosclerosis. Conversely, AIBP gain-of-function resulted in reduced vascular lipid accumulation in zebrafish and in reduced aortic inflammation, hyperlipidemia, and atherosclerosis in mice. These results suggest that AIBP restricts diet-induced metabolic abnormalities and is atheroprotective.

The results of our experiments demonstrate that the AIBP control of diet-induced metabolic abnormalities is an important factor in atheroprotection. Intravenous injections of recombinant AIBP into mice reduced hyperlipidemia-induced inflammation in the aorta, which is considered one of the earliest sites of diet-induced insulin resistance, preceding both liver and WAT (25). A sustained gain-of-function of AIBP, delivered via the AAV2-AIBP, resulted in protection against weight gain, hypertriglyceridemia, and atherosclerosis. Most tellingly, the AIBP knockout did not exacerbate atherosclerosis in the mice fed a normal-fat, high-cholesterol diet, which does not elicit a metabolic response in Ldlr−/− mice (26). However, when fed a HFD, Apoa1bp−/− mice gained more weight and were more glucose-intolerant than C57BL/6 mice, despite consuming equal quantities of food. Similarly, Apoa1bp−/−Ldlr−/− mice fed a high-fat, high-cholesterol diet gained more weight and developed more severe hepatic steatosis compared with Ldlr−/− mice. The resulting exacerbation of hypertriglyceridemia and hypercholesterolemia led to more atherosclerosis in high-fat, high-cholesterol diet-fed Apoa1bp−/−Ldlr−/− mice.

Interestingly, a recent study of mice with reduced expression of ABCA1 in adipocytes and macrophages (27) demonstrated that they share a similar phenotype of weight gain, increased plasma triglycerides, and increased liver cholesterol and triglyceride levels, as well as impaired glucose tolerance (28), found in the AIBP knockout mice. Increased fat mass and insulin resistance are linked to an increase in VLDL synthesis and a reduction in triglyceride-rich lipoprotein uptake (29, 30), both of which may contribute to the high VLDL plasma content in Apoa1bp−/−Ldlr−/− mice. With greater exhibition of metabolic abnormalities, inflammatory macrophage signaling favored, and increased plasma cholesterol, it is expected that Apoa1bp−/−Ldlr−/− mice have significantly increased prevalence of aortic root atherosclerosis.
Systemic low-grade inflammation is an underlying cause of numerous pathologies, including the metabolic syndrome and atherosclerosis. A prevalent source of inflammatory cytokines are tissue macrophages, which become activated by danger-associated molecular patterns (DAMPs) arising in subjects consuming Western-type diets (31). Receptors for many DAMPs and inflammatory cytokines, such as TLR4 and IFNGR, reside in lipid rafts, which provide an ordered microenvironment for receptor dimerization and signaling complex assembly (32, 33). AIBP suppresses inflammatory signaling by enhancing cholesterol efflux from macrophages and thus depleting the lipid raft signaling platform. In AIBP knockout mice, the absence of AIBP results in an increase in the inflammatory milieu in the WAT leading to insulin resistance. This is an important avenue for future research.

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