Interaction of Adipocyte Fatty Acid-binding Protein (AFABP) and JAK2

**AFABP/aP2 AS A REGULATOR OF JAK2 SIGNALING**

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Adipocyte fatty acid-binding protein (AFABP/aP2) facilitates the intracellular solubilization and trafficking of lipids within the aqueous environment of the cell. Studies in the AFABP/aP2 knock-out mouse suggest that the protein may have roles in cellular processes broader than lipid transport. We present herein the finding that AFABP/aP2 interacts with JAK2 in a fatty acid-dependent manner. This interaction was established using yeast two-hybrid analysis, co-immunoprecipitation from adipose tissue, and 3T3-L1 adipocytes as well as in 293 cells overexpressing JAK2 and AFABP/aP2. Mutational analysis of AFABP/aP2 (R126L/Y128F) revealed that fatty acid binding activity is necessary for the interaction and that Asp18 of the helix-turn-helix motif forms a component of the interaction domain. Mutational analysis of JAK2 (Y1007F/Y1008F) revealed that AFABP/aP2 associates with the basal unphosphorylated form of the protein. Interleukin-6, but not interleukin-10, stimulated phosphorylation of STAT3, and induction of SOCS3 mRNA expression were potentiated in a time- and dose-dependent manner in macrophages and has a role in lipid metabolism in both cell types. In adipocytes, AFABP/aP2 mediates lipolysis and lipid re-esterification that in turn affect insulin sensitivity and atherosclerosis, whereas transgenic over expression models demonstrate potentiated characteristics (3–8). The molecular mechanisms that underlie this phenotype have yet to be elucidated but may be due to indirect effects and the availability of lipid ligands for peroxisome proliferator receptor γ or direct effects on AFABP/aP2 interaction with cellular proteins (9–11).

AFABP/aP2 is a member of the cytoplasmic fatty acid-binding protein multigene family (12). FABPs are low molecular mass 15-kDa cytoplasmic proteins that bind fatty acids with high affinity in a 1:1 complex within an interior ligand binding domain (12). They function to solubilize and traffic fatty acids throughout the aqueous cellular environment, thereby increasing the diffusion of molecules between cellular compartments. The family members have 20–70% sequence identity yet maintain super-imposable crystal structures and similar fatty acid affinity and specificity. Fatty acid binding leads to defined, but subtle changes in the protein structure including alterations in the side chain of Phe57 that caps the lipid binding pocket.

The divergent sequence among members confers subtle differences in ligand binding properties between the family members but may also define different protein-protein interaction partners depending upon the cellular context. Indeed, it has been shown that the adipocyte, epithelial, and heart type fatty acid-binding proteins interact with hormone-sensitive lipase, whereas the intestinal and liver isoforms do not (9, 10). AFABP/aP2 is highly expressed in adipocytes and macrophages and has a role in lipid metabolism in both cell types. In adipocytes, AFABP/aP2 has no role in fatty acid influx but mediates lipolysis and lipid re-esterification that in turn affect glucose metabolism (3, 5, 6, 8). In macrophages, the role of AFABP/aP2 in lipid metabolism is undefined, but studies in null macrophages reveal a role in cholesterol metabolism and inflammation (11, 14, 15). The sum total of effects in adipose tissue results in changes in insulin sensitivity and atherosclerosis (3, 5–7, 14–16). The complex nature of the biological effects revealed by the knock-out models supports a role for AFABP/aP2 in cellular processes other than lipid transport. To that end, yeast two-hybrid analysis followed by a combination of studies including titration microcalorimetry, fluorescence resonance energy transfer, and glutathione S-transferase pulldown assays...
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AFABP/aP2 has revealed that AFABP/aP2 interacts with a domain on hormone-sensitive lipase (HSL) defined by amino acid residues 190–200 containing the core sequence motif EXYK (9, 17–20). In addition, AFABP/aP2 has a similar sequence in the helix-turn-helix domain, Asp-Tyr-Met-Lys21, that is necessary for the interaction with HSL (10).

We describe here a novel protein-protein interaction between AFABP/aP2 and JAK2. JAK2 is part of a family of non-receptor-tyrosine kinases which play a critical role in signal transduction from ligands that bind to members of the cytokine receptor superfamily. Upon ligand binding, one or more of the JAK kinases is activated through transphosphorylation and subsequently phosphorylates the receptor and downstream signaling molecules such as the signal transducers and activators of transcription family or STAT proteins (21, 22). JAK2 has 49 potential tyrosyl phosphorylation sites, at least 10 of which have been identified as phosphorylated, and 2 of these sites (Tyr1007 and Tyr1008) are critical for full activation of JAK2 (23–25). In light of this, we have characterized the interaction of JAK2 and AFABP/aP2. Data presented here indicate that the Asp-Tyr-Met-Lys21 region in AFABP/aP2 is critical for this interaction and may represent a common protein-protein interaction domain for FABPs.

**EXPERIMENTAL PROCEDURES**

**Materials**—Zeocin, Genetin, Lipofectamine 2000, and tissue culture reagents were obtained from Invitrogen. Pure oleic acid was from NuChek Prep (Elysian, MN). Antibodies for STAT3 (79D7), phospho-STAT3 Tyr(P)705 (3E2), and JAK2 (D2E12) were from Cell Signaling Technology (Danvers, MA). Antibodies for total phosphotyrosine (pY99) were from Santa Cruz Biotechnology (Santa Cruz, CA). Secondary antibodies, goat anti-mouse IRDye 800CW and goat anti-rabbit IRDye 680, and Blocking Buffer were from Li-cor Biosciences (Lincoln, NE). IL-6 and IL-10 were from PeproTech (Rock Hill, NJ). DNA for pRK5-JAK2 and pRK5-JAK2-Y1007F/Y1008F were kind gifts from Dr. Bruce Witthuhn (University of Minnesota). All other reagents were purchased from Sigma-Aldrich. The University of Minnesota Microchemical Facility carried out DNA sequencing and synthesis of oligonucleotides used for PCR.

**Site-directed Mutagenesis**—Mutations in the proposed AFABP/aP2-JAK2 binding site were made in the template of pCDNA3.0-AFABP/aP2 or pRK5-JAK2 using the QuikChange site-directed mutagenesis technique of Stratagene Cloning Systems (La Jolla, CA). The mutational primers were as follows: E1006A JAK2, 5’-c ttg ccc gag aaa gca tac tac aaa gta aag gag c-3’; K1009A JAK2, 5’-g gag aaa gca tac tac gca gta aag gag cca ggg-3’; K1009D JAK2, 5’-g gag aaa gca tac tac gac gta aag gag cca ggg g-3’; K21A AFABP/aP2, 5’-c ttc gat gat gat gaa gca gaa gta ggt gaa ggt ggc tt gtc gtc gtc gtc gtc g-3’.

**Yeast Two-hybrid**—The yeast two-hybrid screening with JAK2 was done as previously described (26). Briefly, pEG202-JAK2 was used as bait with a rat adipose cDNA library as the prey.

**C8PA Cross-linking**—Transient transfection of C8PA cells, HEK-293 cells stably expressing fatty acid transport protein 1 and perilipin A were used and described previously (19). Briefly, 4 μg of pCDNA3.0-AFABP/aP2, 4 μg of pRK5-JAK2, and 30 μl of Lipofectamine 2000 were used for each 10-cm plate. Transfected plates were fat-loaded 16 h after initial transfection with Dulbecco’s modified Eagle’s medium plus 10% fetal bovine serum containing 300 μM oleate, 100 μM BSA. Twenty hours after fat loading, cells were washed once in phosphate-buffered saline and cross-linked. Cross-linking was done with 0.5% formaldehyde in 10 ml of phosphate-buffered saline at room temperature for 10 min. The reaction was stopped with 1 ml of 1.25 mM glycine for 5 min. The cells were washed twice in phosphate-buffered saline and scraped in radioimmunoprecipitation assay buffer (50 mM Tris, pH 7.5, 50 mM NaCl, 1 mM EDTA, 1 mM EGTA, 50 μM sodium fluoride, 1 mM sodium pyrophosphate, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS) containing protease inhibitors and phosphatase inhibitors. The cell suspension was sonicated twice for 10 s at 4 °C. The cell extract was centrifuged at 13,000 × g for 10 min to pellet cellular debris. The supernatant was used for subsequent Western blotting and immunoprecipitation experiments.

**Western Blotting and Co-immunoprecipitation**—Immunoprecipitation (IP) of JAK2 was done using rabbit anti-JAK2 antibody at 1:100 overnight at 4 °C. Rabbit pre-immune serum was used as the control antibody at the same concentration as the JAK2 antibody, 600 ng/ml. Immunoprecipitations were incubated with protein A-agarose beads, a 12.5-μl bead volume, for 2 h. Beads were washed four times in radioimmunoprecipitation assay buffer and on the last wash transferred to a new tube. Ten μl of radioimmune precipitation assay buffer and 10 μl of loading buffer were used to resuspend the beads, and this mixture was boiled for 20 min. Total immunoprecipitations were loaded on SDS-PAGE gels. Gels were transferred to Immobilon-F polyvinylidene difluoride membrane (Millipore) and blocked in Li-cor blocking buffer for 1 h. Primary antibodies were incubated overnight at 4 °C in 5% BSA, Tris-buffered saline with 0.1% Tween 20 (TBST). After washing in TBST, secondary IRDye antibodies were incubated for 1 h at room temperature in 5% BSA in TBST. Blots were washed in TBST and then rinsed and stored in phosphate-buffered saline. Li-Cor Odyssey was used for detection and quantification. For immunoprecipitation, JAK2, AFABP/aP2, and total phosphotyrosine (pY99) were blotted simultaneously on the same membrane. For signaling, peritoneal macrophages were stimulated with the appropriate cytokine for the indicated times and concentrations in the presence of 10% fetal bovine serum. AFABP/aP2, STAT3, and Tyr705 phospho-STAT3 were blotted simultaneously on the same membrane.

**Macrophage**—Macrophage cell lines were established by J2 retroviral transformation of bone marrow isolated from wild type and AFABP/aP2-EFABP/mal1 double knockout mice (27) using methods previously described (28, 29).

**Quantitative Reverse Transcription-PCR**—Expression of mRNAs was measured by quantitative reverse transcription-PCR. Total RNA was isolated from macrophages using Trizol reagent (Invitrogen) according to manufacturer’s protocol. RNA was treated with DNase I to digest any genomic contamination, and cDNA was synthesized using iScript cDNA synthesis kit (Bio-Rad). iQ SYBRgreen Supermix and MyIQ detec-
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FIGURE 1. Endogenous interaction of AFABP/aP2 and JAK2. Immunoprecipitation with control IgG (C) or anti-JAK2 (Jak) from day 8 3T3-L1 adipocytes and C57B6/J epididymal adipose tissue (WAT) and Western blot for AFABP/aP2. Input lanes represent samples before immunoprecipitation. The blot shown is representative of three independent experiments with similar results. The Western blot shown is three portions of the same blot taken at the same exposure with intervening lanes omitted for clarity.

FIGURE 2. Sequence alignment of JAK2, HSL, and AFABP/aP2 EHYKR motifs. Portions of the amino acid sequences of HSL, JAK2, and AFABP/aP2 that align with the EHYKR motif. All alignment analysis was done using T-Coffee alignment software.

RESULTS

Previous studies using a yeast two-hybrid approach identified AFABP/aP2 as an interaction partner with the HSL (18). Using a similar strategy in the course of investigating JAK2-interacting proteins, we identified AFABP/aP2 as an interaction partner (data not shown). To confirm this interaction in endogenous systems, we carried out co-immunoprecipitation experiments. When JAK2 was immunoprecipitated from adipose tissue of C57Bl/6J mice or from differentiated day 8 3T3-L1 adipocytes and then blotted for AFABP/aP2, specific association was detected (Fig. 1). Immunoprecipitation with control antibodies did not reveal any nonspecific complex formation.

AFABP/aP2 interacts with a domain on HSL containing the core sequence EHYKRNE (17). Analysis of JAK2 and AFABP/aP2 amino acid sequences revealed a similar sequence present in both proteins (Fig. 2). Fluorescence resonance energy transfer analysis has indicated that Asp$^{18}$ of AFABP/aP2 likely forms a salt bond with Lys$^{199}$ of HSL as a key component of the interaction domain. In this context, JAK2 may interact with AFABP/aP2 involving ionic interactions between amino acids of the EHYK domain. Previous studies showed that the interaction of AFABP/aP2 with HSL is fatty acid-dependent (9, 20) and that the orientation of the side chain of Asp$^{18}$ is altered upon ligand binding (30, 31). Considering that most in vitro protein-protein interaction assays utilize one or more chaotropic agents (e.g. high salts, detergents) to reduce nonspecific interactions and that these agents strip fatty acids from the binding pocket of AFABP/aP2, we cannot use conventional immunoprecipitation analysis to evaluate protein-protein interactions. As such, we adopted a cross-linking protocol analogous to chromatin immunoprecipitation to investigate the AFABP/aP2-JAK2 interactions.

To evaluate the AFABP/aP2 interaction with JAK2, we utilized C8PA cells. C8PA cells are HEK-293 cells stably expressing fatty acid transport protein 1 and perilipin A and have the advantage of increased fatty acid uptake and storage and, thus, allow us to probe the JAK2-AFABP/aP2 interaction in an adipocyte-like cell system in the absence of endogenous AFABP/aP2 (19). Cells overexpressing AFABP/aP2 and JAK2 were cross-linked in formaldehyde and lysed, and cell extracts were used for subsequent immunoprecipitation experiments. As shown in Fig. 3, wild type AFABP/aP2 co-immunoprecipitated with JAK2, whereas control IgG does not show any nonspecific interaction. To determine whether the interaction is ligand-dependent, we tested the fatty acid binding mutant R126L/Y128F AFABP/aP2 for interaction with JAK2. In AFABP/aP2, Arg$^{126}$ and Tyr$^{128}$ for H-bonds with the fatty acid carboxylate and are required for high affinity lipid association (31, 32). Fig. 3A shows that R126L/Y128F AFABP/aP2 does not associate with JAK2, indicating that ligand binding to AFABP/aP2 is necessary for this interaction.

The sequence similarity between HSL and JAK2 suggests that the EHYK domain may be important for the JAK2 interac-
interaction. Within JAK2, EYYK1009 is located on the activation loop of the kinase, and phosphorylation of the two tyrosines is critical to full activation (24). To test the importance of phosphorylation and activity of JAK2 on the interaction, we mutated both tyrosines to phenylalanine. As expected, Y1007F/Y1008F JAK2 exhibited decreased tyrosine phosphorylation, suggesting that JAK2 is less active. As shown in Fig. 3A, AFABP/ap2 co-immunoprecipitates more efficiently with Y1007F/Y1008F JAK2 than with wild type JAK2, suggesting that AFABP/ap2 interacts with JAK2 in the basal, unphosphorylated state. To extend the analysis of the EYYK1009 region, we mutated Glu1006 and Lys1009 of JAK2, individually to alanine residues. Analysis of E1006A and K1009A JAK2 revealed decreased total phosphotyrosine levels, suggesting that these mutations affect JAK2 activity in a similar manner as the Y1007F/Y1008F mutant (Fig. 3B). Co-immunoprecipitation experiments show that E1006A and K1009A JAK2 interact more avidly with AFABP/ap2 than does wild type JAK2. The sum of the mutations in the EYYK1009 region suggests that AFABP/ap2 interacts with JAK2 in the basal state and that the EYYK1009 is central to the interaction.

Recent studies have revealed that Asp18, Lys21, and Arg30 of AFABP/ap2 found on the helix-turn-helix domain were necessary for the interaction with HSL and that mutation of these residues rendered AFABP/ap2 either unable to interact or attenuated association with HSL (10). Importantly, mutations at these sites do not affect the AFABP/ap2 ability to bind fatty acid ligand, functionally separating the ligand binding function of AFABP/ap2 from its protein-protein interaction domains (10). Moreover, the loss of HSL association in the D18K AFABP/ap2 mutant can be rescued by corresponding mutation of HSL (K196E). The helix-turn-helix domain also has been shown to undergo main chain and side chain alterations upon ligand binding, suggesting a structural change that coincides with ligand association (31).

To further investigate the helix-turn-helix domain of AFABP/ap2, we performed a charge reversal experiment whereby we mutated Asp18 to Lys in AFABP and Lys1009 to Asp in JAK2. As shown in Fig. 4A, expression of D18K AFABP/ap2 with wild type JAK2 into C8PA cells followed by cross-linking and immunoprecipitation revealed decreased interaction between the two proteins, suggesting that Asp18 and the helix-turn-helix domain of AFABP/ap2 are important for association. Interestingly, K1009D of JAK2 (like K1009A) exhibited decreased total phosphotyrosine of JAK2 and, consistent with the results in Fig. 3B, increased association. When D18K of AFABP/ap2 was co-expressed with K1009D of JAK2, association was returned to basal levels, suggesting that the interaction is ionically based and that the activation loop of JAK2 is the likely site of interaction. However, the site of interaction on AFABP/ap2 with JAK2 is not identical in all respects to the interaction of AFABP/ap2 with HSL. Fig. 4B shows that mutation of Lys21 to Ala (a mutation that blocks AFABP/ap2 interaction with HSL) had no effect on interaction with JAK2. These experiments demonstrate that the requirements for the interaction of AFABP/ap2 with JAK2 clearly overlap but are not identical to those for HSL.

Analysis of the phosphorylation-deficient JAK2 mutants, Y1007F/Y1008F, E1006A, K1009A, and K1009D (Fig. 3), indicates that AFABP/ap2 interacts with JAK2 in the basal state. To understand the physiological implications of this interaction, we made use of macrophages from AFABP/ap2-EFABP/mal1 double knock-out (DKO) mice. DKO mice are resistant to diet-induced obesity, insulin resistance, and atherosclerosis (15, 16). Interleukin-6 and interleukin-10 signaling has been implicated in atherosclerosis development, and a balance between signaling of these two cytokines is necessary for the prevention of atherosclerosis as IL-6 knock-out models in an apolipoprotein E null background have increased atherosclerosis (33–35). JAK2 plays an important role in the signaling mechanism for both of these cytokines in macrophages (36, 37). Once activated, JAK2 recruits and phosphorylates STAT3 at Tyr705 (36). Therefore, we used STAT3 phosphorylation as an indicator of JAK2 activity in wild type and DKO macrophages. Initial assessment of these signaling pathways revealed that treatment of cells with IL-6 or IL-10 for 15 min resulted in Tyr705 phosphorylation of STAT3 (Fig. 5). IL-6 induced Tyr705 phosphorylation of STAT3 was potentiated 4-fold in the DKO cells compared with wild type, whereas IL-10-induced Tyr705 phosphorylation of STAT3 was unaffected (Fig. 5). To more fully characterize the JAK2 signaling via the IL-6/IL-10 cytokines, we evaluated the cytokine concentration dependence at a fixed time (15 min) and the time dependence of STAT3 phosphorylation at a fixed concentration of hormone (100 ng/ml IL-6). As shown in Fig. 6, A and B, wild type cells induced Tyr705 STAT3 phosphorylation at 10 ng/ml IL-6, whereas DKO cells were more sensitive, inducing Tyr705 STAT3 phosphorylation at 1 ng/ml IL-6. The increased sensitivity to IL-6 in the DKO cells was observed at every concentration evaluated, suggesting that AFABP/ap2 attenuates a major determinate of this signaling pathway. In contrast, the IL-10 concentration dependence
revealed no difference between wild type and DKO cells at any concentration tested (Fig. 6, E and F), suggesting that AFABP does not affect IL-10 signaling.

The kinetics of Tyr\(^{705}\) STAT3 phosphorylation exhibited by both wild type and DKO cells show maximally stimulated STAT3 phosphorylation at 15 min with a return to basal levels at 90 min (Fig. 6, C and D). DKO cells show an amplitude effect of the signal rather than duration, suggesting that AFABP/ap2 has an early effect on activation of the pathway but did not play a role in desensitization. Importantly, the mRNA for IL-6 signaling receptors, gp130 and IL-6 receptor, were unchanged in the knock-out macrophages. Knock-out mice have revealed a role of AFABP/ap2 in whole body insulin resistance and atherosclerosis, although the specific molecular mechanisms leading to these broad phenotypes are not well characterized.

The studies presented herein describe a novel interaction between AFABP/ap2 and JAK2. The system also define three important considerations regarding this interaction; the requirement for fatty acids to be bound to AFABP/ap2, JAK2 be in the basal, unphosphorylated state, and mapping of the JAK2 binding site on AFABP/ap2 to a region surrounding the helix-turn-helix domain. Physiological implications for the interaction are revealed by the correlation that AFABP/ap2-EFABP/mal1 knock-out macrophages show potentiated JAK2 signaling.

Using a cross-linking system analogous to chromatin immunoprecipitation, performed in engineered fibroblasts (C8PA cells) that mimic adipocytes to some extent, we have examined the interaction between AFABP/ap2 and JAK2. The system was developed to enable studies of AFABP/ap2 interaction with the hormone-sensitive lipase (20). In those studies retention of either the bound lipid or preservation of the protein-protein interactions was key toward successful complex evaluation. Utilizing a cross-linking strategy, we were able to probe the interaction between AFABP/ap2 and JAK2 without the complication of losing the interaction due to chaotropic agents typically used for co-immunoprecipitation analysis. Moreover, C8PA cells are devoid of any FABP, allowing us to introduce mutants of AFABP and/or JAK2 to define the requirements of specific amino acids. In this system we found that the fatty acid binding mutant R126L/Y128F AFABP/ap2 was unable to interact with JAK2. The ligand dependence of this interaction is likely to be the primary reason that standard protocols for protein-protein interaction analysis do not work for AFABP/ap2, as ligands would be stripped from the protein with detergents used in such procedures. The fatty acid dependence allows AFABP/ap2 to act as a rheostat of intracellular fatty acid levels, regulating its interaction with JAK2. This could have major implications on JAK2 signaling pathways when fatty acid levels are elevated. Fatty acid levels would be increased in the obese condition in both adipocytes and macrophages, resulting in more holo-AFABP/ap2. Under this condition we would expect more AFABP/ap2 interacting with JAK2. Considering AFABP/ap2 has a role in obesity-linked insulin resistance and atherosclerosis, it may be a fatty acid sensor for JAK2 signaling pathways important for disease progression.

Previous work has revealed that AFABP/ap2 interacts with HSL within a sequence EHYKRN199 (17). The EYK motif is also found in AFABP/ap2 (DYMK\(^{21}\)) and, thus, makes it an attractive motif to explore further. Structural studies have revealed that a charge-quartet comprised of Asp\(^{17}\), Asp\(^{18}\), Lys\(^{21}\), and Arg\(^{30}\) in AFABP/ap2 is necessary for the interaction with HSL and that a charge-charge interaction from Asp\(^{18}\) of AFABP/ap2 to Lys\(^{196}\) of HSL is central for this association (10). We show here that AFABP/ap2 interacts with a similar
sequence in JAK2. Interaction was compromised with D18K AFABP/aP2, although the K21A AFABP/aP2 mutant had no effect on the interaction. Importantly, mutation at either of these sites results in similar ligand binding, excluding the possibility that decreased ligand binding is why D18K has decreased interaction. This suggests that the exact amino acids for interaction may be different than with HSL, but the helix-turn-helix domain is important for this association. Single mutations in this domain may not be sufficient to ablate this interaction due to other or more charged residues being important for this interaction with JAK2. Further investigation into this possibility is the subject of future studies.

JAK2 is constitutively active in the C8PA cell system, as indicated by JAK2 phosphotyrosine levels. This has hindered our ability to examine AFABP/aP2 interaction with the basal non-phosphorylated JAK2 in vitro for functional analysis. Tyr1007 and Tyr1008 are critical autophosphorylation sites necessary for
activation loop flip by this kinase, resulting in full activation of JAK2 (24). Mutation of each of the EYYK residues singly or in pairs resulted in a significant reduction in the total phosphoryrosine levels, indicating that all of the mutations affect JAK2 activity in a similar manner. AFABP/aP2 interaction with these mutants was increased, suggesting that AFABP/aP2 interacts with JAK2 in the basal, unphosphorylated state. This would position AFABP/aP2 to attenuate signaling by not allowing JAK2 to autophosphorylate or to inhibit other JAK2 protein-protein interacting partners from binding. It should be noted that in the C8PA cell system ~50% of transfected JAK2 is immunoprecipitated using the JAK2 antibody, and in such immunoprecipitates, about 2% of total AFABP/aP2 is complexed. However, because the JAK2 antibody immunoprecipitates total JAK2 (both activated and non-activated), the relatively small amount of AFABP/aP2 in such immune complexes may represent AFABP/aP2 association with the non-phosphorylated JAK2. Moreover, because our results indicate that only the FABP with a bound fatty acid forms a complex, we anticipate that only a small amount of each protein is associated. Although in macrophages AFABP/aP2 is an abundant protein, we predict that only a small amount of AFABP/aP2 may interact with JAK2 and function as a signaling regulator.

The interaction data suggest that AFABP/aP2, when bound to a fatty acid ligand, interacts with unphosphorylated JAK2. To test the effect of AFABP/aP2 on JAK2 signaling, we made use of the AFABP/aP2-EFABP/mal1 double knock-out macrophages. DKO mice in an apolipoprotein E null background have significantly reduced atherosclerosis on a normal chow diet (15). The finding that AFABP/aP2 null bone marrow transplanted into an apolipoprotein E null animal results in a protection against atherosclerosis, suggests that expression of FABPs in the macrophage is important to atherosclerosis development (7). Development of atherosclerosis is a complex process involving communication between many cell types through cytokine and inflammatory mediators. IL-6 and IL-10 both have anti-atherogenic potential (33–35). Considering the phenotype of the FABP null mice and the possible involvement of JAK2 signaling, we investigated IL-6 and IL-10 signaling in macrophages derived from AFABP/aP2-EFABP/mal1-deficient mice. The results showed a potentiation in IL-6 signaling, whereas IL-10 signaling was unchanged in the DKO macrophages. This difference suggests that AFABP/aP2 has localized effects on JAK2 that may be due to the involvement of other signaling components. Indeed, IL-6 signaling and IL-10 signaling do have immediate upstream differences, such as the involvement of SHP-2 and SOCS3 as negative regulators in IL-6 signaling (13, 36, 40–42). Further analysis of other JAK2-signaling pathways is necessary to determine the specificity of the AFABP/aP2 effect. To this end, studies using AFABP/aP2 knockdown 3T3-L1 adipocytes revealed that the protein had no effect on growth hormone-induced JAK2 signaling (data not shown). JAK2 signaling is very complex and from cytokine to cytokine has different specificities in terms of the STAT proteins activated, other pathways activated such as mitogen-activated protein kinases, and inhibitors and activators such as SHP-2. It will be critical to determine the pathways that AFABP/aP2 affects to understand the elements necessary for regulation of JAK2 signaling in other systems. The inhibitory action of AFABP/aP2 on IL-6-induced STAT3 phosphorylation suggests that its role is at the level of JAK2, the immediate upstream event. These results are consistent with AFABP/aP2 directly inhibiting JAK2 kinase activity through protein-protein interactions.

In sum, we show here a novel interaction between AFABP/aP2 and JAK2. AFABP/aP2 interacts through the helix-turn-helix domain in a ligand-dependent manner with unphosphorylated JAK2 and results in an attenuation of a JAK2 signaling pathway. Taken together, this positions AFABP/aP2 as a fatty acid sensor for JAK2, resulting in the fine-tuning of JAK2 signaling as a consequence of the lipid state of the cell.

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