Characterization of the long pentraxin PTX3 as a TNFα-induced secreted protein of adipose cells

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Abstract **Exposure of preadipocytes to long-chain fatty acids induces the expression of several markers of adipocyte differentiation. In an attempt to identify novel genes and proteins that are regulated by fatty acids in preadipocytes, we performed a substractive hybridization screening and identified PTX3, a protein of the pentraxin family. PTX3 mRNA expression is transient during adipocyte differentiation of clonal cell lines and is absent in fully differentiated cells. Stable overexpression of PTX3 in preadipocytes has no effect on adipocyte differentiation. In line with this, PTX3 mRNA is expressed in the stromal-vascular fraction of adipose tissue, but not in the adipocyte fraction; however, in 3T3-F442A adipocytes, the PTX3 gene can be reinduced by tumor necrosis factor α (TNFα) in a dose-dependent manner. This effect is accompanied by PTX3 protein secretion from both 3T3-F442A adipocytes and explants of mouse adipose tissue. PTX3 mRNA levels are found to be higher in adipose tissue of genetically obese mice versus control mice, consistent with their increased TNFα levels. In conclusion, PTX3 appears as a TNFα-induced protein that provides a new link between chronic low-level inflammatory state and obesity.**—Abderrahim-Ferkoune, A., O. Bezy, C. Chiellini, M. Maffei, P. Grimaldi, F. Bonino, N. Moustaid-Moussa, F. Pasqualini, A. Mantovani, G. Ailhaud, and E-Z. Amri. **Characterization of the long pentraxin PTX3 as a TNFα-induced secreted protein of adipose cells.** ***J. Lipid Res.*** 44: **994–1000.**

Supplementary key words tumor necrosis factor α • cytokines • adipocytes

An increasing body of evidence correlates the obese phenotype with chronically elevated systemic levels of acute-phase reactants and inflammatory cytokines (1–3). These elevated levels may in turn contribute directly or indirectly to the increased incidence of cardiovascular diseases (4). A significant correlation has been found between body mass index and circulating levels of C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor α (TNFα) (5–7). Recent evidence shows that adipocytes secrete these cytokines as well as inflammatory proteins, i.e., serum amyloid A3 and haptoglobin (8–10). Although the role of these reactants has remained largely unknown, TNFα and IL-6 have been shown to inhibit insulin signaling and to induce both hypertriglyceridemia and endothelial activation (11–13). Altogether, these observations suggest strongly that adipose tissue through adipocytes is an important determinant of a chronic low-level inflammatory state (14–16). Clearly, secretion of cytokines in adipose tissue is not confined to adipocytes, as we have previously reported that preadipocytes secrete leukemia inhibitory factor (LIF) transiently at a time when their cognate cell surface receptor (LIF-R) is also expressed (17). The LIF/LIF-R system then promotes adipocyte differentiation via the activation of extracellular signal-regulated kinases that mediate the expression of CCAAT/enhancer binding proteins β and δ (18), and LIF production ceases with the ongoing differentiation process. During the course of our investigations in searching early-expressed, fatty acid-responsive genes, we have identified a novel mRNA that encodes a long form of pentraxin, i.e., PTX3. Up to now, PTX3, also known as TSG14, has been known as a member of the pentraxin gene family that is expressed at extrahepatic sites, predominantly in vascular endothelial cells of heart and skeletal muscle

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Abbreviations: LIF, leukemia inhibitory factor; PPARγ, peroxisome proliferator-activated receptor γ; TNFα, tumor necrosis factor α.

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(19–21). We show herein that, despite the fact that PTX3 expression becomes low, if any, in adipocytes, its expression and secretion are induced dramatically during TNFα exposure. We show also that expression of the PTX3 gene is elevated in mouse models of monogenic obesity, strongly suggesting that PTX3 provides a new link between the chronic low-level inflammatory state and obesity.

MATERIALS AND METHODS

Cell culture

Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 8% fetal bovine serum, 200 U/ml penicillin, and 50 μg/ml streptomycin (standard medium) to confluence (hereafter referred to as Day 0). For differentiation, confluent 3T3-F442A cells were shifted to standard medium supplemented with 5 nM insulin and 2 nM triiodothyronine (differentiation medium). For experiments in serum-free medium, cells were first inoculated in standard medium at a density of 10^3 cells/cm^2 and washed 24 h later with DMEM-Ham's F12 (50:50, v/v). Cells were grown to confluence in 4-F medium (22) consisting of DMEM-Ham's F12 supplemented with insulin (5 μg/ml), transferrin (10 μg/ml), submaxillary gland extract (SMGE) (2 μg/ml), and fibroblast growth factor (25 ng/ml). At confluence, cells were shifted to the same medium in the absence of fibroblast growth factor and SMGE, supplemented with fetuin (100 μg/ml) growth hormone (2 nM), triiodothyronine (0.2 nM), BRL 49653 (rosiglitazone, 10 nM), ascorbate (100 μM), and selenite (20 nM). Confluent 3T3-L1 cells were stimulated to differentiate by addition of hormonal cocktail [0.5 mM 1-methyl-3-isobutylmethyl-xanthine, 0.5 μM dexamethasone, and 170 nM insulin (MDI)] to the standard medium for 2 days and were maintained thereafter in standard medium with only 170 nM insulin. Typically, by Day 6, more than 95% of the cells fully differentiate into adipocytes. Media were changed every other day.

Substractive hybridization

Substractive hybridization of RNA from untreated and fatty acid-treated Ob1771 preadipocyte cells was performed by the use of a substraction kit (Stratagene, The Netherlands) according to the manufacturer's instructions.

Retrovirus production and transduction

BOSC23 cells were transfected with viral DNA at 50% confluence by the use of FuGENE 6 reagent (Roche Molecular Biochemicals, France) as described by the manufacturer. Forty-eight hours after transfection, viral supernatant was harvested, centrifuged, and filtered. Fifty percent confluent 3T3-F442A cells were transduced with viral supernatant diluted with one volume of fresh standard medium in the presence of 6 μg/ml Polybrene. On the following day, cells were split and subjected to neomycin (Sigma) selection (200 μg/ml). Stable cell populations were obtained after 7–10 days of selection. After confluence, cells were maintained in differentiation medium.

Culture of adipose tissue explants

Adipose tissue from epididymal fat pads were dissected under sterile conditions, freed as much as possible from blood capillaries, and bathed in DMEM medium containing antibiotics as above. Adipose tissue was then minced into ~100 mm^3 pieces and incubated in differentiation medium for 2 h to 15 h.

Stromal-vascular and adipocyte fractions were obtained as already described in (23).

Secretion media

Adipose tissue explants or differentiated 3T3-F442A cells were washed twice in PBS (pH 7.4), once with ITT medium (DMEM-Ham's F12 supplemented with 5 nM insulin, 2 nM triiodothyronine, and 10 μg/ml transferring), and then incubated in ITT medium for 2 h. Cytokines were added for 3 h and media were collected. Secretion media were centrifuged for 5 min at 2,000 rpm at 4°C, then proteins were concentrated either by acetone precipitation or by centrifugation with Amicon filters as described by the manufacturer (Millipore, France).

Isolation and analysis of RNA

Total RNA from cells and mouse adipose tissue was extracted using Tri-Reagent™ kit (Euromedex, France) according to manufacturer's instructions and analyzed by Northern blot as described previously (24). Blots were subjected to digital imaging (FujixBAS 1000).

An amount of 1 μg of total RNA, digested with DNaseI (Roche Molecular Biochemicals), was subjected to RT-PCR analysis. The RT reactions were carried out in the presence of pd(N)6 random hexamer (Roche Molecular Biochemicals), 200 units of Moloney murine leukemia virus reverse transcriptase (Promega, France) and supplied buffer, dNTPs (0.5 mM) and RNase inhibitor (4 units) at 37°C for 1 h in a 25 μl reaction volume. Real-time PCR reactions were performed on an ABI Prism 7700 (Perkin-Elmer Applied Biosystem). For each PCR run, a master mixture was prepared on ice with 1× TaqMan Universal PCR Master Mix (Perkin-Elmer Applied Biosystem), 300 nM of each primer except for PTX3 reverse (900 nM), 200 nM probe, and 1 μl of diluted (1:2, v/v) reverse-transcribed cDNA. PCR conditions comprised 2 min at 50°C, an initial denaturation step at 95° for 10 min, and 40 cycles of 15 sec at 95°C, 1 min at 60°C. Gene expression was quantified using the comparative-Ct method. Experiments were performed with triplicate for each data point. TATA-binding protein (TBP) was used as an internal standard. The oligonucleotides of each target of interest, designed using Primer Express software (Perkin-Elmer), were (forward and reverse): PTX3, 5′-GACAACGAAATAGACAATGGACTTCA-3′, 5′-GCGAGTTCTCCAGCATGATGA-3′; and TBP, 5′-ACCCTTCACCAATGACTCCTATG-3′, 5′-ATGATGACTGCAGCAAATCGC-3′. The TaqMan probes were Fam/Tamra-labeled: PTX3, 5′-CACCGAGGACCCCAC-3′ and TBP, 5′-AGCTCTGGAATTGTACCGCAGCTTCAAAATA-3′.

Plasmids

The retroviral construct containing full-length PTX3 cDNA was derived from pSG5-PTX3 and cloned into the *Not*I site of pAkvi-Bipe2 (gift of Dr. K. Kristiansen, University of Odense, Denmark).

Oil red O staining

Culture dishes were washed in PBS (pH 7.4) and cells were fixed in 3.7% formaldehyde for 1 h, followed by staining with Oil Red O for 1 h. Oil Red O was prepared by diluting a stock solution (0.5 g of Oil Red O) (Sigma) in 100 ml of isopropanol with water (60:40, v/v) followed by filtration. After staining, plates were washed twice in water and photographed.

Protein analysis

Whole-cell extracts. Plates were washed twice in 10 mM Tris-HCl buffer (pH 7.5) containing 150 mM NaCl. Cells were directly lysed with a solution containing 2.5% SDS, 10% glycerol, 50 mM Tris-HCl (pH 6.8), 10 mM dithiothreitol, 10 mM β-glycerophosphate, 10 mM NaF, 0.1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, and the complete protease inhibitor mixture (1:50 tablet per ml) (Roche Molecular Biochemicals). Lysis of cells was immediately followed by boiling for 3 min. Lysates were subsequently treated with benzon nuclease (Merck,

France). Whole-cell extracts were stored at −80°C. Protein concentrations were determined by the Bradford method (Bio-Rad, France).

Western blotting. Fifty to one hundred micrograms of protein were loaded in each lane. After SDS-polyacrylamide gel electrophoresis, proteins were blotted onto polyvinylidene difluoride membranes (Amersham Biosciences, France) using a Bio-Rad semidry blotter. Equal loading/transfer was confirmed by Ponceau S staining of membranes. Membranes were blocked overnight in TBS [10 mM Tris-Cl (pH 7.5), 150 mM NaCl] containing 0.1% Tween 20 (TBS-T) and 5% nonfat dry milk. Incubation with primary and secondary antibodies was performed in TBS-T containing 5% nonfat dry milk for 2 h at room temperature. After incubation with antibodies, membranes were washed in TBS-T. Rat anti-human PTX3 was already described (25), and cross-reacted well with the mouse PTX3. Anti-TBP (Santa-Cruz) was used to monitor the equal loading. Secondary antibodies were horseradish peroxidase-conjugated anti-rat (Jackson Laboratories) or anti-rabbit antibodies (Promega). Enhanced chemiluminescence (Amersham Biosciences) was used for detection. Stripping of membranes was done by boiling for 5 min in water.

Materials

Culture media, fetal calf serum, cytokines, and geneticin were from Life Technologies, Inc. (France). Other chemicals were purchased from Sigma and Aldrich (France). Radioactive materials, random priming kit, and nylon membranes were from Amersham Biosciences. BRL 49653 was a kind gift (S. Michel and U. Reicher, Galderma RandD, Sophia-Antipolis, France). Mice (C57BL/6J, ob/ob, db/db) were purchased from Janvier (Le Genest Saint Isle, France) and used at 6–8 weeks of age. Animals were killed by cervical dislocation. All experimental protocols were performed in accordance with the recommendations of the French Accreditation of Laboratory Animal Care.

RESULTS

Identification of PTX3 cDNA and deduced amino acid sequence

To search for new genes that are expressed early in preadipocytes and are responsive to fatty acids, we employed a substractive hybridization technique. Two cDNA libraries were obtained from RNA prepared either from Day 2 postconfluent Ob1771 cells (26) or from RNA of Day 1 postconfluent Ob1771 cells exposed to 300 μM α-linolenate for 24 h. After substractive hybridization screening among various clones, we identified a clone that contained a cDNA fragment of about 1.7 kb. Using this DNA fragment as a probe for Northern blot analysis, a single transcript around 2 kb was observed at higher levels in untreated and at very low levels in fatty acid-treated preadipocytes (**Fig. 1A**). As shown previously (24), aP2 mRNA expression was, in contrast, strongly induced by fatty acid treatment (Fig. 1A). A nearly full-length cDNA clone was subsequently sequenced, and Blastn searching of nucleic acid databases at the National Center for Biotechnology Information to look for sequence homologies (27) identified the cDNA sequence as identical to mouse PTX3 (GenBank™ accession number X83601). The PTX3 gene encodes a 42 kDa glycoprotein with a carboxy-terminal half that shares high homology with the entire sequence of CRP and serum amyloid protein, which are acute-phase proteins of the pentraxin family (28–30), whereas the NH2-terminal part does not show any significant homology with other known proteins.

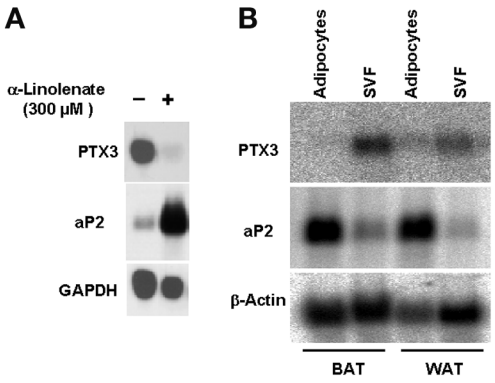

Fig. 1. Effect of fatty acid on PTX3 mRNA expression in preadipocytes and its distribution in adipose tissue. A: One day post-confluent Ob1771 cells were maintained for 24 h in standard medium in the absence (−) or presence (+) of 300 μM α-linolenate. Twenty micrograms of RNA were analyzed as described in Materials and Methods. The results are representative of four independent experiments. B: RNA from stromal-vascular and adipocyte fractions of white adipose tissue (WAT) and brown adipose tissue from six mice was blotted and hybridized with PTX3 probe. The results are representative of two independent experiments.

Expression of PTX3 mRNA during adipocyte differentiation

White and brown adipose tissues were shown to express high and low levels of PTX3 mRNA in the stromal-vascular fraction and the adipocyte fraction, respectively. In contrast to PTX3, aP2 and peroxisome proliferator-activated receptor γ (PPARγ) were predominantly expressed in the adipocyte fraction (Fig. 1B, and not shown). These observations led us to examine PTX3 expression as a function of differentiation of 3T3-F442A (31), 3T3-L1 (32), and Ob1771 cells. As shown in **Fig. 2A**, PTX3 mRNA was already expressed in growing cells but became expressed at higher levels in confluent 3T3-F442A cells, whereas its expression dramatically decreased thereafter. Its kinetics of expression appeared opposite to those of aP2 and PPARγ, which characterize the terminal differentiation process (33, 34). Identical results were obtained with 3T3-L1 (Fig. 2A) and Ob1771 cells (data not shown). PTX3 mRNA expression appeared as a differentiation-related event as the message was maintained in the absence of induction of differentiation of 3T3-L1 cells (Fig. 2A), and a weak and continuous expression was observed in 3T3-C2 cells (data not shown), a fibroblast nonadipocyte clonal line (35). Consistent with this interpretation, in Ob1771 cells exposed to growth hormone or to fatty acids, in order to accelerate the differentiation process, the disappearance of PTX3 mRNA is faster than that observed in untreated cells (data not shown). The same pattern of expression was obtained in 3T3-F442A cells differentiated in a serum-free medium (Fig. 2B). This result excluded nonspecific

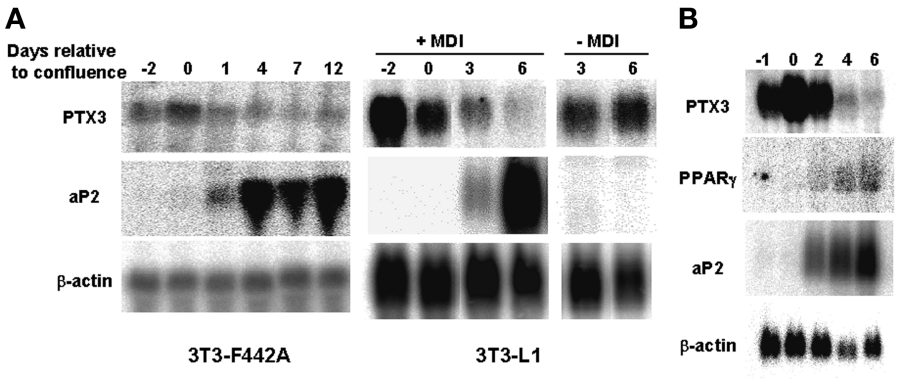

Fig. 2. PTX3 mRNA expression in 3T3-F442A and 3T3-L1 cells during adipocyte differentiation in serum-containing and in serum-free medium. A: RNA was extracted from 3T3-F442A or 3T3-L1 cells that had been maintained in serum-supplemented medium at different intervals relative to confluence. 3T3-L1 cells have been induced to differentiate (+MDI) or not (−MDI) in the presence of an hormonal cocktail (0.5 mM 1-methyl-3-isobutylmethyl-xanthine, 0.5 μM dexamethasone, and 170 nM insulin) for 2 days. B: Total RNA was extracted from 3T3-F442A cells that had been maintained in serum-free medium at different intervals relative to confluence. Twenty micrograms of RNA of each sample were loaded and analyzed by Northern blot as described in Materials and Methods. The results are representative of two independent experiments.

effects of serum components, including cytokines, and favored the existence of a differentiation-specific event. Thus, the finding that PTX3, a member of the long pentraxin family, was transiently expressed in preadipocytes prompted us to examine its role, if any, in adipocyte differentiation.

Differentiation of stable 3T3-F442A cells overexpressing PTX3

Overexpression of PTX3 in 3T3-F442A cells was accomplished by retroviral infection as shown in **Fig. 3** control cells, i.e., cells infected with the empty vector expressed the endogenous PTX3 mRNA at the expected size of 2 kb. Cells infected with the retroviral vector containing the PTX3 coding sequence expressed higher levels of PTX3 protein, and both the 2 kb signal and a 4 kb signal corresponding to the viral transcript were observed (Fig. 3A, B). The expression of the 4 kb mRNA remained unchanged throughout the postconfluent period, consistent with similar levels of PTX3 protein during differentiation (Fig. 3A). As shown in Fig. 3B and C, adipocyte differentiation of PTX3-overexpressing cells was unimpaired compared with that of control cells, as assessed by microscope examination and expression of differentiation-specific markers, excluding a direct role of PTX3 in the differentiation process. However, owing to the involvement of PTX3 in the inflammatory process and its postulated role in the defense against bacterial infections (36–38), studies were performed with 3T3-F442A adipocytes in response to inflammatory cytokines, i.e., TNFα, as PTX3 expression, which is also termed TSG-14, is highly induced by TNFα, and as both human and mouse PTX3 promoters are known to be responsive to TNFα (39, 40).

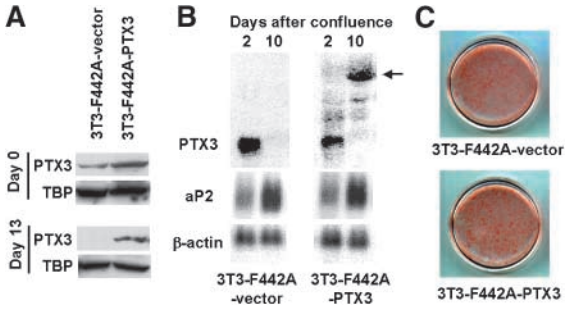

Fig. 3. Ectopic expression of PTX3 and differentiation of 3T3-F442A preadipocytes. Stable 3T3-F442A-vector cells and 3T3-F442A-PTX3 transfectants were maintained after confluence in differentiation medium. A: Total protein was extracted at Days 0 and 13, and 50 μg was analyzed by Western blot. TATA-binding protein (TBP) was used as the internal standard. B: RNA was extracted at Days 2 and 10 after confluence, and 20 μg were analyzed by Northern blot. C: Cells were fixed and stained with Oil red O at Day 10 after confluence and photographed. The results are representative of two independent experiments.

Modulation by TNFα of PTX3 mRNA and protein expression

Upon exposure of differentiated 3T3-F442A cells to TNFα present in the nanomolar range of concentrations, the induction of the PTX3 gene occurred within 1 h. Peak levels were reached within 3 h and decreased somewhat thereafter (**Fig. 4**). Interestingly, IL-6 and LIF were inactive at concentrations up to 1 nM, in contrast to lipopolysaccharide (LPS) which is active at 100 ng/ml. 3T3-F442A cells were able to respond to TNFα at concentrations as low as 0.01 nM (Fig. 4). Likewise, PTX3 protein expression was enhanced following TNFα treatment. PTX3 protein was clearly detectable by Western blot analysis of incubation media from differentiated 3T3-F442A adipocytes as well as from adipose tissue explants (**Fig. 5**). Collectively, these data emphasize that PTX3 is a TNFα-induced protein secreted from adipocytes. As TNFα levels were reported to be elevated in adipose tissue of genetically obese mice, PTX3 levels were examined in these animals.

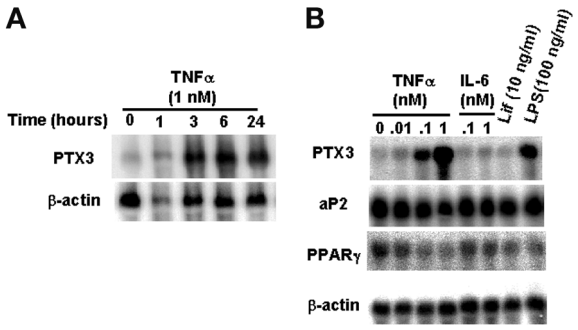

Fig. 4. Effect of cytokines on PTX3 mRNA expression in 3T3-F442A adipocytes. 3T3-F442A cells were maintained after confluence in differentiation medium. At Day 7 after confluence, differentiated cells were (A) treated for different time periods with 1 nM tumor necrosis factor α (TNFα), or (B) treated for 3 h with increasing concentrations of TNFα or interleukin-6 (IL-6) as indicated, or with 10 ng/ml leukemia inhibitory factor or 100 ng/ml lipopolysaccharide (LPS). Twenty micrograms of RNA were analyzed by Northern blot. The results are representative of three independent experiments.

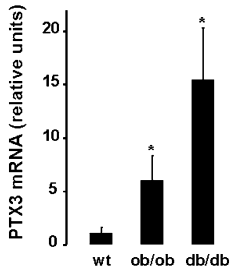

Fig. 6. PTX3 mRNA expression in WAT of genetically-obese mice. Total RNA was extracted from epididymal adipose tissue of wild-type (wt), obese (ob/ob), or obese diabetic (db/db) mice. One microgram of RNA was analyzed by real-time RT-PCR as described in Materials and Methods. The calculations of PTX3 amounts (relative units) were obtained by using the comparative-Ct method. Each point represents measurements from three mice determined in triplicate. TBP served as reference. Asterisk indicates statistically significant differences, $P < 0.05$.

Enhanced expression of PTX3 gene in adipose tissue of genetically obese mice

Real-time RT-PCR analysis of RNA from epididymal fat pads of obese (ob/ob), and obese diabetic (db/db) mice was performed and compared with that of the corresponding control mice. The pattern of expression shown in **Fig. 6** shows a higher level of PTX3 expression in adipose tissue of genetically obese mice versus control mice, although it is difficult to estimate the relative contribution of stromal-vascular cells and adipocytes in each tissue sample. Quantitative analysis allowed an average increase of 5- and 15-fold in ob/ob and db/db mice, respectively, compared with wild-type mice. An increase in the PTX3 level was also observed in Agouti mice compared with control mice (data not shown). However, when one considers *i*) the lack of PTX3 gene expression in the heart, which contains, like adipose tissue, both endothelial cells and various other cell types reported to express PTX3 (fibroblasts, monocytes/macrophages), and *ii*) the enhanced expression of locally produced TNFα in adipose tissue of obese rodents or humans (7, 41), it is likely that adipocytes are responsible for the observed up-regulation of PTX3 gene expression. By microarray techniques, Friedman and coworkers (42) have also reported an increase in PTX3 mRNA levels in adipose tissue of ob/ob mice compared with their lean littermates.

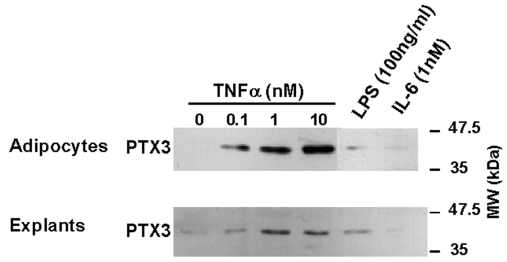

Fig. 5. PTX3 protein secretion from 3T3-F442A adipocytes and adipose tissue explants. Differentiated 3T3-F442A cells (upper panel) or adipose tissue explants (lower panel) were maintained in serum-free medium and treated with increasing concentrations of TNFα as indicated, 100 ng/ml LPS, or 1 nM IL-6 for 3 h. Secretion media were collected and analyzed by Western blot as described in Materials and Methods. The results are representative of two independent experiments.

DISCUSSION

As early events of adipogenesis are critical to gaining insights into subsequent steps, our aim was to identify novel genes and proteins that are differentially regulated by nutrients in preadipocytes. For that purpose, substractive hybridization was used in early confluent preadipocytes treated or not with long-chain fatty acids (24). Unexpectedly, our strategy led to the identification of PTX3, a member of the long form of pentraxin proteins involved in inflammatory processes. The transient expression of PTX3 in the early 3T3-F442A preadipocytes period remains unclear, as its constitutive expression in infected cells shows no impairment of adipocyte differentiation. In this respect, it is of interest to note that myogenic differentiation gives rise to the expression of neuronal pentraxin 1 (NP1), another long form of the pentraxin family whose expression is induced by MyoD (43), and it is tempting to postulate that PTX3 and NP1 share common but still uncharacterized features in the commitment of mesoderm precursor cells to a specific lineage, adipoblast or myoblast, respectively. The identification of the signaling pathways involved in preadipocytes for the transient expression of PTX3 remains unknown. Cytokines would appear as putative effectors, but LIF can clearly be excluded as, despite the fact the LIF/LIF-R system is active in preadipocytes and that LIF-R remains expressed in adipocytes, LIF shows no effect, in contrast to TNFα, on the expression of PTX3 in adipocytes. Of note is the fact that LIF and IL-6, which share a common gp130-related signaling pathway, do not induce activation of the PTX3 gene (44). The expression of PTX3 in preadipocytes can be linked to the

macrophage-like functions of preadipocytes, consistent with the involvement of adipose tissue in immune response during inflammation (45).

PTX3 is a long-form member of the pentraxin family, and we have identified the PTX3 gene as a TNFα-responsive gene leading to the protein secretion. TNFα and IL-1, two major mediators of inflammation, have been reported to induce the transcriptional activation of the PTX3 gene (19, 21, 46). LPS, which raises PTX3 plasma levels after injection in mice, shows a direct effect by activating the PTX3 gene. It is assumed that, as in 3T3-L1 adipocytes, LPS binding to constitutively-expressed Toll-like receptor-4 (TLR4) results in the fast induction of TLR-2 and the synthesis of a set of secretory proteins that would include PTX3 (47). Although cultured adipocytes and adipose tissue explants respond to very low concentrations of TNFα (0.01–1 nM), it remains unclear whether Type 1 or Type 2 TNFα receptors are involved in this response. Further studies using adipocytes lacking each or both of these receptors should clarify this issue (48). Preliminary evidence shows that significant levels of PTX3 are present in mouse plasma, but a possible increase in plasma of genetically obese mice remains to be shown. If it is so, considering the fact that PTX3 binds to C1q (25), which is known to be homologous to adiponectin (49, 50), it is tempting to postulate that higher circulating levels of PTX3 in a chronic low-level inflammatory state may lower the concentration of unbound adiponectin and may participate in the aggravation of the metabolic syndrome observed in obese animals and individuals. Studies in transgenic mice and gene-targeted mice suggest an important role for PTX3 in the regulation of inflammatory reactions and innate immunity (51–54). The study of the development of adipose tissue in these knock-out animals as well as the use of obese TNFα-deficient mice should shed some light on the physiological role of PTX3.

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