Characterisation of receptor-specific TNFα functions in adipocyte cell lines lacking type 1 and 2 TNF receptors

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Abstract Tumour necrosis factor-alpha (TNFα) is a multifunctional cytokine that exerts a myriad of biological actions in numerous different tissues including adipocytes through its two distinct cell surface receptors. To address the role of each TNF receptor in the biological actions of TNFα in adipocytes, we have developed four new preadipocyte cell lines. These were established from wild type controls ($TNFR1^{+/+}R2^{+/+}$) and from mice lacking TNFR1 ($TNFR1^{-/-}$), TNFR2 ($TNFR2^{-/-}$) or both ($TNFR1^{-/-}R2^{-/-}$). All four new cell lines can fully differentiate to form mature adipocytes, under appropriate culture conditions, as judged by cell morphology, expression of multiple adipogenic markers and the ability to mediate agonist-stimulated lipolysis and insulin-stimulated glucose transport. In wild type ($TNFR1^{+/+}R2^{+/+}$) and $TNFR2^{-/-}$ adipocytes, TNFα stimulated lipolysis and inhibited insulin-stimulated glucose transport as well as insulin receptor autophosphorylation. In contrast, these activities were completely lost in the $TNFR1^{-/-}R2^{-/-}$ and $TNFR1^{-/-}$ cells. Taken together, these studies demonstrate that TNFα-induced lipolysis, as well as inhibition of insulin-stimulated glucose transport are predominantly mediated by TNFR1 and that the presence of TNFR2 is not necessary for these functions. This new experimental system promises to be useful in dissecting the molecular pathways activated by each TNF receptor in mediating the biological functions of TNFα in differentiated adipocytes.

Key words: Tumor necrosis factor receptor; Preadipocyte; Lipolysis; Glucose metabolism; Insulin resistance; Adipogenesis

1. Introduction

The biological actions of tumor necrosis factor-α (TNFα) have been studied for over a century. These actions occur in many cell types and include inflammation, mitogenesis, differentiation, immune modulation and antitumor immunity [1]. TNFα has also been implicated as an important modulator of energy metabolism, particularly in adipocytes [2]. In these cells, TNFα regulates the expression of many adipocyte genes and inhibits the differentiation program [3]. It has also been demonstrated to directly interfere with glucose homeostasis and lipid metabolism in adipose tissue and contribute to the development of insulin resistance in obesity and type 2 diabetes [2,4,5].

At the molecular level, the cellular actions of TNFα are mediated by two distinct receptors: type 1 (TNFR1; a 55 kDa peptide in rodents and 60 kDa in humans), and type 2 (TNFR2; 75 kDa in rodents and 80 kDa in humans) [6]. Both these receptors are expressed ubiquitously and oligomerize upon binding of the trimeric ligand. While the extracellular domains of these two receptors exhibit some sequence homology, their intracellular or cytoplasmic domains appear to be quite dissimilar [6]. This is thought to be indicative of the existence of distinct signalling pathways. The biological functions mediated by each TNF receptor have been studied with the aid of type-selective ligands [7–9] or antibodies that act as agonists or antagonists [10]. For example, human TNFα has been reported to be selective for murine TNFR1 (p55) and as a result has been used to demonstrate the involvement of TNFR1 in a variety of cellular systems including adipocytes [7,9,11]. Similarly, human ligand mutants have been developed with differential affinity for hTNFR1 or hTNFR2 [8]. Finally, type selective antibodies, with either agonistic or antagonistic activity have been widely utilized to understand the signalling and biology of the different TNF receptors [11,12]. While these experimental systems have provided a vast amount of valuable information, their use has some limitations, especially in long term studies that arise from differences in affinities and/or stability in different cell types. This has been particularly problematic in studies targeting fat-laden adipocytes. Furthermore, since receptor hetero-complexing may be a prerequisite for some TNFα-induced signals, extracellular targeting of one receptor may not prevent the engagement of its intracellular domains in heterocomplexes and subsequent involvement in signal generation.

Recently, targeted null mutations have been introduced into the murine genes of both TNF receptors [13–15]. These studies have demonstrated that both receptors are critically involved in the establishment of host defense and have provided excellent models for studying the biological capabilities each TNF receptor. The single TNF receptor deficient mice have been critical in demonstrating that TNFR1 plays a dominant role in mediating endotoxic shock, LPS- and TNF-induced toxicity [13,14] but plays a protective role in *L. monocytogenes*-induced infection [13,14]. It is also involved in the development of lymphoid tissue [16] and pathologies such as collagen-induced arthritis [17]. Conversely, it is TNFR2 that suppresses TNF-induced inflammatory responses [15] but also

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Abbreviations: TNFα, tumour necrosis factor; TNFR, tumour necrosis factor receptor; IBMX, isobutyl-methyl xanthine; IR, insulin receptor

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required for TNF-induced T-cell toxicity, tissue necrosis [18], the migration of epidermal Langerhans cells, initiation of cutaneous immune responses [19] and the development of experimental cerebral malaria [20].

In relation to metabolic functions, a number of recent studies using receptor- or ligand-deficient mice have addressed the in vivo role of TNFα signalling in obesity and insulin resistance [21–24]. These studies have demonstrated that the absence of TNFα function affords considerable protection from obesity-related insulin resistance [21,22]. While the dominant mediator of this TNFα function appears to be TNFR1, a role for TNFR2 has not been ruled out. Genetically obese (ob/ob) mice lacking TNFR1, but not TNFR2, showed improved insulin sensitivity [23], a study reported this to be the case only in TNFR2-deficient mice fed a high-fat diet [24]. In an attempt to understand the exact role of each TNF receptor at the cellular and molecular level, we have developed a new in vitro experimental system derived from embryos of TNF receptor knockout mice. The use of adipocyte cell lines with genetic alterations has not been widely utilized due to challenges in developing these experimental systems. This has limited the molecular characterization of the biology of this cytokine particularly in cases where the adipocyte is the primary target. Here, we report our initial characterization of these preadipocyte cell lines, which lack TNFR1, TNFR2, or both, and compare their metabolic responses to wild type control cells with intact receptors.

2. Materials and methods

2.1. Materials and reagents

Recombinant murine TNFα was purchased from Genzyme (Cambridge, MA). 2-Deoxy-D-[1,2-^{3}H]glucose (40 Ci/mmol, 1 mCi/ml) and [α-^{32}P]dCTP (6,000 Ci/mmol, 20 mCi/ml) were from Amersham Life Science, USA and Dupont-NEN (Boston, MA), respectively. The thiazolidinedione, BRL49653 (FW 473.5), was kindly provided by Dr. Hideko Tanaka (Mitsubishi Chemical Corp., Japan).

2.2. Establishment of preadipocyte cell lines

Mice deficient in TNFR1, TNFR2 or both TNFR1 and R2 and the wild type controls were previously reported [21]. Embryos from these mice were eviscerated and used to isolate adherent cells. Fibroblast cell lines were then developed using the previously described 3T3 protocol [25]. This protocol was continued for approximately 15–25 passages, by which time propagating cells had entered and recovered from the growth retardation phase termed 'crisis'. Following crisis each fibroblastic cell line was expanded and multiple aliquots frozen in DMEM containing 10% Cosmic calf serum and 10% DMSO. Eight independent cell lines (two from each genotype) were then tested for their differentiation potential following treatment with the adipogenic agents as described below. Although, four cell lines (one of each genotype) failed to produce any adipocytes even under the strongest adipogenic stimuli, two cell lines (wt and TNFR1$^{-/-}$) produced $>85\%$ adipocytes. The poorly differentiating lineages yielding $<80\%$ adipocytes (TNFR1$^{-/-}$R2$^{-/-}$ and TNFR2$^{-/-}$) were further improved by two consecutive rounds of population cloning [26] producing 30–50 clones from each parental cell line. Each clone was then tested for its adipogenic potential and those producing $>80\%$ adipocytes were further expanded and stored. Finally, the presence and/or absence of each TNF receptor was re-confirmed in each cell line by genotyping [21] and Northern blotting (see below and Fig. 2).

2.3. Cell culture and adipocyte differentiation

Preadipocytes were maintained and propagated in Dulbeco's Modification of Eagle's Media (DMEM), containing high glucose, L-glutamine, pyridoxine HCl, no sodium pyruvate and supplemented with 10% bovine calf serum (Hyclone), 50 U/ml penicillin and 50 μg/ml streptomycin. Differentiation of preadipocytes was achieved by a modification of the procedure described for 3T3-L1 cells [25]. Briefly, cells were seeded and grown to 80–90% confluence in DMEM supplemented with 10% fortified bovine calf serum (FBS or Cosmic calf serum®, Hyclone). Differentiation was then initiated (day 0) by incubation in induction medium (DMEM supplemented with 10% FBS, 50 U/ml penicillin, 50 μg/ml streptomycin, 1 μM dexamethasone, 0.5 mM isobutylmethy xanthine (IBMX), 1 μM BRL49653 and 5 μg/ml insulin). Following a 4-day induction treatment (two 48-h incubations), the medium was changed to a post-induction media (induction media without dexamethasone and IBMX) for an additional 4 days (two 48-h incubations). Thereafter, the medium was replaced every other day with DMEM containing 10% FBS and 5 μg/ml of insulin. Unless otherwise stated, cells were used for experiments on day 16 after the start of induction.

2.4. Determination of stimulated lipolysis

Intracellular lipolysis in fully differentiated adipocytes was assessed by the extent of glycerol release from adherent cells seeded in 35 mm six-well plates. Adipocyte monolayers were washed and incubated in complete serum free medium (with L-glutamine and albumin, Mediatech Cellgrow) ± 10 ng/ml TNFα for 20 h at 37°C. This was followed by a 4-h incubation in fresh complete serum free medium ± 10 ng/ml TNFα or 1 mM dibutyryl-cAMP. The glycerol concentration was determined in heat-inactivated conditioned media using the enzyme-based GPO trinder reagent kit from Sigma (St. Louis, MO, USA). Cell monolayers were then digested in 0.1% SDS for protein quantitation using the BioRad DC protein assay based on the Lowry method (BioRad Laboratories, CA).

2.5. Determination of glucose uptake and insulin-stimulated IR phosphorylation

For glucose uptake experiments, fully differentiated adipocytes were washed and serum starved for 24 h prior to insulin stimulation. Cell monolayers, were then washed three times with warm Krebs–Ringer HEPES buffer (KRH: 120 mM NaCl, 5 mM KCl, 1 mM KH_2PO_4, 0.6 mM $MgSO_4$, 1 mM $CaCl_2$, 20 mM HEPES, pH 7.5 and 0.5% bovine serum albumin) and incubated for 30 min in 1 ml (per 35 mm well) KRH ± 1 μg/ml insulin at 37°C. Isotope-labelled glucose uptake was initiated during the last 10 min by the addition of 2-deoxy-D-[1,2-^{3}H]glucose (1 μCi/ml). The reaction was terminated by immediately removing the medium and washing cell monolayers three times with chilled KRH. Cells were removed in 1 ml SDS (0.1%) and the radioactivity determined in a 0.8 ml aliquot by liquid scintillation counting. The remaining digest was used for protein quantification using the BioRad DC protein assay based on the Lowry method (BioRad Laboratories, CA, USA). Non-specific uptake was determined by incubating with 50 μM cytochalsin B for 10 min prior to the addition of radioactivity [29]. Insulin receptor autophosphorylation was examined in the presence or absence of TNFα (10 ng/ml) as previously described. Immunoblotting was performed using a 1:1000 dilution of a monoclonal anti-phosphotyrosine (PY20) or a 1:300 dilution of the polyclonal anti-murine IRβ antibody (gift from C.R. Kahn), followed by horseradish peroxidase (HRP)-conjugated anti-mouse or anti-rabbit IgG antibodies (1:2500 dilution), respectively. Immunoreactive bands were visualized using an enzyme chemiluminescence (ECL) kit (Amersham Life Science) and pTyr/protein ratios were determined by densitometrical analysis using NIH Image software.

2.6. Statistical analysis

Data is presented as means ± S.E.M. of triplicates. Two-tailed, two-sample, equal variance Student *t*-tests were used to assess statistical significance where $P<0.05$ was the accepted level of statistical significance.

3. Results and discussion

3.1. TNFR-deficient preadipocyte cell lines

To address the role of each TNF receptor in mediating the metabolic actions of TNFα in adipocytes, we have established three preadipocyte cell lines, which carry targeted null mutations in the genes encoding TNFR1, TNFR2, both TNFR1 and TNFR2. A fourth, wild type cell line was also generated with the same genetic background. Prior to differentiation, all

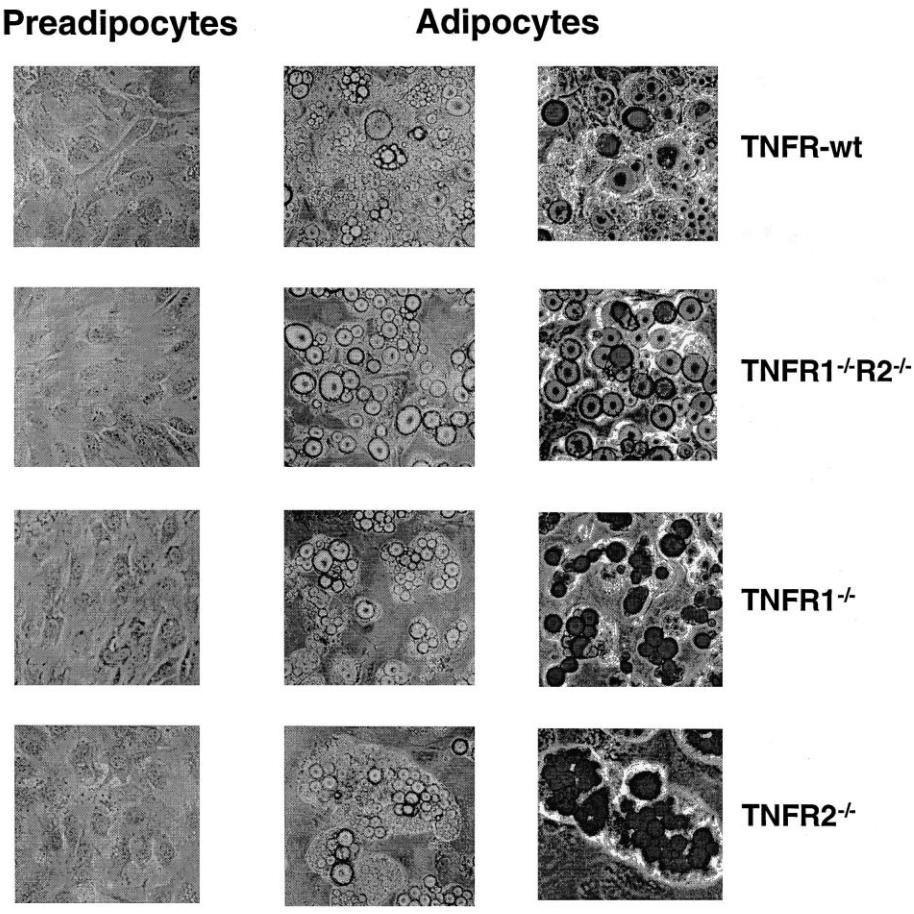

Fig. 1. Morphological features of cultured TNFR-deficient cells. Photomicrographs of TNFR-deficient preadipocytes (left), fully differentiated adipocytes unstained (center) and stained with Oil red O (right). All lines appear to be morphologically indistinguishable from murine preadipocytes and upon stimulation, exhibit typical adipocyte morphology.

of these cells appeared to be morphologically indistinguishable from murine fibroblasts as illustrated in Fig. 1 (left column of photomicrographs). The differentiation potential of each cell type was then tested as described in Section 2. The photomicrographs in Fig. 1 (center and right columns) demonstrate that following adipogenic treatment, all four new cell lines were capable of accumulating lipid droplets (yielding $>90\%$ adipocytes), thereby exhibiting the morphological characteristics of adipocytes. Lipid accumulation in these cells is also readily detectable by Oil Red O staining (Fig. 1, right column). In contrast, when the same cells were grown and maintained at confluency, in medium lacking adipogenic agents, very few ($<0.5\%$), cells adopted the lipid-laden phenotype (Fig. 1, left column).

3.2. Adipocyte-specific gene expression in TNFR deficient cells

During adipocyte differentiation, a number of genes are differentially expressed. Some, such as those involved in lipid synthesis and storage, are related specifically to the adipogenic phenotype [30]. We therefore investigated the expression pattern of several genes in our new preadipocytes during the course of differentiation. Fig. 2 shows the gene expression pattern in TNFR-deficient cells following 16 days of treatment with (+) or without (−) adipogenic agents. In all four cell types, adipogenic treatment clearly resulted in the expression of classic adipocyte specific markers such as aP2 (adipocyte fatty acid-binding protein), PPARγ (peroxisome proliferator-activated receptor γ), C/EBPα (CAAT/enhancer binding protein), Glut4 (insulin sensitive glucose transporter) and adipsin (a protease secreted from adipocytes). Conversely, the maintenance of cells at confluence or growth arrest did not induce the expression of the same genes. The expression levels and patterns of these genes are clearly comparable to those observed in the commonly used preadipocyte cell line 3T3-L1 treated with the same protocol. The same RNA blots were also used to confirm the expression of the TNFR(s) in the respective cell types. As reported previously [23], the level of receptor expression in the single receptor deficient cells appeared comparable to that seen in wild-type cells (Fig. 2). Thus the absence of one TNF receptor does not seem to alter the endogenous expression level of the other.

We next investigated the time course of gene expression throughout the adipogenic treatment and compared this to the same treatment of 3T3-L1 cells. Each of the cell lines exhibited differentiated-dependent gene expression during the 16-day treatment and this gene expression pattern is clearly comparable to that observed in 3T3-L1 cells (data not shown). The level of adipocyte-specific gene expression reached a maximum by day 14, where these cells could be considered mature adipocytes. Therefore, 16-day old adipocytes were used in all the subsequent experiments described in this report. Finally, our data also suggests that the absence of one or both TNFR(s) does not alter the ability or efficiency of these preadipocytes to differentiate into mature adipocytes. This is con-

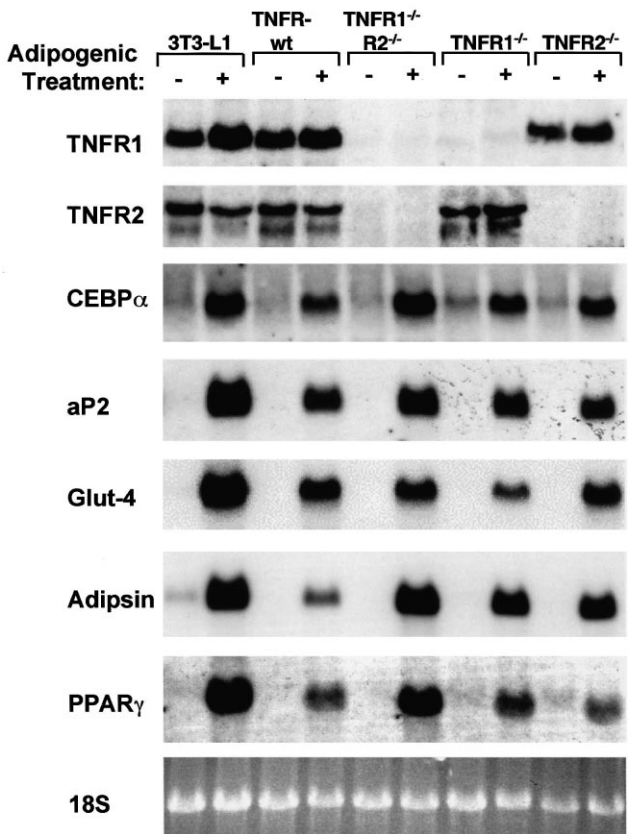

Fig. 2. Adipocyte-specific gene expression during differentiation of TNFR-deficient preadipocytes. Total RNA was extracted from cells of each genotype following 16 day treatment with (+) or without (−) adipogenic agents (as described in Section 2) and expression of the indicated genes examined by Northern blot analysis as previously described [27,28].

sistent with the observation that cultured adipocytes do not express detectable levels of TNFα [28] and mice deficient in one or both of TNF receptors exhibit no obvious alteration in adipose tissue development [23].

3.3. Agonist-stimulated lipolysis in TNFR deficient adipocytes

One of the primary biological functions of adipocytes is to adjust their lipid storage according to the physiological energy requirements of the organism. When extra calories are required, adipocyte intracellular triglyceride stores are metabolized to glycerol and fatty acids for further oxidation, a process termed lipolysis. This process is regulated by adrenergic stimulation and mediated by a cAMP-dependent pathway [31]. To further evaluate the adipocyte phenotype, we next tested the lipolytic capacity of the TNFR deficient adipocytes. Fig. 3A shows that following differentiation, all four of the new preadipocyte cell lines respond to both dibutyryl-cAMP (a membrane permeable, hydrolysis-resistant analogue of cAMP) and the non-specific adrenergic receptor agonist isoproterenol. These data demonstrate that all of the adipocyte cell lines possess the appropriate lipolytic machinery that is required to respond to stimulation directly by dibutyryl-cAMP or via adrenergic receptors.

Since TNFα itself can act as a lipolytic agent in wild-type adipocytes [2,32], we next examined the ability of TNFα to induce lipolysis in each cell type. Fully differentiated adipocytes were treated with or without TNFα (10 ng/ml) for 24 h and the amount of glycerol released into the culture media was determined (Fig. 3B). As expected, TNFα produced a lipolytic response in wild-type adipocytes. This effect was completely absent in adipocytes lacking TNFR1 or both functional receptors but preserved in TNFR2-deficient cells. These experiments demonstrate that TNFα-stimulated lipolysis is intact only in those adipocytes expressing functional TNFR1, indicating that this receptor is the obligatory mediator of this action of TNFα.

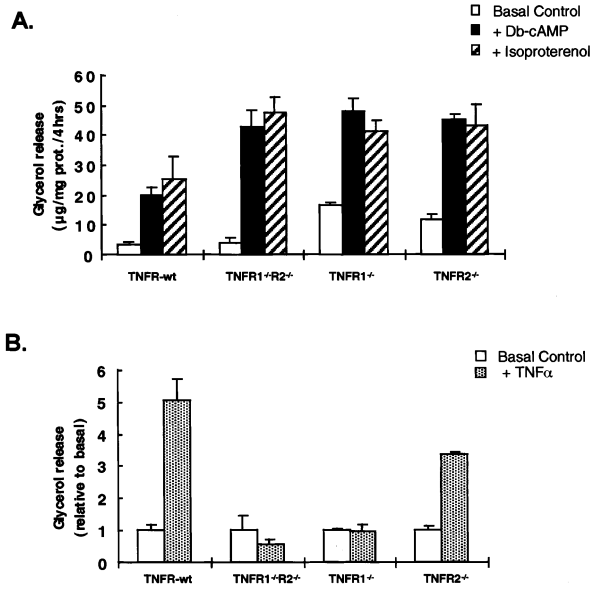

Fig. 3. Stimulated lipolysis in TNFR-deficient adipocytes. Differentiated adipocytes from each genotype were cultured in the absence (basal) or presence of (A) dibutyryl cAMP (1 mM for 4 h) or isoproterenol (1μM for 4 h) or (B) TNFα (10 ng/ml for 24 h). Conditioned media were then collected over the last 4-h incubation and assayed for glycerol content. Values represent means ± S.D. of triplicates. The basal lipolysis values in (B) were 3.5 ± 0.7, 4.1 ± 1.9, 16.4 ± 0.8 and 11.6 ± 1.8 μg glycerol released/mg protein/4 h from TNFR-wt, TNFR1$^{-/-}$R2$^{-/-}$, TNFR1$^{-/-}$ and TNFR2$^{-/-}$ cells, respectively.

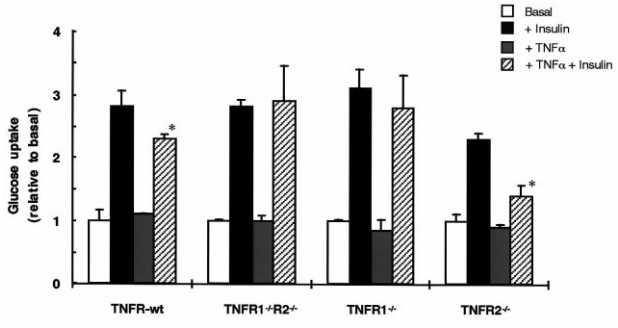

Fig. 4. Effect of TNFα on insulin stimulated glucose uptake in TNFR deficient adipocytes. 2-Deoxy-glucose uptake was determined in differentiated adipocytes of each genotype. TNFα treatment involved a 24 h exposure to 10 ng/ml TNFα followed by stimulation with 1 μg/ml insulin for 30 min. Values represent means ± S.D. of triplicates. The basal glucose uptake in each cell type were 3085 ± 514, 3826 ± 111, 2058 ± 271 and 1157 ± 132 cpm/mg protein for TNFR-wt, TNFR1$^{-/-}$R2$^{-/-}$, TNFR1$^{-/-}$ and TNFR2$^{-/-}$ cells, respectively. * indicates $P < 0.05$ when compared to the insulin-stimulated controls.

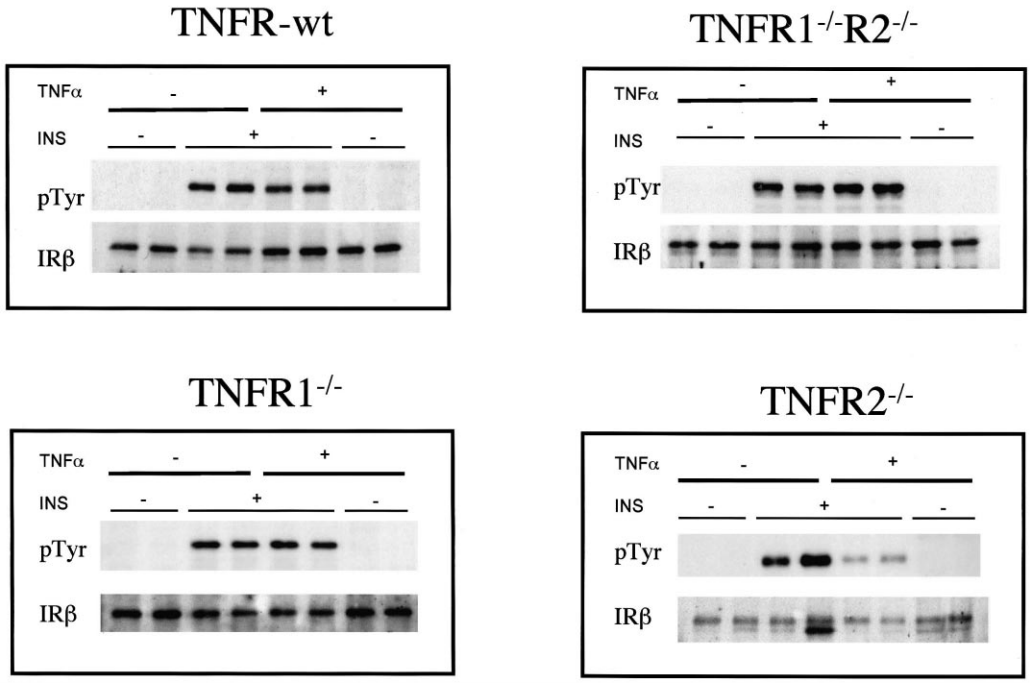

Fig. 5. Effect of TNFα on insulin-stimulated receptor tyrosine phosphorylation in TNFR-deficient adipocytes. Insulin receptors (IR) were immunoprecipitated from control and insulin-stimulated adipocytes that were pre-treated with or without TNFα (10 ng/ml for 72 h). Antibodies specific for phosphotyrosine (pTyr) or insulin receptor beta subunit (IRβ) were used for immunoblotting.

3.4. Insulin-stimulated glucose uptake in TNFR deficient adipocytes

Another well-established action of TNFα in adipocytes, is that of inhibiting insulin-stimulated glucose uptake [2,29,33,34]. To investigate whether this in vitro system could be used to identify the TNFR responsible for mediating this action in adipocytes, we next investigated the effect of TNFα on insulin-stimulated glucose uptake in the TNFR-deficient cell lines. Fully differentiated adipocytes of each genotype were treated with or without TNFα (10 ng/ml for 24 h) and/or insulin (1 μg/ml for 30 min) and the rate of 2-deoxy-D-[1,2-^{3}H]glucose uptake was determined over a 10 min period (Fig. 4). As reported for the 3T3-L1 adipocytes [29], all four of the new adipocyte cell lines clearly responded to insulin by increasing their rate of glucose uptake. This suggests that the insulin signaling pathways, which regulate glucose uptake are also intact in all four cell lines. In these cells, the basal rate of glucose uptake is not significantly altered by the presence of TNFα alone. However, in cells expressing functional TNFR1, namely the wild-type and TNFR2$^{-/-}$ adipocytes, pretreatment with TNFα resulted in significant inhibition of insulin-stimulated glucose uptake (18 and 39% inhibition in wild-type and TNFR2$^{-/-}$ adipocytes, respectively, $P < 0.05$). These results indicate that inhibition of insulin-stimulated glucose transport by TNFα requires the presence of intact TNFR1 signalling.

3.5. Insulin-stimulated IR phosphorylation in TNFR deficient adipocytes

Although TNFα has been demonstrated to inhibit tyrosine phosphorylation of insulin receptor (IR) [33,35], it remains unclear whether this action is solely attributed to the activity of TNFR1 [11,12]. We have therefore investigated the action of this cytokine on IR-phosphorylation in our new cells. Insulin receptors (IR) were immunoprecipitated from differentiated adipocytes of each genotype after treatment with or without TNFα (10 ng/ml) and/or insulin (1.5 μg/ml for 100 s). Fig. 5 illustrates the immunoblots obtained from these immunoprecipitates. Immunoblotting with antibodies specific to IRβ demonstrates that all four cell types express significant levels of insulin receptor. This observation is entirely consistent with the insulin responsiveness shown in Fig. 4. Furthermore, the expression of this protein is not downregulated by TNFα treatment in this experimental system. Probing the same blots with phosphotyrosine (pTyr)-specific antibodies further shows that a marked increase in IR tyrosine phosphorylation following insulin stimulation is evident in all cells. This is also a consistent observation of insulin responsiveness in these cells. Finally, TNFα-induced inhibition of IR-pTyr is apparent only in wild-type (3.9 ± 0.4 versus 2.5 ± 0.3 pTyr/prot signal) and TNFR2$^{-/-}$ (2.8 ± 0.03 versus 1.1 ± 0.4 pTyr/prot signal) adipocytes, but not in TNFR1$^{-/-}$R2$^{-/-}$ (2.5 ± 0.4 versus 2.5 ± 0.4 pTyr/prot signal) or TNFR1$^{-/-}$ (1.9 ± 0.3 versus 1.7 ± 0.3 pTyr/prot signal) cells. This suggests that endogenous levels of TNFR1 alone can mediate inhibition of IR autophosphorylation by TNFα. However, the action of endogenous levels of TNFR2 alone, if any, is undetectable under these experimental conditions.

4. Conclusions

Adipocytes play a critical role in systemic energy homeostasis by producing molecules such as leptin, PAI-1 and several cytokines including TNFα and IL-6 that influence many key metabolic pathways [36]. Interestingly, several of these molecules also regulate the expression and/or production of each other through complex signalling networks [37,38]. Alterations in these aspects of adipocyte biology appear to be

important in several disease states, including obesity, type 2 diabetes and cardiovascular disease. For example, TNFα can regulate leptin production from the adipose tissue and contribute to obesity-associated hyperleptinemia [37]. Similarly, TNFα stimulates PAI-1 expression in adipocytes and appears to be the dominant signal for increased PAI-1 production in obesity and potentially contribute to increased risk for cardiovascular disease [39]. Furthermore, TNFα has well-documented effects on lipid and glucose metabolism by regulating lipolysis, lipid biosynthesis and insulin sensitivity [2,32]. Therefore, understanding the molecular mechanisms of TNFα action in adipocytes might provide important insights into several pathophysiological states. In an attempt to study the biological functions mediated by each TNF receptor in adipocytes, we have developed a new cellular experimental system by establishing preadipocyte cell lines lacking one or both TNF receptors. Biochemical examination of these cells demonstrates that these do differentiate into bona fide adipocytes based on all morphological, molecular and functional characteristics studied. Using these cells, we have also examined the role of TNFR1 and TNFR2 in two critical metabolic activities of TNFα in adipocytes. These studies demonstrate that stimulation of lipolysis, inhibition of insulin receptor signalling and insulin-stimulated glucose uptake by TNFα requires the presence of TNFR1. These observations, together with the existing data obtained through the use of receptor selective ligands or antibodies [7,12] indicate TNFR1 as the primary mediator of TNFα-induced insulin resistance in adipocytes and obesity [23]. In conclusion, these mutant preadipocyte cell lines represent a new experimental system, which should prove to be instrumental in future mechanistic and structure-function studies of TNF receptors in adipocytes.

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References

[1] Rink, L. and Kirchner, H. (1996) Int. Arch. Allergy Immunol. 111, 199–209.
[2] Sethi, J.K. and Hotamisligil, G.S. (1999) Semin. Cell Devel. Biol. 10, 19–29.
[3] Kronke, M., Schutze, S., Scheurich, P. and Pfizenmaier, K. (1991) in: Tumor Necrosis Factor: Structure Function and Mechanism of Action (Aggarwal, B.B. and Vilcek, J., Eds.), pp. 189–216. Marcel Dekker Inc.
[4] Skolnik, E.Y. and Marcusohn, J. (1996) Cytokine Growth Factor Rev. 7, 161–173.
[5] Hotamisligil, G.S. and Spiegelman, B.M. (1994) Diabetes 43, 1271–1278.
[6] Tartaglia, L.A. and Goeddel, D.V. (1992) Immunol. Today 13, 151–153.
[7] Tartaglia, L.A., Weber, R.F., Figari, I.S., Reynolds, C., Palladino Jr., M.A. and Goeddel, D.V. (1991) Proc. Natl. Acad. Sci. USA 88, 9292–9296.
[8] Loetscher, H., Stueber, D., Banner, D., Mackay, F. and Lesslauer, W. (1993) J. Biol. Chem. 268, 26350–26357.
[9] Mackay, F., Rothe, J., Bluethmann, H., Loetscher, H. and Lesslauer, W. (1994) J. Immunol. 153, 5274–5284.
[10] Sheehan, K.C., Pinckard, J.K., Arthur, C.D., Dehner, L.P., Goeddel, D.V. and Schreiber, R.D. (1995) J. Exp. Med. 181, 607–617.
[11] Liu, L.S., Spelleken, M., Rohrig, K., Hauner, H. and Eckel, J. (1998) Diabetes 47, 515–522.
[12] Peraldi, P., Hotamisligil, G.S., Buurman, W.A., White, M.F. and Spiegelman, B.M. (1996) J. Biol. Chem. 271, 13018–13022.
[13] Rothe, J. et al. (1993) Nature 364, 798–802.
[14] Pfeffer, K. et al. (1993) Cell 73, 457–467.
[15] Peschon, J.J. et al. (1998) J. Immunol. 160, 943–952.
[16] Pasparakis, M., Alexopoulou, L., Grell, M., Pfizenmaier, K., Bluethmann, H. and Kollias, G. (1997) Proc. Natl. Acad. Sci. USA 94, 6319–6323.
[17] Mori, L., Iselin, S., De Libero, G. and Lesslauer, W. (1996) J. Immunol. 157, 3178–3182.
[18] Erickson, S.L. et al. (1994) Nature 372, 560–563.
[19] Wang, B., Fujisawa, H., Zhuang, L., Kondo, S., Shivji, G.M., Kim, C.S., Mak, T.W. and Sauder, D.N. (1997) J. Immunol. 159, 6148–6155.
[20] Lucas, R. et al. (1997) Eur. J. Immunol. 27, 1719–1725.
[21] Uysal, K.T., Wiesbrock, S.M., Marino, M.W. and Hotamisligil, G.S. (1997) Nature 389, 610–614.
[22] Ventre, J., Doebber, T., Wu, M., MacNaul, K., Stevens, K., Pasparakis, M., Kollias, G. and Moller, D.E. (1997) Diabetes 46, 1526–1531.
[23] Uysal, K.T., Wiesbrock, S.M. and Hotamisligil, G.S. (1998) Endocrinol. 139, 4832–4838.
[24] Schreyer, S.A., Chua, S.C. and Leboeuf, R.C. (1998) J. Clin. Invest. 102, 402–411.
[25] Green, H. and Meuth, M. (1974) Cell 3, 127–133.
[26] Green, H. and Kehinde, O. (1976) Cell 7, 105–113.
[27] Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294–5299.
[28] Xu, H., Sethi, J.K. and Hotamisligil, G.S. (1998) J. Biol. Chem. 274, 26287–26295.
[29] Wang, C.-N., O'Brien, L. and Brindley, D.N. (1998) Diabetes 47, 24–31.
[30] Tontonoz, P., Hu, E. and Spiegelman, B.M. (1994) Cell 79, 1147–1156.
[31] Honnor, R.C., Dhillon, G.S. and Londos, C. (1985) J. Biol. Chem. 260, 15122–15129.
[32] Feingold, K.R. and Grunfeld, C. (1992) Diabetes 41 (Suppl 2), 97–101.
[33] Hotamisligil, G.S., Murray, D.L., Choy, L.N. and Spiegelman, B.M. (1994) Proc. Natl. Acad. Sci. USA 91, 4854–4858.
[34] Stephens, J.M., Lee, J. and Pilch, P.F. (1997) J. Biol. Chem. 272, 971–976.
[35] Feinstein, R., Kanety, H., Papa, M.Z., Lunenfeld, B. and Karasik, A. (1993) J. Biol. Chem. 268, 26055–26058.
[36] Mohamed-Ali, V., Pinkney, J.H. and Coppack, S.W. (1998) Int. J. Obes. Relat. Metabol. Disord. 22, 1145–1158.
[37] Kirchgessner, T.G., Uysal, K.T., Wiesbrock, S.M., Marino, M.W. and Hotamisligil, G.S. (1997) J. Clin. Invest. 100, 2777–2782.
[38] Samad, F., Uysal, K.T., Wiesbrock, S.M., Pandey, M., Hotamisligil, G.S. and Loskutoff, D.J. (1999) Proc. Natl. Acad. Sci. USA 96, 6902–6907.
[39] Samad, F., Yamamoto, K. and Loskutoff, D.J. (1996) J. Clin. Invest. 97, 37–46.