Thiazolidinediones (TZDs) are widely used for treatment of type 2 diabetes mellitus. Peroxisome proliferator-activated receptor γ (PPARγ) is the molecular target of TZDs and is believed to mediate the apototic effects of this class of drugs in a variety of cell types, including B and T lymphocytes. The finding that TZDs induce lymphocyte death has raised concerns regarding whether TZDs might further impair immune functions in diabetics. To address this issue, we investigated the roles of PPARγ and TZDs in lymphocyte survival. PPARγ was up-regulated upon T cell activation. As previously reported, PPARγ agonists induced T cell death in a dose-dependent manner. However, the concentrations of TZD needed to cause T cell death were above those needed to induce PPARγ-dependent transcription. Surprisingly, at concentrations that induce optimal transcriptional activation, TZD activation of PPARγ protected cells from apoptosis following growth factor withdrawal. The survival-enhancing effects depended on both the presence and activation of PPARγ. Measurements of mitochondrial potential revealed that PPARγ activation enhanced the ability of cells to maintain their mitochondrial potential. These data indicate that activation of PPARγ with TZDs can promote cell survival and suggest that PPARγ activation may potentially augment the immune responses of diabetic patients.

Aging is a major risk factor for many human diseases. The mechanisms underlying the aging process are not well understood, but there is evidence to suggest that it is influenced by genetic and environmental factors. One possible mechanism is the accumulation of damaged proteins, which can lead to the formation of aggregates that are toxic to cells. These aggregates can also trigger inflammation, which can further promote tissue damage. In this study, we investigated the role of protein aggregation and inflammation in aging and found that mice with a mutation in a protein called ATG3b exhibit delayed aging and reduced protein aggregates compared to wild-type mice. This suggests that preventing protein aggregation and inflammation may be a potential strategy for delaying aging.

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Diabetes mellitus is one of the most common noncommunicable diseases. Approximately 100 million people worldwide, including 16 million people in the United States, suffer from diabetes. With about 54,000 deaths per year, it represents the fourth leading cause of death in the United States. The major causes of morbidity and mortality are complications of long-term complications of hyperglycemia, involving heart, kidneys, eyes, nerves, and the immune system. Among the diabetic population, 80–90% are affected by type 2 diabetes, in which impaired tissue sensitivity to insulin is the primary metabolic defect.

Thiazolidinediones (TZDs1; “glitazones”) are a new class of synthetic compounds that potentiate insulin action in the target tissues, alleviate hyperglycemia, and are efficacious in treating type 2 diabetes (see Refs. 1 and 2; reviewed in Ref. 3). At the molecular level, these compounds function as highly specific pharmacologic ligands of peroxisome proliferator-activated receptor γ (PPARγ) (4).

PPARs are members of the nuclear hormone receptor superfamily. There are three isoforms of PPARs, α, δ (also known as β), and γ. Among them, PPARγ is of particular interest, because, in addition to diabetes, it has been implicated in several other significant human pathological conditions, including atherosclerosis, cancer, and inflammation (5, 6). Like other members of the nuclear receptor family, PPARγ serves as a transcription factor. Upon ligand binding, the receptor undergoes a conformational change. This disrupts a corepressor complex and leads to coactivator recruitment and transcriptional activation. PPARγ functions as a heterodimer with retinoid X receptor α, binding to a specific direct repeat DNA sequence and regulating many target genes involved in lipid metabolism.

PPARγ is most abundantly expressed in adipose tissue and colon epithelial cells. It is also broadly expressed in many other tissues including, but not limited to, bone marrow precursors, monocytes/macrophages, lymphocytes, pneumocytes, breast epithelial cells, hepatocytes, and myocytes (7). Studies of the cellular functions of PPARγ have thus far been focused mainly on adipose tissue and macrophages. In adipose tissue, PPARγ promotes adipogenesis by activating transcription of its target genes, such as adipocyte protein 2, lipoprotein lipase, and acyl-CoA synthetase (reviewed in Refs. 8 and 9). In foamy macrophages of the vasculature, PPARγ reduces lipid accumulation by affecting cholesterol flux in the cells through transcriptional regulation of scavenger receptors and reverse cholesterol transporters (10–12).

Although PPARγ is expressed in B and T cells, little is known about its primary functions in lymphocytes (13–15). Recent studies have suggested that PPARγ may play a role in modulating immune functions. It has been shown in human and murine T cells that treatment with PPARγ ligand leads to inhibition of T cell proliferation and a decrease in interleukin-2 (IL-2) production (14, 16). In animal models, ligands of PPARγ, including 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2) and troglitazone, are also effective against inflammatory diseases, al-

1 The abbreviations used are: TZD, thiazolidinedione; PPAR, peroxisome proliferator-activated receptor; 15d-PGJ2, 15-deoxy-Δ12,14-prostaglandin J2; IL, interleukin.

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PPARγ Promotes Cell Survival

leiating rheumatoid arthritis and inflammatory bowel disease (17, 18). It is, however, unclear whether the anti-inflammatory effects of TZD drugs are mediated through PPARγ. Using PPARγ-deficient embryonic stem cells, several groups have shown that in monocytes/macrophages, inhibition of cytokine production and proinflammatory gene expression by both 15d-PGJ2 and TZDs is independent of PPARγ (10, 11). In addition, non-TZD-derived PPARγ agonists do not inhibit the production of proinflammatory cytokines in activated macrophages (19).

In lymphocytes, some studies have reported that treatment of cells with certain PPARγ ligands induces apoptosis in both T and B cells (15, 20), suggesting that PPARγ might be involved in the down-regulation of immune responses through the induction of lymphocyte death. These findings have significant implications, since millions of type 2 diabetic patients are currently taking TZDs to control their hyperglycemia and prevent complications. A serious concern is that TZDs might further impair the already compromised immune functions in these patients. We therefore set out to investigate the roles of PPARγ in lymphocyte survival.

EXPERIMENTAL PROCEDURES

Animals—C57BL/6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME). 2C T cell receptor transgenic/RAG2−/− mice have been previously described (21). All mice were maintained in the University of Pennsylvania Animal Barrier Facilities (Philadelphia, PA). Animals received humane care in compliance with the “Guide for the Care and Use of Laboratory Animals” published by the National Institutes of Health (Bethesda, MD).

Chemicals—Rosiglitazone was obtained as a generous gift from GlaxoSmithKline, and ciglitazone was purchased from Biomol (Plymouth Meeting, PA). Sources of all other reagents are indicated below.

T Cell Purification and Culture—T cells were purified from human peripheral blood or mouse spleen by negative selection using StemSep™ magnetic columns (StemCell Technologies, Vancouver, Canada) following the manufacturer’s instructions. A purity of 95–99% was normally obtained with this method. These cells were then cultured in Dulbecco’s modified Eagle’s medium supplemented with glutamine, penicillin/streptomycin, HEPES buffer, minimal essential medium nonessential amino acids (Invitrogen), β-mercaptoethanol, and 10% fetal bovine serum (Mediatech, Inc., Herndon, VA).

T Cell Stimulation and Proliferation Assays—For T cell stimulation with anti-CD3 (1:250; BD Pharmingen, San Diego, CA) alone or anti-CD3 plus anti-CD28 (37:51; BD Pharmingen), the antibodies were covalently linked to tosyl-activated Dynabeads M-450 (Dynal, Great Neck, NY) according to the manufacturer’s instructions. The antibody-coated beads were then added to purified T cells at a 3:1 bead/cell ratio for the time indicated for each experiment. For T cell stimulation with antigen-presenting cells, 2C T cell receptor transgenic T cells were cultured with irradiated C57BL/6 splenocytes plus 50 μM antigenic peptide SIYRYYGL (Multiple Peptide Systems, San Diego, CA) as described previously (21). For T cell proliferation assays, triplicate T cell cultures were plated at 2.5 × 10^5/ml in the presence of PPARγ agonists or Me2SO. Cells were stimulated with anti-CD3 and anti-CD28-coated beads for 3 days and pulsed with 5 nCi/ul ^[3]H-thymidine for 6–8 h at 37 °C. Cells were harvested with a Tomtec harvester (Hamden, CT) on glass fiber filters and analyzed by a 1205 Betaplate liquid scintillation counter (Wallac, Turku, Finland).

RNA Preparation and RT-PCR—Total RNA was isolated from peripheral T cells 24 h poststimulation using TRizol reagent (Invitrogen) following the manufacturer’s instructions. Reverse transcription was carried out using oligo(dT) primer and Superscript™ II (Invitrogen) according to the manufacturer’s instructions. PCR was performed using AmpliTaQ DNA polymerase and buffers from Applied Biosystems (Branchburg, NJ). The reactions were conducted in a 9600 GeneAmp PCR system (PerkinElmer Life Sciences) using the following conditions: denaturation at 94 °C for 3 min, followed by 25 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 1 min, followed by extension at 72 °C for 5 min. The forward and reverse primers for the amplification of the human PPARγ cDNA are as follows: 5′-TAT CAA GCC CTT CAC TAC TG-3′ and 5′-CTG AGT GCA TTA TGA GAC AT-3′, which generate a 445-bp PCR product.

Western Blot Analysis—To determine levels of protein expression, cells were lysed in radiodinamic precipitation buffer (10 mM Tris-Cl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 1% deoxycholate, 0.1% SDS, 8 μg/ml aprotinin, 2 μg/ml leupeptin, and 170 μg/ml phenylmethylsulfonyl fluoride). For stimulated T cells, lysates from 1 × 10^6 cells were loaded onto an 8% precast Tris-glycine gel (Invitrogen). For FL5.12 cells, lysates containing 20 μg of total protein were loaded. Blots were probed with either anti-PPARγ 561, a rabbit polyclonal antibody against PPARγ (1:1000), or a rabbit polyclonal antibody (H-100) (1:200) purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The blots were then probed with a 1:5000 dilution of Donkey-anti-Rabbit IgG horseradish peroxidase (Amersham Biosciences) and detected by ECL reagents (Amersham Biosciences).

Cell Lines and Culture—FL5.12 cells, an IL-3-dependent hematopoietic cell line, were used for transfection experiments. Cells were transfected with pCDNA3.1-hPPARγ with electroporation as described previously (22). G418-resistant clones transfected with PPARγ were identified by Western blot as described above. Each of the cell survival experiments was performed with at least two PPARγ lines and two empty vector control lines. All cell lines were cultured in complete RPMI supplemented with 10% fetal bovine serum (Mediatech), 0.3 μg/ml recombinant IL-3 (BD Pharmingen), and 1 μg/ml Genetin (Invitrogen).

Luciferase Assay—A reporter construct, acyl-CoA3-TK-LUC (23), and a β-galactosidase expression plasmid were co-transfected by electroporation into a FL5.12-based PPARγ cell line and an empty vector control cell line and an empty vector (pcDNA3.1). Luciferase and β-galactosidase assays (CLONTECH Laboratories, Palo Alto, CA) were performed according to the manufacturer’s instructions. Results of PPARγ transcriptional activity were normalized to β-galactosidase expression. IL-3 withdrawal, Glucose Withdrawal, and Cell Viability Determination—To withdraw IL-3, FL5.12 cells were washed three times in RPMI and resuspended in complete RPMI lacking recombinant IL-3. To limit glucose, cells were washed in glucose-free RPMI three times and resuspended in RPMI supplemented with 10% dialyzed fetal bovine serum (Mediatech) and 50 μM of glucose, which is one-tenth hundredth of the standard glucose used in regular RPMI media. Cell viability was determined by cellular exclusion of 2 μM propidium iodide followed by flow cytometric analysis of 1 × 10^4 events.

Measurement of Mitochondrial Membrane Potentials—12–15 h after IL-3 withdrawal, 2–4 × 10^5 million cells were stained with 20 μM tetramethylrhodamine ethyl ester (Molecular Probes, Inc., Eugene, OR), a potentiometric dye, at 37 °C for 30 min. Cells were then analyzed by flow cytometry.

RESULTS

PPARγ Is Expressed in Peripheral T Lymphocytes and Is Up-regulated upon T Cell Activation—It has been reported that PPARγ is expressed in murine T cell clones and human peripheral T lymphocytes (14, 16). To confirm these findings, PPARγ mRNA levels in purified human peripheral T lymphocytes were assayed under three different activation conditions using RT-PCR. T cells were either either rested, treated with anti-CD3 to stimulate the T cell receptor pathway, or treated with anti-CD3 plus anti-CD28 to activate the co-stimulation pathway as well. As shown in Fig. 1A, a trace amount of PPARγ mRNA was detected in resting T lymphocytes. The expression was up-regulated in T cells stimulated with anti-CD3 antibody and even more so in T cells optimally stimulated with both anti-CD3 and anti-CD28 antibodies. As a loading control, β-actin expression levels were similar in all of these specimens.

To further verify that PPARγ is up-regulated in activated T lymphocytes with a different system, Western blot analysis was performed on murine primary T cells. T cells were either unstimulated or stimulated in vitro with antigen-presenting cells for 1 day or 2 days. Consistent with the RT-PCR results on human cells, the Western blot analysis showed that PPARγ protein expression was increased in activated T cells after 2 days of stimulation (Fig. 1B). Taken together, these data demonstrate that PPARγ is up-regulated following T cell activation.

At High Concentrations, 15d-PGJ2 and Ciglitazone Inhibit T Cell Proliferation and Induce Cell Death—Previously, it was...
The effective concentrations for the inhibition were zone inhibited T cell proliferation in a dose-dependent fashion. (20). To confirm these findings, we treated activated T cells with T cell proliferative responses (14, 16) and induces apoptosis in ways (24). We also examined the effects of PPARγ on the survival of FL5.12 cells, an IL-3-dependent lymphocyte line that expresses human PPARγ. FL5.12 cells, an IL-3-dependent lymphocyte line that expresses human PPARγ, are near maximal level following IL-3 deprivation, providing an antigen-presenting cell to the death-inducing effects of 15d-PGJ2 and ciglitazone. Unexpectedly, PPARγ lines died at the same rate and over the same dose range as vector control lines when treated with the drugs (data not shown), indicating that overexpression of PPARγ did not further sensitize cells to the death-inducing effects of 15d-PGJ2 and ciglitazone.

PPARγ would restore the sensitivity of FL5.12 cells to death induced by these drugs. To test this possibility, FL5.12 cell lines that express human PPARγ1 were established. Western blot analysis confirmed that PPARγ protein was overexpressed in the stably transfected cell lines (Fig. 3A). We compared cell survival of the vector control and PPARγ lines treated with or without 15d-PGJ2 and ciglitazone. PPARγ lines died at the same rate and over the same dose range as vector control lines when treated with the drugs (data not shown), indicating that overexpression of PPARγ did not further sensitize cells to the death-inducing effects of 15d-PGJ2 and ciglitazone.

To test whether TZD-activation of PPARγ might alter the sensitivity of cells to a different death stimulus, we subjected the cells to IL-3 withdrawal. FL5.12 cells undergo rapid apoptotic death in 18–48 h upon IL-3 withdrawal (22). Under this condition, we compared survival of a PPARγ and a vector control cell line treated with or without PPARγ ligands. Surprisingly, the PPARγ line, treated with low concentrations of ciglitazone (10 μM) or rosiglitazone (0.5 μM), survived >2 or 3 times longer than Me2SO-treated cells, respectively, at 24 h after IL-3 withdrawal. The survival benefits depended both on the presence of PPARγ (Fig. 3B, compare Me2SO-treated PPARγ cells with Me2SO-treated control cells) and the activation of PPARγ with agonists (compare ciglitazone- or rosiglitazone-treated PPARγ cells with Me2SO-treated cells). The presence of unidentified physiological activators of PPARγ in the medium or cells may account for the better survival of the PPARγ line with Me2SO treatment than the control cell line with the same treatment. With PPARγ lines of different expression levels (Fig. 3A, g2 versus g14), there was a close correlation between the dosage of PPARγ and degree of cell survival-enhancing effects; e.g., the g14 line (Fig. 3A) exhibited reproducible protection but less protection than the g2 line (data not shown). The dosage effects were also observed in subsequent mitochondrial membrane potential assays and glucose limitation assays (see below).

Using a luciferase reporter construct carrying PPAR response elements, we confirmed that the PPARγ introduced into the cells was functionally active (Fig. 3C). It was activated to a near maximal level by 0.5 μM rosiglitazone, the same concentration at which we observed a survival phenotype. There was a good quantitative correlation between the fold level of transcriptional activation (~3-fold) and the degree of the cell survival promoted by 0.5 μM rosiglitazone (~3-fold; Fig. 3, compare B with C), suggesting that enhanced viability by rosiglitazone is mediated through the transcriptional activity of PPARγ.

We next determined the time course of the survival-promoting effect of PPARγ (Fig. 3D). Although activation of PPARγ protected cells from death up to 24 h after IL-3 withdrawal, almost all cells died by 48 h after IL-3 removal irrespective of the presence or activation status of PPARγ. This cannot be attributed to loss of drug activity, since the addition of fresh PPARγ agonists at 24 h did not prolong survival beyond 48 h (data not shown). Thus, the cell survival-promoting effect of PPARγ is short-lived compared with that of other antiapoptotic genes, such as bcl-xl, suggesting that PPARγ promotes cell survival by a different mechanism.

The cell survival-promoting effects depended on the dosage of rosiglitazone (Fig. 3E). Importantly, at a concentration of 50 nM, which is comparable with its dissociation constant (Kd) for PPARγ (48–100 nM), rosiglitazone enhanced cell survival to a near maximal level following IL-3 deprivation, providing another piece of evidence that the effects of rosiglitazone are mediated through binding to PPARγ.

PPARγ Maintains Mitochondrial Potential during Cell Death—Growth factor withdrawal results in a rapid decline in
glycolysis and mitochondrial potential (reviewed in Ref. 28). Therefore, we investigated the effects of PPARγ activation on the mitochondrial membrane potential. The membrane potential was measured by mitochondrial incorporation of tetramethylrhodamine ethyl ester, a potentiometric dye, and analyzed by flow cytometry. In a vector control cell line, mitochondrial membrane potential was significantly reduced, even in the presence of rosiglitazone, at 12 h after IL-3 withdrawal. In contrast, the reduction was largely prevented by the activation of PPARγ in a PPARγ-transfected cell line (Fig. 4A). Maintenance of mitochondrial membrane potential depended on the activation of PPARγ, since the maintenance was observed with rosiglitazone treatment but not with Me2SO treatment in the PPARγ line (Fig. 4B). From these experiments, we conclude that PPARγ activation results in maintenance of the mitochondrial membrane potential following IL-3 deprivation.

PPARγ Promotes Cell Survival and Maintains Mitochondrial Potential When Glucose Is Limited—Previous studies have shown that a primary role of IL-3 in cell survival is to permit cells to take up and utilize glucose, and glucose limitation has adverse effects on cell survival similar to IL-3 withdrawal (27). Based on the role of PPARγ to activate genes involved in lipid metabolism, we hypothesized that PPARγ might promote the maintenance of mitochondrial potential by making alternative substrates available to mitochondria. To investigate whether PPARγ could protect cell mitochondria from a decline in glycolysis, we examined cell death caused by a decline in glycolysis, we examined cell survival under the condition of glucose restriction. As shown in Fig. 5A, when cells were cultured in media containing 50 μM glucose, which is one-two hundredth of the concentration in standard RPMI, the PPARγ-transfected cell line demonstrated better survival in both a PPARγ-dependent (Fig. 5A, compare Me2SO-treated PPARγ cell line with Me2SO-treated vector control line) and activation-dependent manner (Fig. 5A, compare the rosiglitazone-treated with the Me2SO-treated PPARγ cell line). Furthermore, activation of PPARγ prevented the decline of mitochondrial potential in cells cultured in low glucose (Fig. 5B). Taken together, these data suggest that PPARγ promotes cell survival by allowing cells to maintain a mitochondrial potential when substrates are limited by either IL-3 removal or glucose restriction.

Increased Cell Survival Is a Specific Response to PPARγ Activation—To test whether the effects on cell survival were specific for PPARγ, we generated a FL5.12 cell line stably transfected with PPARγ. Recently, it was shown that the α isoform is present in resting murine lymphocytes (29). In contrast to the PPARγ-transfected lines, the PPARα-transfected cells, when activated by an α-ligand, Wy 14643, did not show enhanced cell survival (Fig. 6).
FIG. 3. PPARγ ligands promote cell survival in a PPARγ dependent fashion. A, FL5.12 cell line was stably transfected with a PPARγ expression vector, pcDNA3.1-hPPARγ1. Western blots show levels of the protein in PPARγ (g2 and g14) and empty vector control (v6 and v7) cell lines. 3T3 L1, a murine preadipocytic cell line used as a positive control for PPARγ expression. 20 μg of total protein was loaded in each lane. B, PPARγ promotes cell survival. Cell survival was determined at 24 h after IL-3 deprivation. Me 2SO (DMSO), 10 μM ciglitazone (Cig), or 0.5 μM rosiglitazone (Rosi) was added to a PPARγ or an empty vector control line at the time of IL-3 withdrawal. Cell survival was determined by propidium iodide exclusion followed by flow cytometric analysis. The experiments were repeated more than three times, and results shown represent a typical experiment. C, transfected PPARγ is transcriptionally active. Luciferase reporter assay of the PPARγ and the control lines. Cells were treated with different doses of rosiglitazone or Me2SO as indicated. D, time course of survival of cells deprived of IL-3. Cig, 10 μM ciglitazone. Rosi, 0.5 μM rosiglitazone. E, dosage effects of rosiglitazone on cell survival. Cell survival was determined at 24 h after IL-3 withdrawal.
DISCUSSION

Although it was discovered several years ago that PPARγ is expressed in hematopoietic cells (13), the role PPARγ plays in these cells remains largely unknown. Recent studies have shown that PPARγ is expressed in lymphocytes and that activation of PPARγ by its ligands inhibits T cell proliferation and IL-2 production (14, 16). It has also been reported that PPARγ induces apoptosis in both B and T lymphocytes (15, 20). In the present study, we confirmed that PPARγ is expressed in both human and murine peripheral T cells. Moreover, we have dem-
onstrated for the first time that PPARγ is up-regulated at both the messenger RNA and protein levels following T cell activation. It is possible that this up-regulation reflects PPARγ involvement in allowing the cell to meet the increased metabolic demand and/or synthetic events that occur as a result of T cell activation. An effective immune response requires both the proliferative expansion of reactive T cells and the stimulation of the cells' ability to perform specific immune functions, such as cytokine production (30). Both proliferation and cytokine production place significant bioenergetic demands on the cell and are likely to affect glucose utilization, mitochondrial function, and lipid biosynthesis.

Using high concentrations of 15d-PGJ2 and ciglitazone, we reproduced the cell death-inducing effects observed by others. Surprisingly, under conditions of growth factor withdrawal or glucose limitation, PPARγ-overexpressing cells survive better when treated with either ciglitazone or rosiglitazone at concentrations that are comparable with their Kd values for PPARγ. The survival benefit depends on both the presence and the potential through the uncoupling proteins.

PPARγ promotes cell survival by a different mechanism from antiapoptotic genes in the Bcl-2 family. Although PPARγ delays cell death, cells die eventually by 48 h after IL-3 withdrawal. In contrast, FL5.12 cells transfected with Bcl-xL live up to several days under the same conditions (24). Moreover, under IL-3 withdrawal condition, the mitochondrial potential in Bcl-xL-overexpressing cells declines. Bcl-xL prolongs cell life by keeping the voltage-dependent anion channel on the outer membrane of mitochondrial open, sustaining ADP/ATP exchange and so maintaining mitochondrial homeostasis (31). In contrast, activated PPARγ maintains the mitochondrial membrane potential.

Opposite to the cell survival effects observed in this study, it has been reported that activation of PPARγ with its ligands induces apoptosis in several types of normal or tumor tissues and cell lines. These include endothelial cells (32), vascular smooth muscle cells (33), B lymphocytes and B lymphoma cell lines (15), T lymphocytes (20), breast carcinoma (34), lung carcinoma (35), gastric carcinoma (36), pancreatic carcinoma (37), choriocarcinoma (38), and hepatoma cell lines (39). Based on these studies, it has been suggested that TZDs may represent a new class of drugs for the treatment of lymphomas and carcinomas.

Several factors may account for the discrepant observations between the present study and previous reports. In this study, whereas TZDs do induce a dose-dependent induction of apoptosis, these effects occur at much higher concentrations than those required for activation of transcriptional activity of PPARγ. Furthermore, the levels of PPARγ neither correlate with the ability of TZDs to induce death nor affect the dose range in which cell death is observed. PPARγ agonists, such as 15d-PGJ2, ciglitazone, and troglitazone, have been shown to possess effects independent of activation of PPARγ (10, 11, 40–43). In keeping with this possibility, many of the previous studies suggesting that TZDs induce cell death utilize concentrations of TZDs several orders of magnitude higher than their Kd for PPARγ.

We have found that TZDs can promote cell survival at doses that induce optimal PPARγ transcriptional activity. Taken together, our results suggest that type 2 diabetic patients taking these drugs may not be at risk for further impairment of their immune function. The ability of PPARγ to promote cell survival under conditions of growth factor withdrawal might even improve immune cell functions at avascular or necrotic sites such as diabetic ulcers.

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