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Cytokines (interferon-γ and tumor necrosis factor–α)-induced nuclear factor–κB activation and chemokine (C-X-C motif) ligand 10 release in Graves disease and ophthalmopathy are modulated by pioglitazone

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Abstract

Until now, the following are not known: (1) the mechanisms underlying the induction of chemokine (C-X-C motif) ligand 10 (CXCL10) secretion by cytokines in thyrocytes; (2) if pioglitazone is able, like rosiglitazone, to inhibit the interferon (IFN)-γ–induced chemokine expression in Graves disease (GD) or ophthalmopathy (GO); and (3) the mechanisms underlying the inhibition by thiazolidinediones of the cytokines-induced CXCL10 release in thyrocytes. The aims of this study were (1) to study the mechanisms underlying the induction of CXCL10 secretion by cytokines in GD thyrocytes; (2) to test the effect of pioglitazone on IFNγ-inducible CXCL10 secretion in primary thyrocytes, orbital fibroblasts, and preadipocytes from GD and GO patients; and (3) to evaluate the mechanism of action of thiazolidinediones on nuclear factor (NF)–κB activation. The results of the study (1) demonstrate that IFNγ + TNFα enhanced the DNA binding activity of NF-κB in GD thyrocytes, in association with the release of CXCL10; (2) show that pioglitazone exerts a dose-dependent inhibition on IFNγ + TNFα–induced CXCL10 secretion in thyrocytes, orbital fibroblasts, and preadipocytes, similar to the effect observed with rosiglitazone; and (3) demonstrate that thiazolidinediones (pioglitazone and rosiglitazone) act by reducing the IFNγ + TNFα activation of NF-κB in Graves thyrocytes. To the best of our knowledge, this is the first study showing that cytokines are able to activate NF-κB in Graves thyrocytes and a parallel inhibitory effect of pioglitazone both on CXCL10 chemokine secretion and NF-κB activation. Future studies will be needed to verify if new targeted peroxisome proliferator-activated receptor–γ activators may be able to exert the anti-inflammatory effects without the risk of expanding retrobulbar fat mass.

1. Introduction

We have recently shown [1] that, in primary cultures of thyrocytes, retrobulbar fibroblasts, and retrobulbar preadipocytes from Graves ophthalmopathy (GO) patients (p), the stimulation with interferon (IFN)-γ or tumor necrosis factor (TNF)–α + IFNγ induced chemokine (C-X-C motif) ligand 10 (CXCL10) release. However, until now, the mechanisms underlying the induction of CXCL10 secretion by cytokines in thyrocytes are still unknown.

Treatment of thyroid follicular cells, orbital fibroblasts, or preadipocytes with a pure peroxisome proliferator-activated receptor–γ (PPARγ) activator, rosiglitazone, at near-therapeutic doses significantly inhibited IFNγ-stimulated CXCL10 secretion, strongly suggesting that PPARγ might be involved in the regulation of IFNγ-induced chemokine expression in human thyroid autoimmunity and GO [1]. Altogether, these evidences suggest that PPARγ activators might attenuate the recruitment of activated T cells at sites of Th1-mediated inflammation.

Moreover, it has been suggested that the increased orbital fat tissue observed in GO may be a consequence of the overexpression of PPARγ caused by the inflammatory process. With regard to this, a recent case report described

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a type 2 diabetes mellitus patient who experienced exacerbation of GO with expansion of the orbital fat during treatment with the PPARγ agonist pioglitazone [2]. In cultured retrobulbar preadipocytes, PPARγ agonists caused a 2- to 13-fold increase, whereas a PPARγ antagonist caused a 2-7-fold reduction, in adipogenesis [2]. Other studies [3,4] have confirmed the adipogenetic potential of PPARγ agonists on orbital preadipocytes, suggesting that PPARγ antagonists could provide a novel therapy for GO-p in the active stage of the disease.

However, until now, it is not known if pioglitazone is able, like rosiglitazone, to inhibit the IFNγ-induced chemokine expression in human thyroid autoimmunity and GO; and the mechanisms underlying the inhibition by thiazolidinediones (TZDs) of the cytokines-induced CXCL10 release in thyrocytes have not been identified.

The aims of this study were (1) to study the mechanisms underlying the induction of CXCL10 secretion by cytokines in Graves thyrocytes, (2) to test the effect of PPARγ activation by pioglitazone on IFNγ-inducible CXCL10 secretion in primary cultures of cells obtained from the main tissues involved in the pathogenesis of GD and GO (thyrocytes, orbital fibroblasts, and preadipocytes), and (3) to evaluate the mechanism of action of pioglitazone on nuclear factor (NF)–κB activation.

2. Materials and methods

The effects of IFNγ, TNFα, and PPARγ agonists (pioglitazone and rosiglitazone) on the release of CXCL10 were investigated in primary cultures of human thyroid follicular cells, fibroblasts, and preadipocytes. Furthermore, the effect of pioglitazone on NF-κB activation in the thyroid cells was evaluated by electrophoretic mobility shift assay (EMSA).

2.1. Thyroid follicular cells

Surgical thyroid tissue was obtained from 5 GO-p, euthyroid at the time of surgery. Thyroidectomy was advised to GO-p (4 women, 1 man; age, 34, 42, 45, 51, and 56 years; antithyroid peroxidase antibody (AbTPO) [normal values [vn] <40 U/L], 12, 34, 43, 58, and 107; thyrotropin receptor antibody (TRAb) [vn <1 U/L], 3, 11, 21, 22, and 39) mainly because of relapse of hyperthyroidism after a previous methimazole course in the presence of a large goiter and/or thyroid nodules. In addition, normal thyroid tissue was obtained from 5 patients (3 undergoing parathyroidectomy; 2, laryngeal intervention). All study subjects gave their informed consent to the study, which was approved by the local ethical committee. Thyrocytes were prepared as reported previously [5,6]. The specimens were minced with scissors and digested with collagenase (1 mg/mL; Roche, Mannheim, Germany) in RPMI 1640 (Whittaker Bioproducts, Walkersville, MD) for 1 hour at 37°C. Semidigested follicles were removed, sedimented for 2 minutes, washed, and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) (Seromed Biochrom, Berlin, Germany), 2 mmol/L glutamine, and 50 μg/mL penicillin/streptomycin at 37°C and 5% CO_2 in plastic 75-cm^2 flasks (Sarstedt, Verona, Italy).

2.2. Fibroblast and preadipocyte cell cultures

Orbital adipose/connective tissue explants were obtained from 5 patients (5 women; age, 39, 46, 51, 55, and 56 years; AbTPO [vn <40 U/L], 31, 41, 48, 71, and 214; TRAb [vn <1 U/L], 2, 22, 27, 37, and 48) undergoing orbital decompression for severe GO during the inactive phase of the disease (all previously treated with antithyroid medication and systemic corticosteroids [7,8], euthyroid at the time of surgery [3 on levothyroxine after thyroidectomy]; none treated with orbital radiotherapy). All study subjects gave their informed consent to the study, which was approved by the local ethical committee. The GO tissue samples were minced and placed directly in plastic culture dishes, allowing preadipocyte fibroblasts to proliferate as described previously [3,9]. Fibroblasts were propagated in medium 199 containing 20% FBS (Gibco, Invitrogen, Paisley, United Kingdom), penicillin (100 U/mL), and gentamycin (20 μg/mL) in a humidified 5% CO_2 incubator at 37°C and maintained in 75-cm^2 flasks with medium 199 containing 10% FBS and antibiotics. To initiate adipocyte differentiation, orbital fibroblasts were grown to confluence in 6-well plates in medium 199 with 10% FBS. Differentiation was carried out as reported previously [3]; cultures were changed to serum-free Dulbecco modified Eagle medium/Ham F-12 (1:1; Sigma, St Louis, MO) supplemented with biotin (33 μmol/L), pantothenic acid (17 μmol/L), transferrin (10 μg/mL), triiodothyronine (0.2 nmol/L), insulin (1 μmol/L), carbaprostacyclin (0.2 μmol/L; Calbiochem, La Jolla, CA), and—for the first 4 days only—dexamethasone (1 μmol/L) and isobutylmethylxanthine (0.1 mmol/L). The differentiation protocol was continued for 10 days, during which time the medium was replaced every 3 to 4 days. In separate experiments, fibroblasts derived from the same patients' orbital tissues were maintained for the same period in medium lacking several of the components necessary for complete adipocyte differentiation (ie, carbaprostacyclin, dexamethasone, and isobutylmethylxanthine).

Control fibroblasts and preadipocytes were obtained from unaffected dermal tissues of the same patients. Orbital preadipocyte fibroblast cultures [3,9] were plated in 1-well culture chamber slides (Nalge Nunc International, Rochester, NY) in medium 199 containing 10% FBS, grown to confluence, and subjected to either the differentiation protocol or nondifferentiation conditions. Cells were washed twice with 1× phosphate-buffered saline, fixed in 10% formalin overnight at room temperature, and rinsed in 60% isopropanol before staining with filtered 0.21% oil red O in isopropanol/water for 1 hour. Washed cells were stained with Mayer hematoxylin solution (Sigma, MHS-32)

for 5 minutes and rinsed with tap water before being visualized using an Olympus IX50 light microscope (Olympus Corp, Tokyo, Japan) and photographed at ×20.

2.3. CXCL10 secretion assay

For CXCL10 secretion assays, cells were seeded in 96-well plates at a concentration of 30 000 cells per milliliter in a final volume of 100 μL per well in growth medium. After 24 hours, the growth medium was removed; and cells were accurately washed in phosphate-buffered saline and incubated in phenol red and serum-free medium. Cells were incubated (24 hours) with IFNγ (R and D Systems, Minneapolis, MN; 500, 1000, 5000, and 10 000 U/mL) and 10 ng/mL TNFα (R and D Systems), alone or in combination [10]. The concentration of TNFα was selected in preliminary experiments to yield the highest responses. After 24 hours, the supernatant was removed and kept frozen at −20°C until CXCL10 assay.

To investigate the effect of PPARγ activators on IFNγ-induced chemokine secretion, cells were stimulated (24 hours) with IFNγ (1000 U/mL) and TNFα (10 ng/mL) in the absence or presence of increasing concentrations (0, 0.1, 1, 5, 10, and 20 μmol/L) of the PPARγ agonists rosiglitazone (Glaxo, Welwyn, United Kingdom) or pioglitazone; and conditioned media were assayed by enzyme-linked immunosorbent assay for CXCL10 concentrations. All experiments were repeated 3 times with the 3 different cell preparations.

2.4. Enzyme-linked immunosorbent assay for CXCL10

The CXCL10 levels were measured in culture supernatants and using commercially available kits (R and D Systems). The mean minimum detectable dose was 1.67 pg/mL for CXCL10; the intra-and interassay coefficients of variation were 3.0% and 6.9% for CXCL10. Samples were assayed in duplicate. Quality control pools of low, normal, or high concentration for all parameters were included in each assay.

2.5. Nuclear extracts preparation and EMSA

Thyroid cells were seeded in cell culture dishes at a concentration of 200 000 cells per milliliter in a final volume of 10 mL. Afterward, cells were treated (1 hour) with IFNγ (1000 U/mL) and TNFα (10 ng/mL) in the absence or presence of 10 or 20 μmol/L rosiglitazone or pioglitazone.

Nuclear extracts were prepared and processed for EMSA as previously described [11].

2.6. Data analysis

Values are given as mean ± SD for normally distributed variables; otherwise, as median and interquartile range. Mean group values were compared by using 1-way analysis of variance (ANOVA) for normally distributed variables; otherwise, by the Mann-Whitney *U* or Kruskal-Wallis test. Proportions were compared by the χ^2 test. Post hoc comparisons on normally distributed variables were carried out using the Bonferroni-Dunn test.

3. Results

In primary thyrocyte cultures, CXCL10 was undetectable in the supernatant. Interferon-γ dose-dependently induced CXCL10 release (CXCL10: 0, 77 ± 11, 211 ± 13, 284 ± 27, and 391 ± 43 pg/mL, respectively, with IFNγ 0, 500, 1000, 5000, and 10 000 IU/mL; ANOVA, $P < .001$), whereas TNFα alone had no effect. However, the combination of TNFα and IFNγ had a significant synergistic effect on CXCL10 secretion (1389 ± 125 vs 224 ± 51 pg/mL with IFNγ alone, $P < .0001$). Treatment of thyrocytes with pioglitazone (Fig. 1A) or rosiglitazone (Fig. 1B), added at the time of IFNγ and TNFα stimulation, dose-dependently inhibited CXCL10 release. Pioglitazone or rosiglitazone alone had no effect and did not affect cell viability or total protein content (data not shown). The data obtained with thyrocytes from normal thyroid tissue were not statistically different from those obtained from GO-p (data not shown).

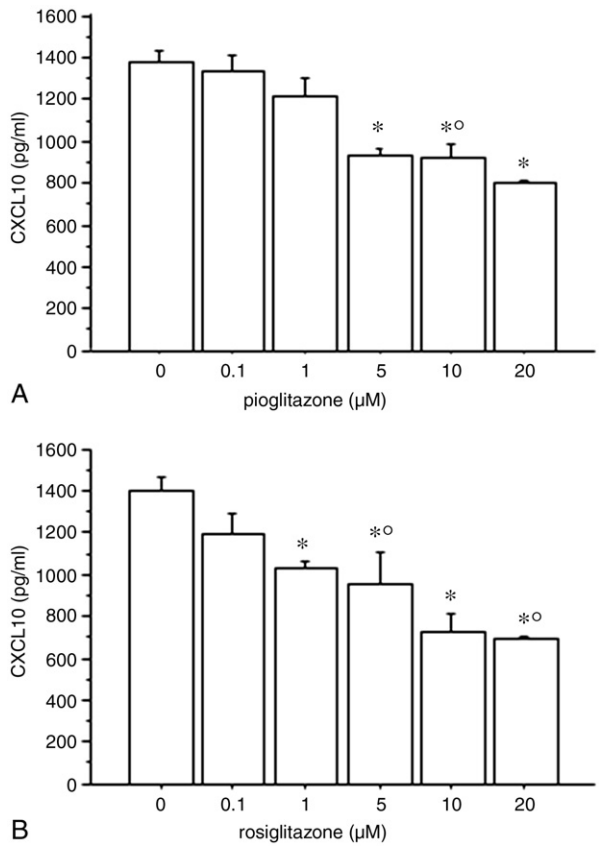

Fig. 1. Increasing doses of pioglitazone (A) or rosiglitazone (B) (0.1, 1, 5, 10, and 20μmol/L) inhibit CXCL10 release from thyrocytes stimulated with IFNγ (1000 U/mL) and TNFα (10 ng/mL) ($P < .0001$ by ANOVA). Bars are mean ± SEM. *$P < .05$ or less vs 0. °Not significantly different from the preceding dose by Bonferroni-Dunn test.

The pattern of results seen in cultured thyrocytes was essentially repeated in retrobulbar fibroblasts and preadipocytes. Thus, in both fibroblasts (CXCL10: 0, 143 ± 16, 248 ± 25, 329 ± 28, and 481 ± 49 pg/mL, respectively, with IFNγ: 0, 500, 1000, 5000, and 10 000 IU/mL; ANOVA, $P < .001$) and preadipocytes (CXCL10: 0, 127 ± 17, 378 ± 25, 405 ± 45, and 527 ± 56 pg/mL, respectively, with IFNγ: 0, 500, 1000, 5000, and 10 000 IU/mL; ANOVA, $P < .001$), IFNγ dose-dependently induced CXCL10 release. The combination of TNFα and IFNγ had a significant synergistic effect on CXCL10 secretion (in fibroblasts: 1316 ± 218 vs 259 ± 119 pg/mL with IFNγ alone, $P < .0001$; in preadipocytes: 1547 ± 158 vs 366 ± 34 pg/mL, $P < .0001$). Treatment of retrobulbar fibroblasts (Fig. 2A, B) and preadipocytes (Fig. 3A, B) with pioglitazone or rosiglitazone, added at the time of IFNγ and TNFα stimulation, dose-dependently inhibited CXCL10 release. The data obtained with fibroblasts or preadipocytes from unaffected dermal tissues of the same patients were not statistically different from those obtained with their retrobulbar fibroblasts and preadipocytes (data not shown).

The effect of TZDs on NF-κB activation in the thyroid cells has been evaluated by EMSA (Fig. 4) to explain the inhibition exerted by PPARγ agonists on CXCL10 secretion. Thyroid cells did not show a constitutive activation of NF-κB (lanes 1-2), and the treatment with IFNγ and TNFα ("IFNg + TNFa") enhanced the DNA binding activity of NF-κB (lanes 3-4). The treatments with pioglitazone (20 μmol/L) significantly reduced the IFNγ + TNFα activation of NF-κB (lanes 5-6). Rosiglitazone had a similar effect (data not shown).

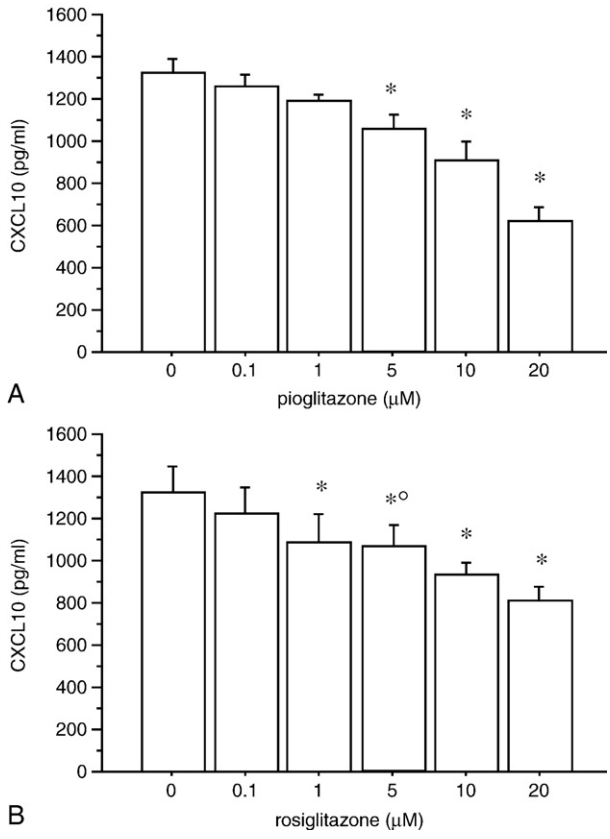

Fig. 2. Increasing doses of pioglitazone (A) or rosiglitazone (B) (0.1, 1, 5, 10, and 20.μmol/L) inhibit CXCL10 release from fibroblasts stimulated with IFNγ (1000 U/mL) and TNFα (10 ng/mL) ($P < .0001$ by ANOVA). Bars are mean ± SEM. *$P < .05$ or less vs 0. °Not significantly different from the preceding dose by Bonferroni-Dunn test.

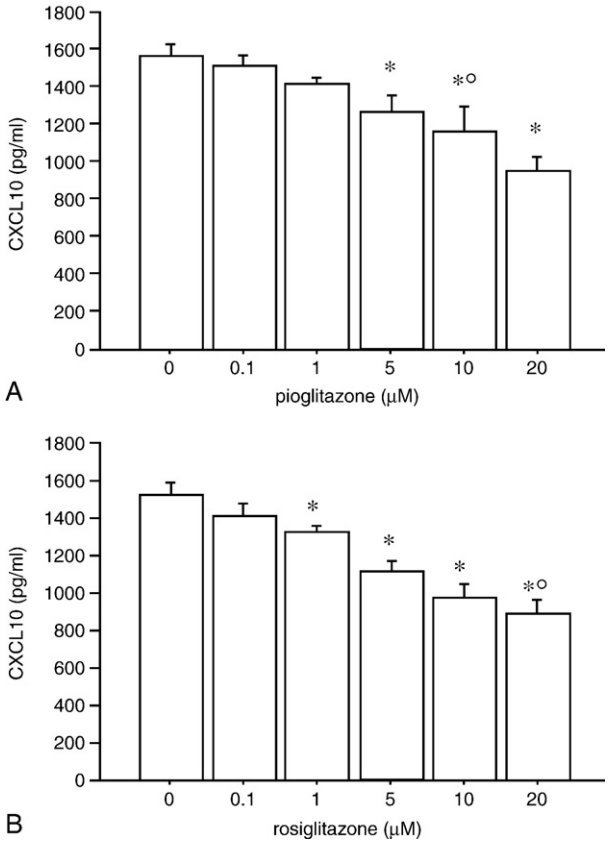

Fig. 3. Increasing doses of pioglitazone (A) or rosiglitazone (B) (0.1, 1, 5, 10, and 20μmol/L) inhibit CXCL10 release from preadipocytes stimulated with IFNγ (1000 U/mL) and TNFα (10 ng/mL) ($P < .0001$ by ANOVA). Bars are mean ± SEM. *$P < .05$ or less vs 0. °Not significantly different from the preceding dose by Bonferroni-Dunn test.

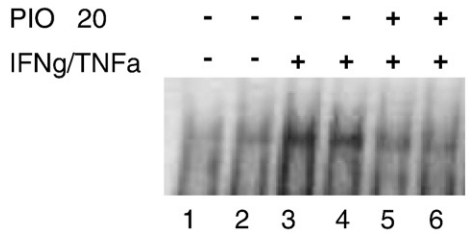

Fig. 4. Electrophoretic mobility shift assay of thyroid cells nuclear extracts. Thyroid cells did not show a constitutive activation of NF-κB (lanes 1-2), and the treatment with IFNγ and TNFα ("IFNg + TNFa") enhanced the DNA binding activity of NF-κB (lanes 3-4). The treatments with pioglitazone (20 μmol/L) significantly reduced the IFNg + TNFa activation of NF-κB (lanes 5-6).

4. Discussion

The results of the present study (1) demonstrate that IFNγ and TNFα enhanced the DNA binding activity of NF-κB in Graves thyrocytes, in association with the release of CXCL10 by the same cells; (2) confirm that IFNγ and TNFα are able to induce the CXCL10 release by GO orbital fibroblasts and preadipocytes; (3) show that the PPARγ agonist pioglitazone exerts a dose-dependent inhibition on IFNγ + TNFα–induced CXCL10 secretion in thyrocytes, orbital fibroblasts, and preadipocytes, similar to the effect observed with rosiglitazone; and (4) demonstrate that TZDs (pioglitazone and rosiglitazone) act by reducing the IFNγ + TNFα activation of NF-κB in Graves thyrocytes. To the best of our knowledge, this is the first study showing that cytokines are able to activate NF-κB in Graves thyrocytes and a parallel inhibitory effect of pioglitazone both on CXCL10 chemokine secretion and NF-κB activation.

In our study, IFNγ and TNFα enhanced the DNA binding activity of NF-κB in Graves thyrocytes, in association with the release of CXCL10 by the same cells. These findings are in agreement with the results observed by Marx et al 2000 [10] that showed that IFNγ and TNFα induced NF-κB in endothelial cells, suggesting that NF-κB activation has a pivotal role in the induction of CXCL10 release by target cells.

The finding of increased levels of CXCL10 in active GO is in agreement with previous studies showing an involvement mainly of Th1 cytokines in GD and GO [12–16]. In particular, it has been shown that, in GO, the active phase is characterized by the presence of proinflammatory and Th1-derived cytokines, whereas other cytokines, among them Th2-derived cytokines, do not seem to be associated with a specific stage of GO [17]. Furthermore, the Th1 involvement in GO has been recently confirmed by the finding that thyrocytes and retrobulbar cell types from patients with GO participate in the self-perpetuation of inflammation, releasing CXCL9 and CXCL11 chemokines when stimulated with IFNγ and TNFα [18].

This is in agreement with our previous study [1] that suggests that CXCL10 is transiently involved in the active phase of GO, when the inflammatory process is sustained by Th1-mediated immune responses. A switch from a Th1 to Th2 phenotype appears to occur in long-standing GO, in line with a previous report showing that lymphocytes obtained from orbital tissue of GO-p had a prevalent Th1 profile, whereas patients with remote-onset hyperthyroidism had a large majority of Th2 lymphocytes [14]. This phenomenon has been reported in other long-standing autoimmune diseases, for example, in systemic sclerosis [19]. The prognostic value of increased, or rising, CXCL10 levels in patients with GO remains to be established.

The difference between active and inactive GO is the presence of a lymphocytic infiltrate [20,21]; therefore, the increased production of CXCL10 might be sustained by orbital lymphocytes. However, our in vitro studies demonstrate that CXCL10 can be produced by nonlymphoid cells in the orbit. In fact, both fibroblasts and preadipocytes from GO-p were induced to secrete CXCL10 by stimulation with increasing doses of IFNγ. Stimulation of fibroblasts or preadipocytes with TNFα alone was not able to induce chemokine secretion; but combinations of IFNγ and TNFα synergistically increased CXCL10 secretion, similarly to what was observed in human thyrocytes [5] and endothelial cells [10]. Interferon-γ–inducible CXC chemokines can be produced by several types of normal mammalian cells, such as colon epithelial cells [22], islet cells [23], dendritic cells [24], and others. However, these cells are not able to produce the CXC chemokines in basal condition, but only after the stimulation by cytokines, such as IFNγ and TNFα, which are secreted in a Th1-type inflammatory site, such as the orbit at the beginning of GO, by Th1-activated lymphocytes.

Altogether, these results are compatible with the view that production of IFNγ and TNFα by Th1-activated lymphocytes at the orbital level induces CXCL10 secretion by orbital fibroblasts and preadipocytes; in turn, the chemokine induces migration of Th1 lymphocytes into the orbit, thereby perpetuating the autoimmune cascade. This process has been suggested to be involved in the initiation and the perpetuation of the inflammation in several autoimmune diseases [1,5,10,22–24] and, on the basis of our results, can be applied to the orbit, too, in GO.

Peroxisome proliferator-activated receptor–γ ligands attenuate activity of inflammatory bowel disease in animal models [22,25], and clinical trials have shown that they ameliorate inflammation in patients with ulcerative colitis [26]. Peroxisome proliferator-activated receptor–γ anti-inflammatory effect has been shown also in experimental autoimmune encephalomyelitis [27], arthritis [28], and psoriasis [29]. Some of the anti-inflammatory effects of PPARγ ligands result from direct actions on cells of the innate and adaptive immune system [30,31]. In macrophages, they inhibit activation and production of inflammatory cytokines such as TNFα, interleukin (IL)-1b, and IL-6 [32,33]. In dendritic cells, PPARγ agonists downregulate the synthesis of chemokines involved in the recruitment of T lymphocytes [24].

Peroxisome proliferator-activated receptor–γ has recently been shown to modulate inflammatory responses in endothelial cells [22,24].

With regard to the mechanism of these actions, several anti-inflammatory mechanisms of PPARγ have been suggested, including inhibition of NF-κB, AP1, and STAT transcription factors [34,35]. A recent study demonstrated that some of these effects are PPARγ independent [36]. Peroxisome proliferator-activated receptor–γ also regulates inflammation by blocking gene transcription through “transrepression.” A recent report demonstrated a PPARγ ligand–dependent stimulation of PPARγ that leads to its recruitment to repressor complexes in the promoter regions of inflammatory genes regulated by NF-κB, preventing their release and inhibiting proinflammatory

gene expression [37]. Furthermore, PPARγ ligands inhibit T-lymphocyte proliferation and reduce the production of IFNγ, TNFα, and IL-2 [30,38,39].

Moreover, PPARγ activators may act on CXCL10 through different pathways. Rosiglitazone and troglitazone have been shown to be able to decrease CXCL10 promoter activity and inhibit protein binding to the 2 NF-κB sites [10]. The results of our study in Graves thyroid cells show that the NF-κB activation by IFNγ + TNFα is inhibited by the PPARγ agonist pioglitazone according to the inhibitory effect observed by Marx et al [10] with other PPARγ agonists in endothelial cells.

However, pioglitazone may act on CXCL10 through different pathways, for example, reducing CXCL10 protein levels in a dose-dependent manner at concentrations (nanomoles per liter) that did not affect messenger RNA levels or NF-κB activation [22].

The anti-inflammatory potential of PPARγ may be relevant to GO. In fact, the expression of the PPARγ gene is higher in orbital adipose/connective tissue from patients in the active stage of GO than in tissues from controls or inactive GO-p [40]; and its activity may be involved in the regulation of IFNγ-induced chemokine expression.

Peroxisome proliferator-activated receptor–γ activators might attenuate the recruitment of activated T cells at sites of Th1-mediated inflammation. Our data fully support this hypothesis. Treatment of thyroid follicular cells, orbital fibroblasts, or preadipocytes with PPARγ activators, such as pioglitazone or rosiglitazone, at near-therapeutic doses significantly inhibited IFNγ-stimulated CXCL10 secretion, strongly suggesting that PPARγ might be involved in the regulation of IFNγ-induced chemokine expression in human thyroid autoimmunity and GO.

Thus, the increased orbital fat tissue observed in GO may be a consequence of the overexpression of PPARγ caused by the inflammatory process.

Adipogenesis has been suggested to be a mechanism for stanching chronic inflammation [41]. Orbital adipocytes express immunoreactive and functional thyrotropin receptor; and a positive correlation between thyrotropin receptor, PPARγ, and other adipocytic differentiation markers has been observed in tissues from GO-p [4]. Although adipogenesis serves to abate inflammation, the associated increase in orbital tissue mass is undesirable; and despite anti-inflammatory actions of PPARγ, its proadipogenic functions in the orbit might worsen the disease, contra-indicating the use of agents activating this pathway in GO [2]. Several case reports have described development of exophthalmos in patients receiving TZDs treatment of type 2 diabetes mellitus [2,41,42]. In particular, a patient with stable and inactive GO experienced aggravated disease with orbital fat expansion after pioglitazone therapy [1,2,41].

In cultured retrobulbar preadipocytes, PPARγ agonists caused a 2- to 13-fold increase, whereas a PPARγ antagonist caused a 2-7-fold reduction, in adipogenesis [2]. Other studies [3,4] have confirmed the adipogenetic potential of PPARγ agonists on orbital preadipocytes, suggesting that PPARγ antagonists could provide a novel therapy for GO-p in the active stage of the disease.

Peroxisome proliferator-activated receptor–γ modulators with selective activities would be required if PPARγ activation is to be targeted for the therapy for GO. Identification of "selective PPARγ modulators," or SPPARgMs, has been sought as a better therapy for type 2 diabetes mellitus [43,44]. In this context, designing partial PPARγ agonists that display insulin-sensitizing activity but lack adipogenic properties might be attractive [44].

For example, metaglidasen has been shown in vitro to act as a partial PPARγ agonist/antagonist [45]; compared with rosiglitazone, metaglidasen is less adipogenic in primary human adipocytes and in mouse 3T3-L1 adipocytes. In rodent models of insulin resistance, both metaglidasen and another SPPARgM, PAT5A, increased insulin sensitivity similarly to rosiglitazone, with only weak adipogenic potential [43–45]. The development of SPPARgMs to target insulin probably will permit to target the anti-inflammatory properties of PPARγ in the future [43].

In conclusion, we have shown that IFNγ and TNFα activate NF-κB in Graves thyrocytes, in association with the release of CXCL10. Furthermore, pioglitazone, a PPARγ agonist that has been implicated in the exacerbation of GO, is able to suppress the release of chemokines, induced by IFNγ, from thyrocytes and retrobulbar cell types in GO and acts by reducing the IFNγ + TNFα activation of NF-κB in thyrocytes. Further experimental in vitro and animal studies will be needed to verify if new targeted PPARγ activators may be able to exert the anti-inflammatory effects without the risk of expanding retrobulbar fat mass.

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