Autoimmunity Reviews 18 (2019) 673–678

Contents lists available at ScienceDirect

Autoimmunity Reviews

journal homepage: www.elsevier.com/locate/autrev

Differential modulation of CXCL8 versus CXCL10, by cytokines, PPAR-gamma, or PPAR-alpha agonists, in primary cells from Graves' disease and ophthalmopathy

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ARTICLE INFO

Keywords:
CXCL8
CXCL10
Graves' disease
Graves' ophthalmopathy
Thyrocytes
Fibroblasts

ABSTRACT

Background: Thyrocytes secrete CXC chemokines, particularly (C-X-C motif) ligand (CXCL)8 and CXCL10; its physiopathological significance remains unclear. This study investigates the modulation of the secretion of CXCL8 vs. CXCL10, in human primary cells cultures of thyroid follicular cells (TFC) in Graves' disease (GD), and fibroblasts (OF) or preadipocytes (OP) from Graves' ophthalmopathy (GO).

Methods: Cells were initially incubated with different concentrations of tumor necrosis factor (TNF)α (1, 5, 10 ng/mL). Then, CXCL8 and CXCL10 were measured in the supernatants of TFC, OF or OP cells basally and after 24 h of treatment with interferon (IFN)γ (1000 IU/mL) and/or TNFα (10 ng/mL), in presence/absence of the peroxisome proliferator activated receptor (PPAR)γ agonist pioglitazone (0, 0.1, 1, 5, 10, 20 μM), or the PPARα agonist fenofibrate (5, 10, 50, 100 μM).

Results: CXCL8, not CXCL10, was detected in basal conditions in TFC, OF and OP. CXCL8 secretion increased dose-dependently with increasing concentrations of TNFα. CXCL10 secretion was significantly stimulated by IFNγ ($P < 0.01$) and not by TNFα, whereas CXCL8 was induced by TNFα ($P < 0.01$), and inhibited by IFNγ ($P < 0.01$) in TFC, OF and OP. Combining TNFα and IFNγ, the IFNγ-induced CXCL10 secretion was synergistically increased ($P < 0.01$) while the TNFα-induced CXCL8 secretion ($P < 0.01$) was reversed in all cell types. Pioglitazone had no significant effect on the secretion of CXCL8 stimulated by TNFα, while inhibited CXCL10. Fenofibrate, in presence of IFNγ plus TNFα, dose-dependently inhibited both CXCL10 and CXCL8 release.

Conclusion: We first show that TFC, OF, and OP secrete CXCL8 and CXCL10 differentially, sustained by specific proinflammatory cytokines or their combination. This could reflect a different role of the two chemokines in the course of the disease, as CXCL10 could be associated with the initial phase of the disease when IFNγ is preponderant, while CXCL8 could be associated with a later chronic phase of the disease, when TNFα prevails.

1. Introduction

(C-X-C motif) ligand (CXCL)8/IL-8 is a chemokine produced by macrophages and other cell types such as epithelial cells [1], and endothelial cells [2]. In humans, the IL-8 protein is encoded by the *IL-8* gene [3].

CXCL8 is a member of the CXC chemokine family. The genes encoding this and the other ten members of the CXC chemokine family form a cluster in a region mapped to chromosome 4q [3]. CXCL8 binds to receptors of the surface membrane; the most important are the G protein-coupled serpentine receptors C-X-C motif receptor (CXCR)1 [4], and CXCR2 [4]. Expression and affinity to CXCL8 are different for the two receptors (CXCR1 > CXCR2). Toll-like receptors are the receptors of the innate immune system, and recognize antigen patterns [like lipopolysaccharides (LPS) in gram negative bacteria]. CXCL8 is secreted and is an important mediator of the immune reaction in the innate immune system response.

CXCL8, also called neutrophil chemotactic factor, has two primary

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https://doi.org/10.1016/j.autrev.2019.05.004
Received 17 January 2019; Accepted 26 January 2019
Available online 04 May 2019
1568-9972/

functions. It induces chemotaxis in target cells, mainly neutrophils but also other granulocytes, and leads to their migration toward the site of infection. CXCL8 is also known to be a potent promoter of angiogenesis and it is often associated with inflammation. As an example, it has been cited as a proinflammatory mediator in gingivitis [5] and psoriasis.

Recently, it has been investigated whether the secretion of CXCL8 and CXCL10 chemokines by normal human thyrocytes depends on specific proinflammatory stimuli [6]. CXCL8 but not CXCL10 was detected in basal conditions. The two chemokines showed differences in their response to proinflammatory cytokines. Indeed, a significant CXCL10 release was induced by interferon (IFN)γ and not -tumor necrosis factor (TNF)α, whereas CXCL8 was secreted in response to TNFα being inhibited by IFNγ. The combination of TNFα plus IFNγ synergistically increased the IFNγ-induced CXCL10 secretion, and reversed the TNFα-induced CXCL8 secretion [6]. The role of CXCL8 in Graves' disease (GD) and ophthalmopathy (GO) remains to be investigated.

This study evaluates the modulation, by cytokines, of the secretion of CXCL8, in comparison to CXCL10, in human primary cell cultures of thyroid follicular cells (TFC) in GD, and in fibroblasts (OF) or preadipocytes (OP) from GO.

2. Materials and methods

The effects of IFNγ, TNFα and peroxisome proliferator activated receptor (PPAR)γ or PPARα agonists on the release of CXCL8, in comparison to CXCL10, was evaluated in human primary cell cultures of GD thyrocytes, and GO fibroblasts, and preadipocytes. All subjects participating to the study gave their informed consent; the study was approved by the local ethical committee of the University of Pisa.

2.1. Thyroid follicular cells

Surgical thyroid tissue was obtained from 6 patients with GO, euthyroid at the time of surgery. Thyroidectomy was adviced owing to a relapse of hyperthyroidism in these patients with a large goiter and/or thyroid nodules, after the therapy with methimazole. Normal thyroid tissue was collected from 6 patients (3 undergoing parathyroidectomy, 3 to laryngeal intervention), too.

Thyrocytes were prepared as reported previously [7,8]. Surgical tissues were fragmented and digested at 37 °C for 1 h with collagenase (1 mg/mL; Roche Diagnostics, Almere, The Netherlands) in RPMI 1640 (Gibco, ThermoFisher Scientific, Waltham, MA, USA). The cells were then centrifuged for 2 min and seeded in medium RPMI 1640 with 10% fetal bovine serum (FBS) (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany), 50 μg/mL penicillin/streptomycin, and 2 mM glutamine at 37 °C and 5% CO_2.

2.2. Fibroblasts and preadipocytes

Retrobulbar connective/adipose tissue samples were collected from 6 patients during orbital decompression owing to severe GO, in the inactive phase [subjects had been earlier administered with antithyroid drugs and systemic corticosteroids, and were euthyroid when operated (3 of them were treated with levothyroxine after surgery); orbital radiotherapy had been conducted in none of them]. GO tissue samples were fragmented and put into plastic culture dishes, in order to let preadipocyte fibroblasts to proliferate [7,8].

Cells were spread in medium 199 with 20% FBS, gentamycin (20 μg/mL), and penicillin (100 IU/mL) at 37 °C and 5% CO_2, and maintained in medium 199 in presence of antibiotics and 10% FBS.

The growth of retrobulbar cells was continued till reaching the confluence in medium 199 with 10% FBS in 6-well plates, to induce adipocyte differentiation. Differentiation was conducted as previously shown [9]: cells were grown in serum-free DMEM/Ham's-F-12 (1:1; Sigma-Aldrich) with pantothenic acid (17 μM), carbaprostacyclin (cPGI2; 0.2 μM; Calbiochem, La Jolla CA), biotin (33 μM), insulin (1 μM), transferrin (10 μg/mL), triiodo-thyronine (0.2 nM), and, for the first 4 days only, isobutylmethylxanthine (IBMX; 0.1 mM) and dexamethasone (1 μM). Differentiation went on for 10 days, replacing the medium every 3–4 days. Meanwhile, retrobulbar fibroblasts obtained from the same patients were kept in medium without IBMX, dexamethasone, and cPGI2, required for adipocyte differentiation.

Control cultures were established from normal dermal tissues from the same patients.

Retrobulbar preadipocyte fibroblasts [9,10] were seeded in medium 199 with 10% FBS in 1-well culture chamber slides (Nalge Nunc International, Rochester, NY), and once reached the confluence maintained in differentiation or nondifferentiation protocol. Cells were washed and stained with 0.21% Oil Red O in isopropanol/water, and then with Mayer's hematoxylin solution, as previously reported [8]. Cells were then observed with a light microscope (Olympus IX50) and photographed at x20.

2.3. CXCL8 and CXCL10 secretion assay

For CXCL8 and CXCL10 secretion assays, 3000 cells were seeded in 96-well plates in growth medium. After 24 h, the growth medium was removed, cells were accurately washed in PBS, and incubated in phenol red and serum-free medium.

To evaluate whether TNFα was able to modulate the secretion of CXCL8, cells were incubated (24 h) with different concentrations of TNFα (R&D Systems, Minneapolis, MN, USA; 1, 5, 10 ng/mL).

Cells were then incubated with IFNγ (R&D Systems; 1000 IU/mL) and 10 ng/mL TNFα (R&D Systems), alone or in combination, for 24 h [11]. The concentration of IFNγ was chosen to obtain the highest responses according to previously conducted experiments (7, 8), and the concentration of TNFα was chosen from the previous curve of dose-dependence. Supernatants were collected after 24 h and maintained at −20 °C until chemokine levels evaluation.

To evaluate the effect of PPARγ or PPARα agonists, cells were treated for 24 h with TNFα (10 ng/mL) and IFNγ (1000 IU/mL) in presence/absence of increasing concentrations of the PPARγ agonist, pioglitazone (PGZ, Glaxo, Welwyn, UK; 0, 0.1, 1, 5, 10, 20 μM), or the PPARα agonist fenofibrate (Sigma-Aldrich; 5, 10, 50, 100 μM). Conditioned medium was assayed by ELISA for chemokines concentrations. All experiments were repeated 3 times with the 3 different cell preparations.

2.4. Cell cultures and PGZ or fenofibrate treatment

Cultures of thyrocytes were treated (24 h) with 0.1, 1, 5, 10, or 20 μM PGZ or 5, 10, 50, 100 μM fenofibrate. Control cultures were grown (24 h) in the same medium containing vehicle (absolute ethanol, 0.47% v/v) without PGZ or fenofibrate. Some cultures were observed by phase contrast microscopy with an Olympus IX50.

After 10 days, fibroblasts and preadipocytes were treated with 0.1, 1, 5, 10, or 20 μM PGZ or 5, 10, 50, 100 μM fenofibrate, for 24 h in the same medium with only vehicle (absolute ethanol, 0.47% v/v) without PGZ or fenofibrate, for 24 h. Some cultures were observed by phase contrast microscopy with an Olympus IX50. Other cultures were observed after staining with Oil Red O. The effect of PGZ or fenofibrate was evaluated in cells under differentiation protocol (after 10 days) and in cells grown in non-differentiated conditions [7,8,12].

For quantification of total protein in cell preparations, lysis and homogenization were performed and the sample was immediately assayed for its protein concentration by conventional methods.

2.5. ELISA for CXCL10 and CXCL8

CXCL8 and CXCL10 levels were measured in serum and culture supernatants with commercially available kits (R&D Systems).

The mean minimum detectable dose of CXCL8 was 3.3 pg/mL. The intra- and inter-assay coefficients of variation were 3.2 and 6.6%, respectively.

The mean minimum detectable dose was 1.54 pg/mL for CXCL10; the intra- and inter-assay coefficients of variation were 3.2% and 6.6% for CXCL10.

Each sample was tested in duplicate. For all parameters, quality control pools of low, normal, or high concentration were evaluated in each assay.

2.6. Statistics

Normally distributed variables are expressed as mean ± SEM, otherwise as median and [interquartile range]. For normally distributed variables ANOVA was used to compare mean group values, otherwise by the Mann-Whitney *U* or Kruskal-Wallis test. χ^2 test was used to compare proportions and Bonferroni-Dunn test for *post-hoc* comparisons on normally distributed variables.

3. Results

3.1. CXCL8

3.1.1. Normal thyrocytes

In basal conditions, the secretion of CXCL8 (range 45.2–152.4 pg/mL) was measured in all preparations of cultured thyroid cells (Fig. 1A). The secretion of CXCL8 was increased dose-dependently by different concentrations of TNFα (1, 5, 10 ng/mL), with the highest response obtained with 10 ng/mL TNFα (basal 105 ± 26 pg/mL vs. TNFα 845 ± 241 pg/mL; $P < 0.01$) (Fig. 1A).

On the other hand, IFNγ (1000 IU/mL) significantly inhibited the basal secretion of CXCL8 (IFNγ 32 ± 19 pg/mL vs. basal; $P < 0.05$) (Fig. 2A). IFNγ when added in combination with TNFα led to a significant reversal of the stimulating effect of TNFα (TNFα + IFNγ 387 ± 150 pg/mL vs. TNFα 845 ± 241 pg/mL; $P < 0.05$) (Fig. 2A). IFNγ did not completely reverse the stimulating effect of TNFα on the secretion of CXCL8, because the concentration of this chemokine was still significantly higher than in basal conditions (TNFα + IFNγ vs. basal; $P < 0.01$). PGZ had no significant effect on the secretion of CXCL8 stimulated by TNFα (823 ± 194 pg/mL vs. TNFα; $P > 0.05$). Treatment of thyrocytes with fenofibrate, in presence of IFNγ plus TNFα, dose-dependently inhibited the CXCL8 secretion (204 ± 63 pg/mL vs. TNFα; $P < 0.05$).

3.1.2. Graves' thyrocytes

In basal conditions, the secretion of CXCL8 (range 57–177 pg/mL) was measured in all preparations of cultured Graves' cells (Fig. 1B). The secretion of CXCL8 was increased dose-dependently by different concentrations of TNFα (1, 5, 10 ng/mL), with the highest response obtained with 10 ng/mL TNFα (basal 111 ± 25 pg/mL vs. TNFα 956 ± 150 pg/mL; $P < 0.01$) (Fig. 1B).

On the other hand, IFNγ (1000 IU/mL) significantly inhibited the basal secretion of CXCL8 (IFNγ 39 ± 15 pg/mL vs. basal; $P < 0.05$) (Fig. 2B). IFNγ when added in combination with TNFα led to a significant reversal of the stimulating effect of TNFα (TNFα + IFNγ 400 ± 184 pg/mL vs. TNFα 956 ± 150 pg/mL; $P < 0.05$) (Fig. 2B). IFNγ did not completely reverse the stimulating effect of TNFα on the secretion of CXCL8, because the concentration of this chemokine was still significantly higher than in basal conditions (TNFα + IFNγ vs. basal; $P < 0.01$). PGZ had no significant effect on the secretion of CXCL8 stimulated by TNFα (911 ± 162 pg/mL vs. TNFα; $P > 0.05$). Treatment of Graves' thyrocytes with fenofibrate, in presence of IFNγ plus TNFα, dose-dependently inhibited the CXCL8 secretion (217 ± 86 pg/mL vs. TNFα; $P < 0.05$). The basal and stimulated CXCL8 was not significantly different in normal control thyroid cells and in Graves' thyrocytes.

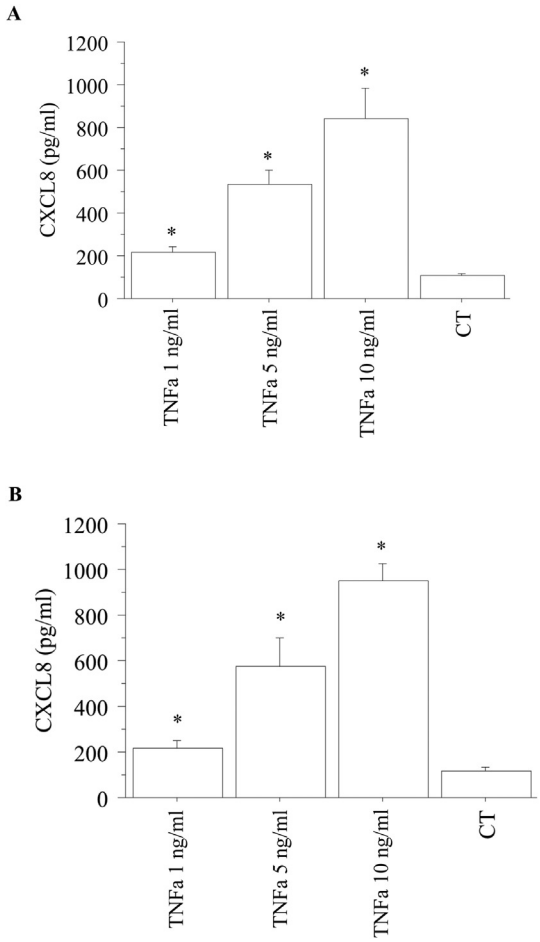

Fig. 1. Stimulation of CXCL8 secretion by TNFα. (A) The secretion of CXCL8 (range 45.2–152.4 pg/mL) was measured in all preparations of cultured normal thyroid cells in basal conditions (CT). CXCL8 secretion was significantly stimulated by increasing doses of TNFα (TNFa; 1, 5, 10 ng/mL), with the highest response obtained with 10 ng/mL TNFα ($P < 0.01$, by ANOVA). Bars are the mean ± SEM. (B) The secretion of CXCL8 (range 57–177 pg/mL) was measured in all preparations of cultured Graves' thyrocytes in basal conditions (CT). The secretion of CXCL8 was increased dose-dependently by different concentrations of TNFα (TNFa; 1, 5, 10 ng/mL), with the highest response obtained with 10 ng/mL TNFα ($P < 0.01$, by ANOVA).

3.1.3. Graves' orbital fibroblasts

In basal conditions, the secretion of CXCL8 (range 69–203 pg/mL) was measured in all preparations of cultured Graves' orbital fibroblasts (Fig. 3A). The secretion of CXCL8 was increased dose-dependently by different concentrations of TNFα (1, 5, 10 ng/mL), with the highest response obtained with 10 ng/mL TNFα (basal 176 ± 22 pg/mL vs. TNFα 1278 ± 177 pg/mL; $P < 0.01$) (Fig. 3A).

On the other hand, IFNγ (1000 IU/mL) significantly inhibited the basal secretion of CXCL8 (IFNγ 65 ± 12 pg/mL vs. basal; $P < 0.05$) (Fig. 4A). IFNγ when added in combination with TNFα led to a significant reversal of the stimulating effect of TNFα (TNFα + IFNγ 753 ± 161 pg/mL vs. TNFα 1278 ± 177 pg/mL; $P < 0.05$) (Fig. 4A). IFNγ did not completely reverse the stimulating effect of TNFα on the secretion of CXCL8, because the concentration of this chemokine was still significantly higher than in basal conditions (TNFα + IFNγ vs. basal; $P < 0.01$). PGZ had no significant effect on the secretion of CXCL8 stimulated by TNFα (1201 ± 133 pg/mL vs. TNFα; $P > 0.05$). Treatment of Graves' orbital fibroblasts with fenofibrate, in presence of IFNγ plus TNFα, dose-dependently inhibited the CXCL8 secretion (565 ± 94 pg/mL vs. TNFα; $P < 0.05$). The basal and stimulated CXCL8 was not significantly different in normal dermal fibroblasts (data

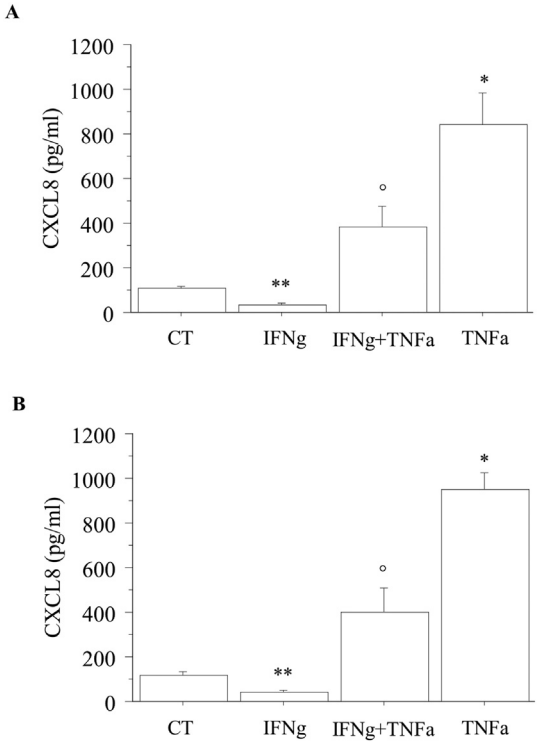

Fig. 2. Secretion of CXCL8 by IFNγ and/or TNFα from normal thyroid follicular cells (A) and from Graves' thyrocytes (B). The secretion of CXCL8 was increased by TNFα (10 ng/mL) alone [TNFa; * = $P < 0.01$ vs. basal (CT) by ANOVA]. On the other hand, IFNγ (1000 IU/mL) significantly inhibited the basal secretion of CXCL8 [IFNg; ** = $P < 0.05$ vs. basal (CT) by ANOVA]. IFNγ when added in combination with TNFα led to a significant reversal of the stimulating effect of TNFα [IFNg + TNFa; ° = $P < 0.05$ vs. TNFα by ANOVA]. IFNγ did not completely reverse the stimulating effect of TNFα on the secretion of CXCL8, because the concentration of this chemokine was still significantly higher than in basal conditions (TNFα + IFNγ vs. basal; $P < 0.01$). Bars are the mean ± SEM.

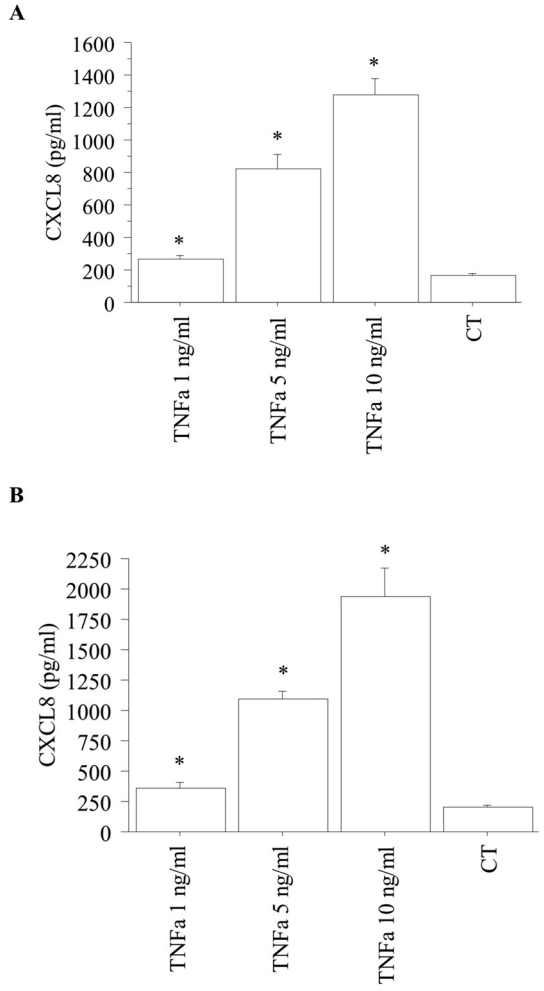

Fig. 3. Stimulation of CXCL8 secretion by TNFα. (A) The secretion of CXCL8 (range 69–203 pg/mL) was measured in all preparations of cultured Graves' orbital fibroblasts in basal conditions (CT). CXCL8 secretion was significantly stimulated by increasing doses of TNFα (TNFa; 1, 5, 10 ng/mL), with the highest response obtained with 10 ng/mL TNFα ($P < 0.01$, by ANOVA). (B) The secretion of CXCL8 (range 77–258 pg/mL) was measured in all preparations of cultured Graves' orbital preadipocytes in basal conditions (CT). The secretion of CXCL8 was increased dose-dependently by different concentrations of TNFα (TNFa; 1, 5, 10 ng/mL), with the highest response obtained with 10 ng/mL TNFα ($P < 0.01$, by ANOVA). Bars are the mean ± SEM.

not shown) and in Graves' orbital fibroblasts.

3.1.4. Graves' orbital preadipocytes

In basal conditions, the secretion of CXCL8 (range 77–258 pg/mL) was measured in all preparations of cultured Graves' orbital preadipocytes (Fig. 3B). The secretion of CXCL8 was increased dose-dependently by different concentrations of TNFα (1, 5, 10 ng/mL), with the highest response obtained with 10 ng/mL TNFα (basal 196 ± 41 pg/mL vs. TNFα 1947 ± 386 pg/mL; $P < 0.01$) (Fig. 3B).

On the other hand, IFNγ (1000 IU/mL) significantly inhibited the basal secretion of CXCL8 (IFNγ 82 ± 15 pg/mL vs. basal; $P < 0.05$) (Fig. 4B). IFNγ when added in combination with TNFα led to a significant reversal of the stimulating effect of TNFα (TNFα + IFNγ 987 ± 166 pg/mL vs. TNFα 1947 ± 386 pg/mL; $P < 0.05$) (Fig. 4B). IFNγ did not completely reverse the stimulating effect of TNFα on the secretion of CXCL8, because the concentration of this chemokine was still significantly higher than in basal conditions (TNFα + IFNγ vs. basal; $P < 0.01$). PGZ had no significant effect on the secretion of CXCL8 stimulated by TNFα (1899 ± 302 pg/mL vs. TNFα; $P > 0.05$). Treatment of Graves' orbital preadipocytes with fenofibrate, in presence of IFNγ and TNFα, dose-dependently inhibited the CXCL8 secretion (469 ± 84 pg/mL vs. TNFα; $P < 0.05$). The basal and stimulated CXCL8 was not significantly different in normal dermal preadipocytes (data not shown) and in Graves' preadipocytes.

3.2. CXCL10

In primary thyrocyte cultures from patients with GD, CXCL10 was not measurable in the supernatant. CXCL10 secretion was stimulated dose-dependently by IFNγ, while TNFα alone had no effect. The treatment with TNFα (10 ng/mL) plus IFNγ (1000 IU/mL) synergistically stimulated the secretion of CXCL10 (1511 ± 232 vs. 236 ± 61 pg/mL with IFNγ alone, $P < 0.0001$). Treating thyrocytes with PGZ, with IFNγ plus TNFα, dose-dependently inhibited CXCL10 release [13], such as the treatment with fenofibrate [14]. PGZ, or fenofibrate, 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 patients with GO (data not shown).

The results obtained in primary thyrocytes were substantially the same in retrobulbar fibroblasts and preadipocytes. IFNγ dose-dependently stimulated the CXCL10 secretion in fibroblast and preadipocyte cells. The treatment with TNFα (10 ng/mL) plus IFNγ (1000 IU/mL) synergistically stimulated the secretion of CXCL10 (in fibroblasts: 1543 ± 197 vs. 232 ± 111 pg/mL with IFNγ alone, $P < 0.0001$; in preadipocytes: 2341 ± 387 vs. 211 ± 87 pg/mL, $P < 0.0001$). Treating retrobulbar fibroblasts and preadipocytes with PGZ, with IFNγ

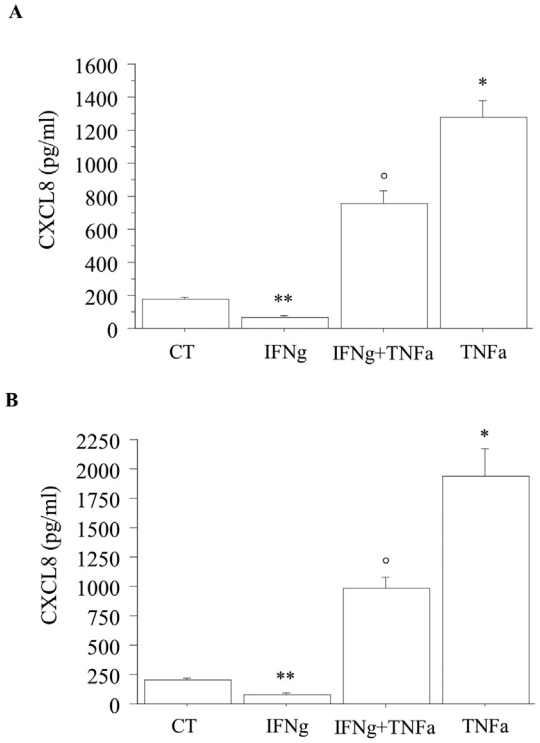

Fig. 4. Secretion of CXCL8 by IFNγ and/or TNFα from Graves' orbital fibroblasts (A) and from Graves' orbital preadipocytes (B). The secretion of CXCL8 was increased by TNFα (10 ng/mL) alone [TNFa; * = $P < 0.01$ vs. basal (CT) by ANOVA]. On the other hand, IFNγ (1000 IU/mL) significantly inhibited the basal secretion of CXCL8 [IFNg; ** = $P < 0.05$ vs. basal (CT) by ANOVA]. IFNγ when added in combination with TNFα led to a significant reversal of the stimulating effect of TNFα [IFNg + TNFa; ° = $P < 0.05$ vs. TNFα by ANOVA]. IFNγ did not completely reverse the stimulating effect of TNFα on the secretion of CXCL8, because the concentration of this chemokine was still significantly higher than in basal conditions (TNFα + IFNγ vs. basal; $P < 0.01$). Bars are the mean ± SEM.

plus TNFα, dose-dependently inhibited CXCL10 release [15], such as the treatment with fenofibrate [15]. The results in fibroblasts or preadipocytes from normal dermal tissues of the same patients did not significantly differ from those obtained in their retrobulbar fibroblasts and preadipocytes (data not shown). Total protein content or cell viability were not affected by PGZ, or fenofibrate, that also did not induce adipogenic changes (evaluated by Oil Red O) in either cell types (after 24 h of treatment) (data not shown).

4. Discussion

The results confirm that CXCL8 is secreted by normal thyroid follicular cells [6,16], and first show that it is modulated in a similar way in thyrocytes from patients with GD such as in fibroblasts and preadipocytes from patients with GO, and that TNFα dose-dependently induced CXCL8 release. Even if CXCL8 was the first chemokine to be demonstrated in the supernatants of cultured human thyroid cells [16], the role of CXCL8 in thyroid diseases remains scanty. It was demonstrated [17] that the gene encoding for CXCL8 was hyper-expressed in thyroid cancer specimens as compared with normal thyroid samples, whereas similar expression levels were found in non-neoplastic specimens of thyroiditis as compared with normal thyroid samples. Whether the basal production of CXCL8 has any biological significance is not known at present. The recent demonstration that two important regulators of thyrocyte function and growth, such as iodide and TSH, are positively associated with the mRNA encoding for CXCL8, supports the hypothesis that CXCL8 has a role in thyroid homeostasis [18–20]. Future studies specifically aimed at elucidating the biological significance of CXCL8 in thyrocytes appear necessary.

Interestingly, IFNγ does stimulate the secretion of CXCL10 while it inhibits that of CXCL8; while TNFα by itself has no effect on the secretion of CXCL10, whereas it increases the secretion of CXCL8. This differential modulation of CXCL10 and CXCL8 chemokines may reflect a different role of the two chemokines in the course of the disease, as IFNγ is associated with the initial phase of the disease, while TNFα is associated with the chronic phase of the disease. IFNγ, by stimulating the intrathyroidal secretion of CXCL10, which in turn recruits T helper (Th)1 lymphocytes secreting IFNγ, would also play a role in the initial phase of the classical model of hypothyroidism [21–23], which results from the loss of thyroid follicles due to massive lymphocytic infiltration [24,25], and in the active phase of GD [26,27].

By contrast TNFα, which is secreted by monocytes, macrophages, and resident cells, is a pleiotropic proinflammatory cytokine involved in systemic inflammatory disease states [28]. The increased levels of CXCL10 in active GO [8] agree with previous papers reporting an involvement mainly of Th1 cytokines in GD and GO [29–33] and in other autoimmune diseases [34,35].

In fact, the active phase of GO seems to be characterized by the presence of proinflammatory and Th1-derived cytokines, while others (as Th2-derived cytokines) are not supposed to be associated with a specific stage of GO [36]. Our data in fibroblasts from patients with GO are similar to those obtained in fibroblasts from patients with other chronic inflammatory diseases [37,38]. Furthermore, our data confirm and reinforce the results of another study that showed GO progression and inflammmation are associated to types of fibroblasts and amount of CXCL8 production in these cells [39].

No study, to the best of our knowledge, has evaluated previously CXCL8 in preadipocytes of whatever origins. Interestingly, PPARγ agonists that have recently been shown to modulate inflammatory responses in various cell types and in thyrocytes [7,8,11,40,41], have no effect on the CXCL8 chemokine secretion induced by TNFα, suggesting the PPARγ agonists modulation of chemokine secretion acts through different molecular pathways than those involved in the secretion of CXCL10.

Furthermore the effect on chemokines of biological therapies needs to be evaluate [42,43].

In conclusion, we have shown that TNFα by itself has no effect on the secretion of CXCL10, whereas it increases dose-dependently the secretion of CXCL8 in Graves' thyrocytes, and in GO fibroblasts and preadipocytes, while IFNγ does stimulate the secretion of CXCL10 but it inhibits that of CXCL8. This differential modulation of CXCL10 and CXCL8 chemokines may reflect a different role of the two chemokines in the course of the disease, as CXCL10 could be associated with the initial phase of the disease when IFNγ is preponderant, while CXCL8 could be associated with a later chronic phase of the disease, when TNFα prevails.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Take home messages

- This study shows that TNFα by itself has no effect on the CXCL10 secretion, while it increases dose-dependently the secretion of CXCL8 in GD, and in GO fibroblasts and preadipocytes; whereas IFNγ stimulates the secretion of CXCL10 but it inhibits the one of CXCL8.
- This differential modulation of CXCL10 and CXCL8 chemokines could reflect a different role of the two chemokines during the course of the disease, as CXCL10 could be associated with the initial phase of the disease when a Th1 immune response (induced by

IFNγ) is preponderant, while CXCL8 could be associated with a later chronic phase of the disease, when there is a switch to a Th2 prevalent immune response (induced by TNFα).

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