"N-acetylcysteine and 15 deoxy-{delta}12,14-prostaglandin J2 exert a protective effect against autoimmune thyroid destruction in vivo but not against interleukin-1{alpha}/interferon {gamma}-induced inhibitory effects in thyrocytes in vitro."

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Abstract

Reactive oxygen species (ROS) are crucial for thyroid hormonogenesis, and their production is kept under tight control. Oxidative stress (OS) is toxic for thyrocytes in an inflammatory context. In vitro, Th1 pro-inflammatory cytokines have already been shown to decrease thyroid-specific protein expression. In the present study, OS level and its impact on thyroid function were analyzed in vitro in Th1 cytokine (interleukin [IL]-1alpha/interferon [IFN] gamma)-incubated thyrocytes (rat and human), as well as in vivo in thyroids from nonobese diabetic mice, a model of spontaneous autoimmune thyroiditis. N-acetylcysteine (NAC) and prostaglandin, 15 deoxy-(Delta12,14)-prostaglandinJ2 (15dPGJ2), were used for their antioxidant and anti-inflammatory properties, respectively. ROS production and OS were increased in IL-1alpha/IFN-gamma-incubated thyrocytes and in destructive thyroiditis. In vitro, NAC not only reduced ROS production below control levels, but further decreased the expression of ...

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N-Acetylcysteine and 15 Deoxy-Δ^{12,14}-Prostaglandin J2 Exert a Protective Effect Against Autoimmune Thyroid Destruction in Vivo but Not Against Interleukin-1α/Interferon γ-Induced Inhibitory Effects in Thyrocytes in Vitro

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Reactive oxygen species (ROS) are crucial for thyroid hormonogenesis, and their production is kept under tight control. Oxidative stress (OS) is toxic for thyrocytes in an inflammatory context. In vitro, Th1 proinflammatory cytokines have already been shown to decrease thyroid-specific protein expression. In the present study, OS level and its impact on thyroid function were analyzed in vitro in Th1 cytokine (interleukin [IL]-1α/interferon [IFN] γ)-incubated thyrocytes (rat and human), as well as in vivo in thyroids from nonobese diabetic mice, a model of spontaneous autoimmune thyroiditis. N-acetylcysteine (NAC) and prostaglandin, 15 deoxy-Δ^{12,14}-prostaglandin J2 (15dPGJ2), were used for their antioxidant and anti-inflammatory properties, respectively. ROS production and OS were increased in IL-1α/IFNγ-incubated thyrocytes and in destructive thyroiditis. In vitro, NAC not only reduced ROS production below control levels, but further decreased the expression of thyroid-specific proteins in addition to IL-1α/IFNγ-inhibitory effects. Thus, besides ROS, other intracellular intermediaries likely mediate Th1 cytokine effects. In vivo, NAC and 15dPGJ2 reduced OS and the immune infiltration, thereby leading to a restoration of thyroid morphology. It is therefore likely that NAC and 15dPGJ2 mainly exert their protective effects by acting on infiltrating inflammatory cells rather than directly on thyrocytes. (Am J Pathol 2010, 177:219–228; DOI: 10.2353/ajpath.2010.091253)

Thyrocytes continuously produce H₂O₂ and various reactive oxygen species (ROS) that are physiologically required for normal thyroid hormone synthesis. To control the toxicity resulting from ROS, thyrocytes possess several protective mechanisms. During thyroid hormone synthesis, H₂O₂ is produced in a limited area at the apical membrane and is immediately consumed in the peroxidation reaction catalyzed by thyroperoxidase (TPO). When ROS are produced in higher amounts, they are systematically eliminated by potent antioxidant systems such as peroxiredoxins, catalase, and glutathione peroxidases.1–5 Thus, a basal ROS production, which we define as oxidative load, is required to safeguard thyroid hormone synthesis, as recently demonstrated.6 Likewise, an harmless oxidative stress (OS) may also be important for cell division during goiter formation when thyrocytes are facing iodine deprivation.7

The context is quite different in the case of thyroid inflammation. Thus, in models of thyroiditis (transient or permanent), high amounts of ROS are produced and may become toxic.8–13 Using one of these models, we recently showed that increased OS associated with a strong inflammatory reaction can be controlled by 15 deoxy-Δ^{12,14}-prostaglandin J2 (15dPGJ2),2 an anti-inflammatory prostaglandin14 that prevents OS-induced cytotoxicity.15 Iodine administration to goitrous thyrocytes produces an inflammatory reaction that is transient in most cases. However, in individuals genetically prone to develop autoimmune thyroiditis, this transient inflam-
mation may become permanent, thereby evolving toward destructive thyroiditis. A model of destructive thyroiditis can be obtained in nonobese diabetic (NOD) mice. In this model of Hashimoto’s-like thyroiditis, the ongoing inflammatory reaction relies on pro-inflammatory Th1 cytokines that inhibit the expression of thyroid-specific proteins such as thyroglobulin, TPO, Na+/I− symporter (NIS), and dual oxidases (Duox). Mechanisms responsible for this inhibition are not yet known. In autoimmune processes targeting other cell types, such as pancreatic β cells, Th1 cytokine effects are mediated by nitric oxide (NO). In human, but not in rat thyrocytes, NO has also been identified as mediating the inhibitory actions of Th1 cytokines, but only partially. Intracellular factors other than NO should therefore mediate Th1 cytokine-induced inhibitory effects. ROS may represent, among others, alternative candidates, as suggested by previous studies. For instance, Th1 cytokines are known to increase ROS generation in the respiratory tract. Up to now, nothing is known about the eventual involvement of ROS as intracellular mediators of Th1 cytokine-induced inhibitory actions in thyrocytes.

In the present study we aimed to evaluate the impact of Th1 cytokines (interleukin [IL]-1α/interferon [IFN] γ) on ROS production and how they may influence thyroid cell function in vitro. Likewise, the role played by OS in thyroiditis was analyzed in vivo in the aforementioned NOD mouse model of spontaneous autoimmune thyroiditis. In both in vitro and in vivo models, the roles of ROS were evaluated by using N-acetylcysteine (NAC), a potent antioxidant, and 15dPGJ2 for its anti-inflammatory properties. We also investigated how antioxidant systems behave in these conditions.

Materials and Methods

Cell Cultures

PCCL3 cells, a continuous line of nontransformed rat thyroid follicular cells, were a gift of Dr. F. Miot (Université Libre de Bruxelles, Institut de recherche interdisciplinaire en biologie humaine et moléculaire, Brussels, Belgium). They were grown to 80% to 90% confluence in RPMI-1640 medium (BRL-Gibco, Paisley, UK) supplemented with 5% newborn calf serum, penicillin (50 U/ml), streptomycin (50 μg/ml), fungizone (5 μg/ml; BRL-Gibco), 1 mU/ml thyroid stimulating hormone, 10 μg/ml insulin, and 5 μg/ml transferase (Sigma, Bornem, Belgium), in a humidified atmosphere (5% CO₂). Recombinant rat IL-1α (2 ng/ml, Chemicon International, Temecula, CA) and recombinant rat IFN-γ (100 U/ml, Chemicon International) were added for three additional days, in combination or not with NAC (1 mmol/L; Sigma) or 15dPGJ2 (2.5 μmol/L, Sigma) in the same medium containing 0.5% newborn calf serum and 1 mU/ml thyroid stimulating hormone. NAC, 15dPGJ2, or vehicle was added 2 hours before the cytokine cocktail. As a control, NAC or 15dPGJ2 were added on thyroid cells in the absence of cytokines.

Human thyroid tissues from patients who underwent thyroid surgery for benign multinodular goiter were obtained from the anatomopathology department after patients gave their informed consent. Thyrocytes were isolated according to Nilsson et al and suspended in modified Earle’s medium without phenol red containing 5% newborn calf serum, penicillin (50 U/ml), streptomycin (50 μg/ml), and fungizone (2.5 μg/ml; BRL-Gibco). They were plated in 6-well plates (50 μg DNA/well) or in multichamber glass slide (Nunc International, Naperville, IL; 7 μg DNA/chamber) and cultured in a humidified atmosphere (5% CO₂) with 1 μU/ml thyroid stimulating hormone. After 1 week, cells were incubated for three additional days with cytokines in combination or not with NAC (1 mmol/L) or 15dPGJ2 (2.5 μmol/L), as described for PCCL3 cells. All experiments were repeated at least twice.

ROS Production

Thyrocytes were incubated in multichamber glass slides in appropriate medium. ROS production was measured by using a fluorescent dye, 2’7’ dichlorofluorescin diacetate (DCFH-DA; Molecular Probes, Paisley, UK). PBS-washed (pH 7.4) thyroid cells were incubated in Krebs-Ringer HEPES medium, pH 7.4, containing DCFH-DA (25 μM) at 37°C for 1 hour. The excess of dye was removed by two washes with PBS. Cells were stained with Hoechst for 20 minutes and rinsed in PBS. Cover slides were mounted in fluorescent mounting medium (DakoCytomation, Carpinteria, CA) for microscopic observation. ROS production was visualized on a fluorescent microscope equipped with a digital camera.

Viability Assay

Cell viability was assessed by using the Alamar blue assay (Biosource International, Camarillo, CA), as previously described.

Apoptosis Detection

Caspase activity was measured by using a CaspASE fluoroscein isothiocyanate-VAD-fmk in situ marker (Promega, Madison, WI), which binds activated caspases, according to the manufacturer’s instructions. Briefly, cells were incubated with 20 μmol/L fluorescein isothiocyanate-VAD-fmk at 37°C for 20 minutes. Cells were then washed twice with PBS, fixed in 10% buffered formalin for 30 minutes, and rinsed with PBS. Coverslides were mounted in fluorescent mounting medium for microscopic observation. Cells treated with staurosporine (5 μmol/L; Sigma) were used as positive control.

Nitrite Assay

Nitrite accumulation in the medium of human thyrocytes was measured by the Griess reaction by using a commercially available kit (Promega).
Table 1. Forward and Reverse Primers and Annealing Temperatures Used

<table>
<thead>
<tr>
<th>Target</th>
<th>Primer forward</th>
<th>Primer reverse</th>
<th>Annealing temperature, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actin</td>
<td>5′-CAGCGGACTGCTTGGAGCCT-3′</td>
<td>5′-GGCGGTAGAAGATCGGCAAGAAGA-3′</td>
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<tr>
<td>Duox</td>
<td>5′-GGCGGACTGCTTGGAGCCT-3′</td>
<td>5′-GGCGGACTGCTTGGAGCCT-3′</td>
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<tr>
<td>Duox1</td>
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<td>5′-GGCGGACTGCTTGGAGCCT-3′</td>
<td>60</td>
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<tr>
<td>Duox2</td>
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<td>60</td>
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<tr>
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<tr>
<td>PRDX3</td>
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<tr>
<td>PRDX5</td>
<td>5′-GGCGGACTGCTTGGAGCCT-3′</td>
<td>5′-GGCGGACTGCTTGGAGCCT-3′</td>
<td>60</td>
</tr>
</tbody>
</table>

Real-Time RT-PCR

Cells were suspended in TriPure isolation reagent (Roche Diagnostics GmbH, Mannheim, Germany), and total RNA was purified according to the manufacturer’s protocol. Reverse transcription was performed by incubating 2 μg RNA with 200 U Moloney Murine Leukemia Virus reverse transcriptase (Invitrogen, Merelbeke, Belgium) in the recommended buffer containing 1 μl RNasin (Promega), 0.5 mmol/L dNTP (Promega), 2 μmol/L oligodT (Sigma), and 10 mmol/L dithiothreitol (20 μl final volume) overnight at 42°C. CDNA was diluted 1:5 in water for use in real time PCRs.

CDNAs (2 μl) were mixed with 500 nmol/L of each selected primer (Table 1) and SYBR Green reaction mix (BioRad, Herts, UK) in a final volume of 25 μl. Reactions were performed by using a iCycler apparatus (BioRad) as follows: 95°C for 1 minute, followed by 40 cycles of 95°C for 15 seconds, annealing temperature for 45 seconds (Table 1), and 81°C for 15 seconds. Amplification levels were normalized to that of β-actin expression.

Western Blotting

Thyrocytes were suspended in Laemmli buffer (50 mmol/L Tris-HCl, pH 6.8; 2% SDS, and 10% glycerol), containing a protease inhibitor cocktail (Sigma), and were sonicated during 30 seconds. Protein concentration was determined by using a bicinchoninic acid protein assay kit (Pierce, Rockford, IL). Duox (antibody provided by F. Miot, IRIBHN, Brussels), TPO (antibody provided by J. Ruf, Université de la Méditerranée, Marseille, France), catalase (Sigma), peroxiredoxin 3 and 5 (PRDX3, PRDX5; antibodies provided by B. Apeles, Université catholique de Louvain, Louvain La Neuve), and β-actin (Sigma) Western blotting were performed as previously described.22 Proteins (30 μg/lane) were heated at 95°C for 5 minutes in the loading buffer (Laemmli buffer containing 100 mmol/L dithiothreitol and 0.1% bromophenol blue), separated by 8% SDS-polyacrylamide gel electrophoresis, and transferred onto a nitrocellulose membrane (Hybond ECL, Amersham Biosciences, Rosenthaal, The Netherlands). Membranes were blocked for 1 hour at room temperature in PBS (pH 7.4), 5% nonfat dry milk, 0.1% Tween, and incubated overnight at 4°C with the primary antibody at a dilution of 1:4000 (Duox, TPO), 1:10,000 (PRDX3, PRDX5), or 1:2000 (catalase, β-actin).

Membranes were incubated for 1 hour at room temperature with EnVision (1:200, DakoCytomation) peroxidase-labeled secondary antibody and visualized with enhanced chemiluminescence (SuperSignal West Pico, Pierce) on CLXposure TM films (Pierce). Western blots were scanned and quantified by densitometry using the NIH Scion Image Analysis Software (NIH, Bethesda, MD). Values were normalized by reporting the signal intensity to β-actin expression.

Immunofluorescence

Thyrocytes were cultured in multichamber glass slides in appropriate medium. Thyrocytes were fixed for 30 minutes in 4% paraformaldehyde, rinsed once with PBS, permeabilized for 15 minutes in a PBS-Triton 1% solution at room temperature, and washed with PBS supplemented with 1% bovine serum albumin. Cells were then incubated overnight with PRDX5 primary antibody (1:75) at room temperature. After being washed in PBS, fluorescein isothiocyanate-conjugated secondary antibody was added for 1 hour at room temperature at a dilution of 1:30 (anti-rabbit; DakoCytomation). Coverslides were mounted in fluorescent mounting medium for microscopic observation.

Animals and Treatments

Three-month-old female NOD mice, under a standard diet and kept under semibarrier conditions, were originally obtained from Professor Wu (Beijing, China) and inbred since 1989 (Proefdierencentrum, Leuven, Belgium). Animals were injected intraperitoneally with a saline solution of NAC (100 mg/kg/day) or with a saline solution of 15dPGJ2 (40 μg/kg/day) for 4 days. NMRI mice were used as control. Mice were housed and handled according to Belgian Regulation of Laboratory Animal Welfare.

Preparation of Tissue Samples for Microscopy and Morphometric Analysis

Five animals of each group were anesthetized with pentothal, and thyroid lobes were dissected. One thyroid lobe was fixed in paraformaldehyde (4% in PBS)
Data were expressed as mean ± SEM, n = 6 for all experiments. Each experiment was repeated at least twice. Statistical analyses were performed by using analysis of variance followed by Tukey-Kramer Multiple Comparison Test (GraphPad InStat, San Diego, CA), or by unpaired t-test. P < 0.05 was considered as statistically significant.

Results

IL-1α/IFN-γ Increase Intracellular ROS Production without Affecting Cell Viability In Vitro: Differential Effects of NAC and 15dPGJ2

Although Th1 cytokines are known to induce ROS production in various cell types, this has not been yet described in thyrocytes. IL-1α/IFN-γ-induced ROS, and nitrite production was therefore analyzed both in rat and human thyroid cells.

In rat PCCL3 control cells, ROS detected by DCFH-DA fluorescence were observed as granules within the cytoplasm (Figure 1A). The staining was greatly enhanced in Th1 cytokine-treated cells (Figure 1B), whereas ROS were detected both in the cytoplasm and in nuclei. In cells treated with NAC alone, and in accordance with NAC anti-oxidant properties, ROS fluorescence was strongly reduced compared with control cells (Figure 1C). In cells co-incubated with IL-1α/IFN-γ together with NAC, ROS fluorescence was below control levels (Figure 1D). By contrast, 15dPGJ2 influenced ROS production, neither in control cells (data not shown) nor in Th1 cytokine-treated cells (Figure 1E). Similar results were obtained in human primary cells (data not shown).

According to previous results, nitrite levels, the stable end-product of NO generation, were low in media from control human thyroid cells, but greatly enhanced in Th1 cytokine-treated cells (Figure 1F). When cells were co-incubated with IL-1α/IFN-γ and NAC or 15dPGJ2, nitrite levels were significantly reduced as compared with Th1-treated cells, but remained higher than in control cells (Figure 1F).

Cell viability was not affected, and no change in apoptosis was detected whatever the treatment used, indicating that the observed effects were not resulting from cell death (data not shown).
IL-1α/IFNγ Induce a Down-Regulation of Thyroid Cell Function In Vitro: Differential Effects of NAC and 15dPGJ2

As already reported, IL-1α/IFNγ induced a down-regulation of the thyrocyte function, as indicated by decreased Duox (Duox1 and Duox2), TPO, and NIS expression, at mRNA or protein levels in rat PCCL3 and human cells (Figure 2; Supplemental Figure S1, see http://ajp.amjpathol.org). Our data also confirm that Duox protein and mRNA expression was strongly decreased in PCCL3 cells incubated with NAC alone. When incubated together with IL-1α/IFNγ, NAC further aggravated Th1-induced inhibitory effects (Figure 2, A and B). In human thyroid cells, NAC alone or in combination with Th1 cytokines significantly reduced Duox protein and mRNA expression without additional effect on Th1-induced down-regulation of Duox protein expression (Supplemental Figure S1, A and B, see http://ajp.amjpathol.org).

The distinct analysis of Duox1 and Duox2 expression in PCCL3 cells showed a different pattern. NAC influenced neither basal nor Th1-induced down-regulation of Duox1 mRNA expression (Figure 2C), but induced a decrease in Duox2 mRNA expression and further impaired Th1 cytokine-induced down-regulating effects (Figure 2D).

As for Duox and already reported, NAC alone induced a down-regulation of TPO mRNA and protein expression both in PCCL3 and human thyroid cells (Figure 2E; Supplemental Figure S1, C and D, see http://ajp.amjpathol.org). In cells incubated together with NAC and IL-1α/IFNγ, an additive effect of their respective inhibitory action was observed.

Although NAC alone had no effect on NIS mRNA, it further aggravated IL-1α/IFNγ-induced NIS down-regulation, at least in human cells (Figure 2F; Supplemental Figure S1E, see http://ajp.amjpathol.org). Both in PCCL3 and human cells, 15dPGJ2, when administered alone or together with Th1 cytokines, had no specific effect on Duox nor on NIS expression (Figure 2A–D and F; Supplemental Figure S1A, B, and E, see http://ajp.amjpathol.org). The only isolated modification we observed was on TPO mRNA expression in PCCL3 cells (Figure 2E).

The Expression and Intracellular Localization of PRDX3 and PRDX5 Antioxidant Enzymes Are Strongly Influenced by Th1 Cytokines, NAC, and 15dPGJ2

PRDX5 and PRDX3 protein expression in PCCL3 cells was significantly increased by IL-1α/IFNγ (Figure 3, A

Effects of NAC and 15dPGJ2 on Thyroiditis 223
AJP July 2010, Vol. 177, No. 1
The subcellular localization of PRDX5 also changed. In control cells, PRDX5 was expressed only as granules in the cytoplasm (Figure 3D). In Th1 cytokine-treated cells, PRDX5 expression not only increased in the cytoplasm, but also lighted up in the nuclei (Figure 3E). NAC treatment differentially regulated both PRDXs. NAC alone or together with Th1 cytokines reduced PRDX5 expression (Figure 3, A and F), but increased the expression of PRDX3 (Figure 3B). 15dPGJ2 significantly increased PRDX5 and PRDX3 expression in PCCL3 without affecting their expression induced by Th1 cytokines, or their localization (Figure 3A, B, and G). Other antioxidant enzymes reacted differently, as catalase protein expression remained stable in all experimental conditions (Figure 3C).

The Strong Increase of Both OS and Inflammatory Reaction in the NOD Mouse Model of Spontaneous Thyroiditis Is Down-Regulated by NAC and 15dPGJ2

To study the role of OS in thyroiditis in vivo, we used the NOD mouse model of spontaneous thyroiditis. At 3 months, 100% of mice exhibited an inflammatory infiltrate in the thyroid together with variable stages of tissue destruction. Instead of large and regular follicles filled with colloid and lined by cuboidal epithelial cells as observed in normal thyroids, the thyroids of NOD mice consisted of small follicles with narrowed follicular lumina lined by thicker cell walls. Signs of cell destruction were observed in some areas: dead cells were observed in follicular lumina, some follicles were completely destroyed, and the interstitium was massively occupied by inflammatory cells (Figure 4A). These observations were confirmed by the morphometric analysis that showed a decrease in the relative volume of the colloid together with an increase in the relative volume of both epithelium and interstitium (Figure 4B).

4-HNE, a toxic product resulting from lipid peroxidation, was used as a OS marker. Although 4-HNE immunostaining was fairly detected in thyroids from NMRI mice, it strongly increased in NOD mice, with localization in thyrocytes, as well as in some interstitial cells (Figure 5).

In NAC-treated NOD thyroid glands, OS was drastically reduced, as confirmed by the strong decrease in 4-HNE expression, although remaining higher than in NMRI mice (Figure 5). Follicles were larger and more regular in size. Follicular lumina were filled with colloid and lined by flattened epithelial cells. Although the inflammatory reaction was still observed in the interstitium, it was markedly less pronounced compared with thyroids of untreated NOD mice (Figure 4A). Hence, the colloid relative volume was significantly increased, but remained lower than in NMRI mice. The relative volume of the epithelium decreased to values similar to NMRI mice. The relative volume of the interstitium also decreased, but remained higher than in NMRI mice (Figure 4B).

As with NAC, the follicles of NOD mice treated with 15dPGJ2 were larger and more regular in size. Their
In this study, we show for the first time that, in vivo, thyroiditis is associated with increased toxic OS and that, in vitro, Th1 pro-inflammatory cytokines induce intracellular ROS production in rat and human thyrocytes. This intracellular accumulation of ROS is quite different from that observed under physiological conditions. Hence, in a normal thyroid, H$_2$O$_2$ is produced by Duox in a limited area called thyroxisome that is located at the apical pole of the cell in microvilli. H$_2$O$_2$ is either consumed during the hormone synthesis process or detoxified by potent antioxidant systems, thereby being harmless for cells. The outcome for thyrocytes is quite different when ROS are heavily produced in cells incubated with Th1 cytokines. ROS may then become toxic, being detected both in the cytoplasm and in nuclei. In previous studies, we have shown that Th1 cytokines induce a down-regulation of Duox, and a reduction in the production of extracellular H$_2$O$_2$ in the FRTL5 cell line (unpublished data). It is therefore likely that Th1 cytokine-induced ROS in thyrocytes are not generated from Duox enzymes, but instead from a source that remains to be discovered.

Another question that remains to be sorted out concerns the exact nature of ROS produced when thyrocytes are incubated with Th1 cytokines. The DCFH-DA probe used in our study is not sensitive enough to distinguish H$_2$O$_2$ from other ROS, likely more toxic, including peroxynitrate and hydroxyl radicals. It is, however, clear that the nature of ROS will be determinant in terms of cell survival, some of them being more deleterious than others. Among reactive oxygen species, reactive nitrogen species, especially NO, are known to be induced by IL-1α/IFNγ in human thyrocytes and are partially responsible for the inhibitory effects of Th1 cytokines on thyrocytes. In addition, although NAC partially reduces the production of nitrite, ROS immunofluorescence detected by DCFH-DA fluorescence is completely abolished. Thus, ROS other than NO are likely produced in Th1 cytokine-incubated thyrocytes.

In vivo, the production of ROS was evaluated indirectly by measuring the induction of OS. In NOD mice, both toxic OS and inflammatory reaction affecting the whole thyroid gland were observed, as described in other models such as osteoarthritis, autoimmune encephalomyelitis, and lung diseases. Here, OS results from ROS produced by thyrocytes themselves facing Th1 cytokines (intrafollicular OS), but also from inflammatory cells colonizing the interstitium (extrafollicular OS). A way to explain the toxicity of OS in thyroiditis in NOD mice is the induction of intracellular adhesion molecule-1 by ROS. In vitro, the paradigm is not exactly the same as precursor of glutathione, NAC may decrease cell survival, some of them being more deleterious than others. Among reactive oxygen species, reactive nitrogen species, especially NO, are known to be induced by IL-1α/IFNγ in human thyrocytes and are partially responsible for the inhibitory effects of Th1 cytokines on thyrocytes. In addition, although NAC partially reduces the production of nitrite, ROS immunofluorescence detected by DCFH-DA fluorescence is completely abolished. Thus, ROS other than NO are likely produced in Th1 cytokine-incubated thyrocytes.

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as NAC reduces the inflammatory reaction, thereby protecting indirectly thyrocytes against the cell destruction induced by the extrathyroidal autoimmune reaction. This could be due to the ability of NAC to decrease the inflammation by inhibiting various cytokines (tumor necrosis factor-α, IFNγ, IL-8, and IL-6) and/or to restore the cellular redox-status and to modulate the activity of redox sensitive cell signaling pathways such as nuclear factorκB that regulates pro-inflammatory genes. The absence of NAC effects directly on thyrocytes in vivo compared with the in vitro experimental conditions could also be due to differences in terms of local concentration of NAC, probably reaching lower concentrations in thyroids when administered systemically in vivo than when added in vitro directly to cultured thyrocytes.

The effects of 15dPGJ2 were different from those of NAC, at least in vitro, as no reduction of ROS production was observed. By contrast, in vivo, as for NAC, OS was decreased, thereby allowing the recovery of a near normal thyroid morphology. It is not the first time that such protective effects are observed as they have been already reported in a nonautoimmune model of iodine-induced thyroid involution. This is likely due to the ability of 15dPGJ2 to inhibit the expression of a variety of pro-inflammatory factors including cyclooxygenase-2, NOS2, and several cytokines (IL-6, IL-12, and tumor necrosis factor-α). 15dPGJ2 may also modulate or inhibit the nuclear factorκB system and activate the mitogen activated protein kinase pathway through PPARγ-dependent and independent mechanisms. Because 15dPGJ2 has no direct effect on thyroid cells in vitro, but is able to reduce inflammation in vivo, we suggest that 15dPGJ2 may favorably influence OS in the thyroid gland by acting directly on infiltrating inflammatory cells.

In this present study we report for the first time a differential regulation of Duox1 and Duox2 mRNA levels in PCCL3 rat thyroid cells. The respective roles of these two proteins encoded by two different genes remain unclear. Rigutto et al. have reported in PCCL3 cells that Duox1 alone is able to generate H2O2 and that the amount of Duox1 present is sufficient to generate enough H2O2. By contrast, other arguments suggest that in humans, thyroid Duox2 is the main H2O2 generator. A study in the respiratory tract epithelium demonstrated that IL-4, a Th2 cytokine, increases Duox1 mRNA expression and that IFNγ, a Th1 cytokine, markedly induces Duox2 mRNA expression. In our study, the results were quite different. Here, Th1 cytokines inhibited both Duox genes. On the other hand, the antioxidant NAC negatively influenced only Duox2 mRNA. Thus, Duox2 expression seems to require a minimal oxidative load to be adequately expressed, whereas Duox1 expression does not depend on the thyroid cell ROS content. Obviously, further investigations are required to clarify the exact underlying mechanisms.

In conclusion, our results confirm that the maintenance of a minimal oxidative load, as in control cells, is essential to safeguard thyroid cell function. In addition, ROS are not the sole intracellular mediators of Th1 cytokine-induced inhibitory effects of thyroid cell function in vitro. In vivo, both the antioxidant NAC and the anti-inflammatory prostaglandin 15dPGJ2 protect the thyroid against toxic...
OS mainly by acting on infiltrating inflammatory cells, thereby contributing to the reduction of the extracellular toxic OS. The intracellular OS remains under the control of efficient intracellular antioxidant systems, thereby allowing the thyroid cell function and morphology to recover.

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Effects of NAC and 15dPGJ2 on Thyroiditis 227

AJP July 2010, Vol. 177, No. 1


