

Oxidative Stress in the Thyroid Gland: From Harmlessness to Hazard Depending on the Iodine Content

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In basal conditions, thyroid epithelial cells produce moderate amounts of reactive oxygen species (ROS) that are physiologically required for thyroid hormone synthesis. They are not necessarily toxic because they are continuously detoxified either in the process of hormone synthesis or by endogenous antioxidant systems. Using a rat model of goiter formation and iodine-induced involution, we found that compared with control thyroids, the oxidative stress, assessed by the detection of 4-hydroxynonenal, was strongly enhanced both in hyperplastic and involuting glands. The level of antioxidant defenses (glutathione peroxidases and peroxiredoxins) was also up-regulated in both groups, although somewhat less in the latter. Of note, increased oxidative stress came along with an inflammatory reaction, but only in involuting glands, suggesting that although antioxidant systems can adequately buffer a heavy load of ROS in goiter, it is not necessarily the case in

involuting glands. The effects of 15-deoxy- $\Delta^{12,14}$ -prostaglandin J2 (15dPGJ2), an endogenous ligand of peroxisome proliferated-activated receptor γ (PPAR γ) with antiinflammatory properties, were then investigated in involuting glands. This drug strongly reduced both 4-hydroxynonenal staining and the inflammatory reaction, indicating that it can block iodine-induced cytotoxicity. When experiments were carried out with the PPAR γ antagonist, bisphenol A diglycidyl ether, 15dPGJ2-induced effects remained unchanged, suggesting that these effects were not mediated by PPAR γ . In conclusion, thyroid epithelial cells are well adapted to endogenously produced ROS in basal and goitrous conditions. In iodine-induced goiter involution, the increased oxidative stress is accompanied by inflammation that can be blocked by 15dPGJ2 through PPAR γ -independent protective effects. (*Endocrinology* 149: 424–433, 2008)

THYROID EPITHELIAL CELLS are constantly exposed to reactive oxygen species (ROS). ROS are physiologically necessary and even intimately associated with thyroid hormone synthesis (1). Nevertheless, when they are too much produced, they may become toxic. Hence, they have been associated with large cellular destruction and inflammation in various models of iodine-induced thyroid involution (2–10). Besides obvious short-term functional consequences such as transient hypothyroidism, the acute inflammatory reaction, considered as an early step of autoimmune reaction (11), may sometimes evolve toward chronic thyroiditis, as observed in autoimmune predisposed animals, such as nonobese diabetic mice (NOD) (6, 7). This link between iodine and autoimmune consequences has been recently emphasized in a survey in China that showed that excessive iodine intake is associated with a higher prevalence of Hashimoto's disease and hypothyroidism (12). It is therefore crucial for thyrocytes to be efficiently protected against exces-

sive ROS production; otherwise, it would not be possible for these cells to be kept alive and, obviously, to function properly. Thus, to face the oxidative challenge and survive, thyrocytes have developed protective systems that limit the toxicity of endogenously and naturally produced ROS. They include antioxidant enzymes such as superoxide dismutases, catalase, glutathione peroxidases, and peroxiredoxins (10, 13–16).

One could propose that iodine-induced toxicity in iodine-deficient thyrocytes occurs when endogenous antioxidant systems are overwhelmed by ROS produced in excess, thereby leading to cellular destruction that in turn triggers inflammation. Although the link between oxidative stress and inflammation has been clearly established in iodine-induced involuting glands, this seems not to be the case in goitrous glands. It has not even been proven yet that the oxidative stress is indeed increased in hyperplastic glands, although high is the likelihood (17). In addition, there are no data on how antioxidant systems are exactly regulated in the thyroid during goiter formation and involution. To answer these questions, we looked at the oxidative status in thyrocytes and the expression and/or function of antioxidant systems in a rat model of goiter formation and involution. We also investigated whether iodine-induced cytotoxicity can be impeded by pharmacological means known to induce anti-inflammatory effects. For this purpose, experiments were carried out with 15-deoxy- $\Delta^{12,14}$ -prostaglandin J2 (15dPGJ2),

First Published Online September 20, 2007

Abbreviations: AEC, 3-Amino-9-ethylcarbazole; BADGE, bisphenol A diglycidyl ether; DAB, 3-3'-diaminobenzidine tetrahydrochloride; 15dPGJ2, 15-deoxy- $\Delta^{12,14}$ -prostaglandin J2; GPx, glutathione peroxidase; HID, high-iodine diet; 4-HNE, 4-hydroxynonenal; PPAR γ , peroxisome proliferated-activated receptor γ ; PRDX5, peroxiredoxin 5; ROS, reactive oxygen species; RT, room temperature.

Endocrinology is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

a member of the cyclopentenone family of prostaglandins that exerts antineoplastic, antiviral, and antiinflammatory properties (18). Because 15dPGJ2-induced effects are at least partly mediated by peroxisome proliferated-activated receptor γ (PPAR γ) activation (18, 19), we also investigated the expression of PPAR γ in the thyroid gland and its possible involvement in 15dPGJ2-induced protective effects.

Materials and Methods

Animals and treatments (Fig. 1)

Hyperplastic goiter was induced in 6-wk-old female Wistar rats (UCL, Brussels, Belgium) by feeding a low-iodine diet (<20 μg iodine/kg; Animolabo, Brussels Belgium) supplemented with 0.25% 6-*n*-propyl-2-thiouracil (Sigma Chemical Co., St. Louis, MO) for 19 d and low-iodine diet alone for an additional 2 d. Involution of hyperplasia was obtained by feeding a normal iodine diet associated with daily ip injections of 100 μg iodide [high-iodine diet (HID)] or of 20 μg T₄ (Sigma) for 3 d. The time course and the design of this experimental model are based on experience acquired over the last 25 yr (5–10, 20–22). To validate the experimental model and verify its reproducibility compared with previously published papers, the weight of thyroids as well as TSH plasma levels were recorded (Fig. 2, A and B). The prostaglandin-treated involution groups received 25 $\mu\text{g}/\text{kg}$ 15dPGJ2 (Sigma) for 4 d before being killed (ip in saline solution). The bisphenol A diglycidyl ether (BADGE) group received 5 mg/kg BADGE (ip, saline solution with 2% dimethylsulfoxide; Fluka, Buch, Switzerland) 30 min before each ip injection of 15dPGJ2. The different doses and the route of drug administration were chosen based on papers previously reported (23, 24). Control groups received normal diet and tap water. Animals were killed under thiopental anesthesia. Blood was collected, and plasma was stored at -20°C until use. Rats were maintained in accordance with the principles of laboratory animal welfare. Each experimental setting was repeated twice.

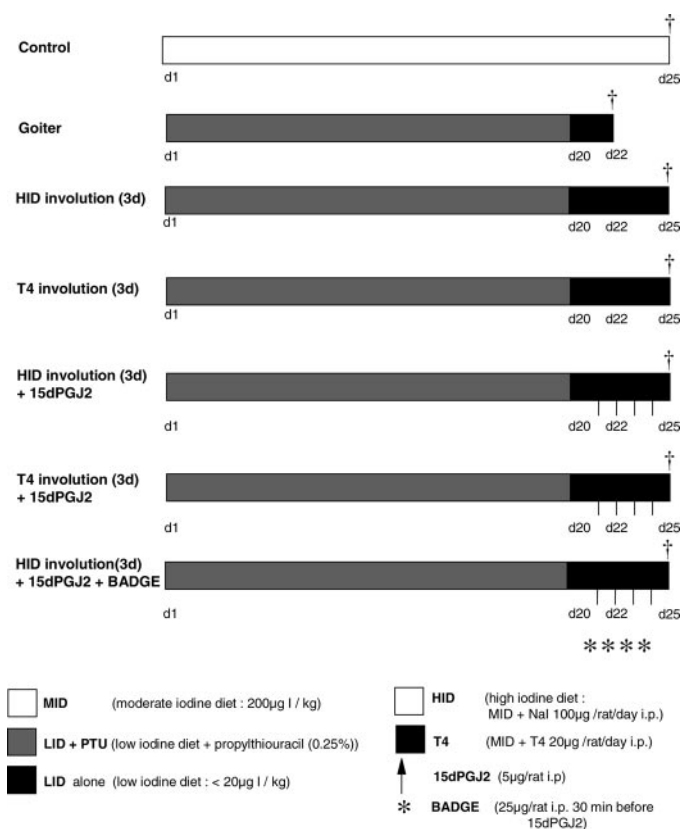


FIG. 1. Design of the experimental procedure.

Morphology and morphometric analyses

Five animals per group were used for the morphological study. Thyroids were dissected and weighed. One lobe was immersed for 2 h in a 2.5% glutaraldehyde solution. After fixation, thyroid fragments were rinsed in cacodylate buffer, postfixed for 90 min in 1% osmium tetroxide, dehydrated in alcohol solutions, and embedded in LX112 resin (Ladd Research Industries, Burlington, VT). Semi-thin sections (0.5 μm) were cut and stained with toluidine blue. The second lobe was fixed in formaldehyde and embedded in paraffin. Sections (5 μm) were stained with hematoxylin-eosin or used for immunohistochemistry.

TSH level assay

Plasma TSH levels were measured in duplicate by RIA using a specific kit for detection of rat TSH (Amersham Biosciences, Little Chalfont, UK). All values are expressed as means \pm SEM. The statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test (GraphPad Instat, San Diego, CA).

Immunohistochemistry

CD68, PPAR γ 2, 4-hydroxynonenal (4-HNE), and peroxiredoxin 5 (PRDX5) immunostaining was performed on 5- μm -thick paraffin sections laid on Super Frost glass slides (Menzel-Glaser, Braunschweig, Germany). Sections were dewaxed and rehydrated, and endogenous peroxidase was quenched with 1% H₂O₂ for 30 min. Except for PRDX5 detection, tissue sections were pretreated in a microwave in citrate buffer (pH 6.6) for one cycle of 3 min at 750 W, followed by four cycles of 3.5 min, each at 350 W. All sections were washed with PBS supplemented with 1 or 5% BSA (PBS-BSA) and thereafter incubated in PBS-BSA containing 2 or 5% normal goat serum at room temperature (RT). Sections were incubated with the first antibody (CD68, PPAR γ 2, 4-HNE, or PRDX5) at RT (see Table 1). The binding of antibodies was detected using a second antibody conjugated to a peroxidase-labeled polymer (EnVision detection; DakoCytomation, Carpinteria, CA) or a biotinylated second antibody for 30 min followed by an avidin-biotin peroxidase complex for 30 min (Vectastain ABC kit; Vector Laboratories, Burlingame, CA). The peroxidase activity was revealed with 3-amino-9-ethylcarbazole (AEC) substrate (DakoCytomation) or 3-3'-diaminobenzidine tetrahydrochloride (DAB; Sigma). Sections were counterstained with Mayer's hematoxylin, rinsed, and mounted in Faramount Aqueous mounting medium (DakoCytomation). To verify the binding specificity, some sections were incubated with the second antibody alone or with a preimmune serum. Positive cells for CD68 (monocytes and macrophages) were counted under a light microscope on the whole section, and the number was reported per square millimeter of section. All values are expressed as means \pm SEM. The statistical analysis was performed using ANOVA followed by Tukey-Kramer multiple comparisons test (GraphPad Instat).

Western blot analysis

Five animals from each group were used for protein detection by Western blotting. After dissection, thyroid lobes were quickly frozen in liquid nitrogen and stored at -80°C . Frozen lobes were homogenized in PBS (pH 7.4) supplemented with 0.05% Triton X-100 and protease inhibitor cocktail (Sigma). The protein concentration was determined using BCA protein assay kit (Pierce, Rockford, IL). Proteins (30 μg /lane) were denatured by heating homogenates at 95°C for 5 min in the loading buffer [50 mM Tris HCl (pH 6.8), 100 mM dithiothreitol, 2% SDS, 0.1% bromophenol blue, and 10% glycerol]. Proteins were separated on 15% SDS-PAGE and transferred to a nitrocellulose membrane (Hybond ECL; Amersham Biosciences, Roosendaal, The Netherlands). Membranes were blocked with 5% nonfat dry milk in PBS containing 0.1% Tween 20 for 1 h at RT and then incubated overnight at 4°C either with a polyclonal rabbit anti-PRDX5 antibody (1:10,000) or with a polyclonal rabbit anti- β -actin (1:2000) on the same membrane. Membranes were washed, incubated for 1 h at RT with peroxidase-labeled secondary antibody (1:20,000; Amersham), and visualized with enhanced chemiluminescence detection (SuperSignal WestPico; Pierce) on CL-Xposure films (Pierce). Western blots were scanned and quantified using the NIH Scion Image Analysis Software (National Institutes of

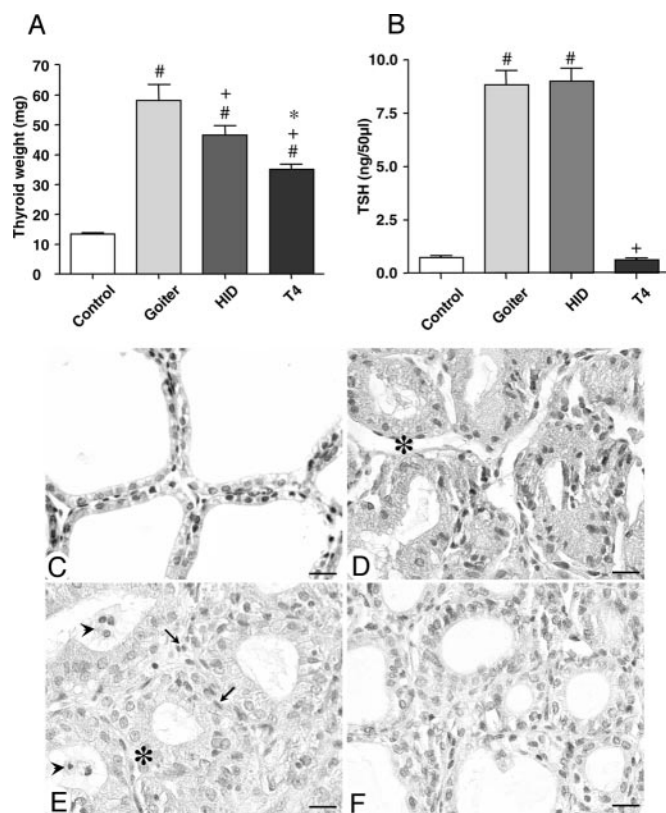


FIG. 2. Thyroid weight (A), TSH plasma levels (B), and thyroid morphology (C–F) in the experimental model of goiter formation and of iodine and T_4 -induced involution. In goitrous thyroids (D), blood vessels were markedly enlarged (*star*), epithelial cells were hypertrophic, and follicular lumina were narrow. In HID-involving thyroids (E), blood vessels were constricted (*star*), follicular lumina were widened, and some necrotic cells were present in lumina (*arrowheads*). The interstitium was infiltrated by inflammatory cells (*arrows*). In T_4 -involving thyroids (F), follicles were regular, and their lumina were filled with colloid. There was neither cellular debris nor necrotic cells in the follicular lumina. Scale bars, 20 μ m. #, $P < 0.05$ vs. control; +, $P < 0.05$ vs. goiter; *, $P < 0.05$ vs. HID.

Health, Bethesda, MD). All values are expressed as means \pm SEM. The statistical analysis was performed using unpaired t test (GraphPad Instat).

Glutathione peroxidase activity

Five animals from control, goiter, HID-involution, and T_4 -involvement groups were used for the determination of glutathione peroxidase (GPx) activity. Thyroids were homogenized in imidazole-sucrose buffer (0.2 M, pH 7) and centrifuged at 1600 rpm. Reduced glutathione (10 mM; Sigma), potassium phosphate buffer (0.1 M, pH 7), EDTA (1.8 mM), NaN_3 (1.8

mM), NADPH (2.5 mM; Roche Diagnostics, Mannheim, Germany), oxidized glutathione (GSSG-reductase; Roche Diagnostics) were added to supernatants and incubated for 10 min at 37 $^{\circ}$ C. GPx activity was determined by following the decrease in absorbance at 340 nm induced by the addition of *tert*-butylhydroperoxide. The activity of glutathione peroxidase was measured as nanomoles of NADPH oxidized per min and a blank was subtracted from each value. Results are expressed as milliunit of activity per micrograms of DNA. The statistical analysis was performed using ANOVA followed by Tukey-Kramer multiple comparisons test (GraphPad Instat).

Results

The administration of iodine to goitrous rats induces glandular oxidative stress and inflammation

After 21 d of goitrogenic treatment, the thyroid weight was significantly increased compared with control animals (Fig. 2A). The 4-fold increase in plasma TSH levels confirmed the functional hypothyroid status of the rats (Fig. 2B). Compared with control thyroids (Fig. 2C), blood vessels were enlarged and narrow follicular lumina were lined by hypertrophic epithelial cells (Fig. 2D). The weight of iodine-induced involuting glands was significantly reduced compared with goiter (Fig. 2A). TSH plasma levels remained elevated, suggesting that hormone synthesis did not initiate because of a Wolff-Chaikoff effect (Fig. 2B). The follicular lumina were slightly enlarged and contained more colloid. Blood capillaries were constricted, and scattered dead or fragmented cells were observed in follicular lumina. The interstitium was infiltrated with inflammatory cells (Fig. 2E). The weight of T_4 -induced involuting glands was lower than iodine-treated goitrous rats (Fig. 2A). TSH plasma levels were similar to control values, indicating the absence of a Wolff-Chaikoff effect (Fig. 2B). The follicles were larger and regular in size. In addition, lumina were filled with colloid and lined by cuboidal epithelial cells. An inflammatory reaction was also observed in the interstitium but strikingly less pronounced compared with iodine-induced involution (Fig. 2F). All these morphological and functional features were in line with our previously reported papers (1, 5, 8–10, 20–22).

4-HNE, a toxic product resulting from lipid peroxidation, was used as a marker for the oxidative stress (25). A very weak 4-HNE immunostaining was detected in control thyroids (Fig. 3A). It was stronger in goiters and in iodine-induced involution (Fig. 3, B and C). In goiters, its pattern of expression was granular (Fig. 3B). In iodine-induced involution, the staining was more diffuse (Fig. 3C). In T_4 -induced involution, it was as weak as in control animals (Fig. 3D).

TABLE 1. Experimental conditions for immunohistochemistry

Antibody	First antibody	Second antibody	Revelation substrate
PRDX5 (polyclonal rabbit; B.K., UCL)	Rabbit polyclonal, dilution 1/200, incubation time 1 h	EnVision rabbit (DakoCytomation)	AEC (DakoCytomation)
PPAR γ 2 (Sigma)	Rabbit polyclonal, dilution 1/100 in PBS-BSA 10%, incubation time overnight	EnVision rabbit (DakoCytomation)	AEC (DakoCytomation)
CD68 (Serotec, Oxford, UK)	Mouse monoclonal biotinylated, dilution 1/50, incubation time overnight		Avidin-biotin peroxidase complex (Vector) and DAB
4-HNE (Calbiochem, Darmstadt, Germany)	Rabbit polyclonal, dilution 1/800, incubation time overnight	EnVision rabbit (DakoCytomation)	AEC (DakoCytomation)

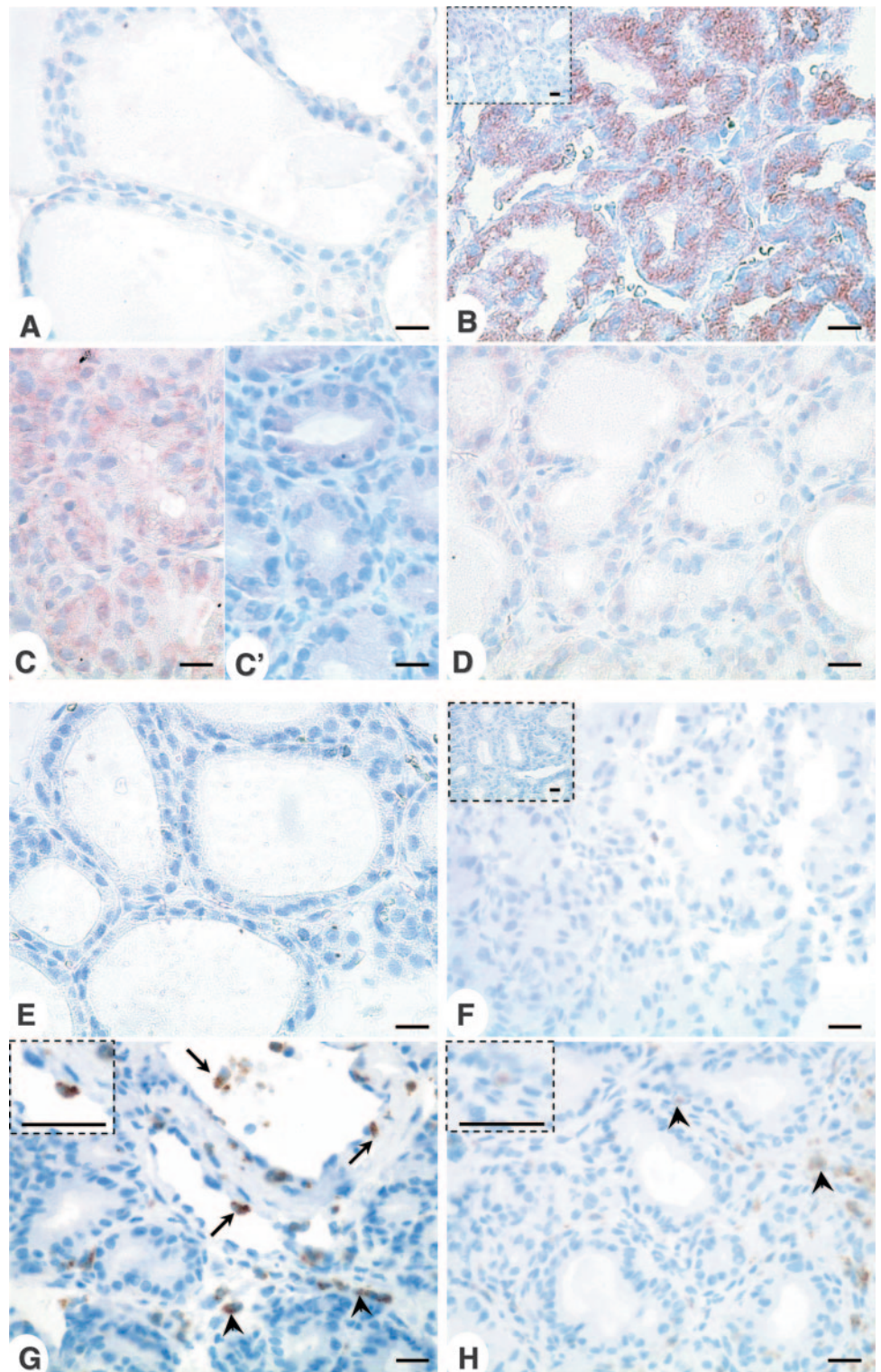


FIG. 3. Immunohistochemical detection of 4-HNE and CD68. 4-HNE immunodetection in control (A), goiter (B), HID-induced involution (C), HID-induced involution with 15dPGJ2 (C'), and T₄-induced involution (D). 4-HNE was barely present in the control thyroid (A). It was highly expressed in thyrocytes from goitrous rats (B); its expression remained high after HID administration (C) but was low when giving T₄ (D). When HID-involution rats were treated with 15dPGJ2, 4-HNE staining strongly decreased (C'). Negative controls were performed by omitting the primary antibody (*inset* in B; *scale bar*, 50 μm). CD68 immunodetection in control (E), goiter (F), HID-induced involution (G) and T₄-induced involution (H). CD68 expression was increased in HID-induced involution (G) and T₄-induced involution (H) compared with control (E) and goiter (F), where it was absent. In HID-induced involution, positive cells were mostly localized in the interstitium (*arrowheads*) as well as in capillary lumina and arterial walls (*arrows* and *insets*; *scale bar*, 20 μm). Negative controls were performed by omitting the primary antibody (*inset* in F; *scale bar*, 50 μm). *Scale bars*, 20 μm.

An antibody directed against CD68 was used to identify infiltrating interstitial cells as monocytes and macrophages (26). There were very few of these cells in the thyroid of normal and goitrous rats (Figs. 3, E and F, and Fig. 4). By contrast, many positive cells were found in the thyroid of iodine-treated goitrous rats (Figs. 3G and 4). They were mostly localized in the interstitium, but also in

capillaries, vascular lumina, and arterial walls. CD68-positive cells were also detected in T₄-treated goitrous rats (Fig. 3H). Their number was slightly lower than in iodine-induced involution, but the difference was not significant (Fig. 4). In contrast, CD68 staining was quite faint, indicating that the expression of this transmembrane glycoprotein was weak (Fig. 3H).

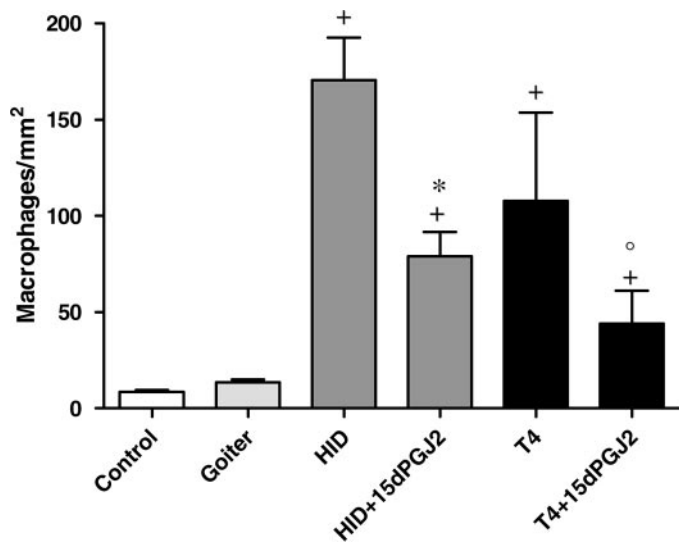


FIG. 4. Number of macrophages. Positive CD68 cells were counted and expressed as number of positive cells per square millimeter (mean \pm SEM, $n = 9$). +, $P < 0.05$ vs. goiter; *, $P < 0.05$ vs. HID; °, $P < 0.05$ vs. T_4 .

The expression of antioxidant enzymes is strongly enhanced in goitrous and involuting thyroid glands

The great induction of the oxidative stress observed in goitrous and iodine-treated animals prompted us to look at the functional and morphological status of antioxidant defenses. For this purpose, we analyzed the activity or the level of expression of two important enzymatic antioxidant systems, namely GPxs and PRDX5, that were previously shown to be highly regulated in the thyroid (13, 14).

GPx activity was significantly increased in the thyroid of goitrous animals and slightly but significantly reduced after iodine and T_4 treatments. In T_4 -treated rats, GPx activity was further decreased compared with iodine-treated goitrous rats (Fig. 5).

PRDX5 expression analyzed by Western blot was low in control animals but significantly increased in hyperplastic and involuting glands (Fig. 6). There was no statistical difference between the last three experimental groups. The immunohistochemical analysis revealed differences in the subcellular localization of the protein. It was weakly expressed in the cytoplasm of control thyrocytes (Fig. 7A). In accordance with the Western blot analysis, the signal was stronger in goitrous thyroids (Fig. 7B). Numerous nuclei were labeled. In iodine-treated animals, the staining was less pronounced and more diffuse throughout the cytoplasm as well as in numerous nuclei and inflammatory cells (Fig. 7C). In T_4 -treated animals, PRDX5 expression was slightly greater, and its cellular localization was predominantly cytosolic and sometimes nuclear (Fig. 7D).

15dPGJ2 reduces both oxidative stress and inflammation in involuting thyroid glands but does not modify PRDX5 expression

In both involution groups treated with 15dPGJ2, follicular lumina were enlarged and the inflammatory reaction was less developed compared with groups without prostaglan-

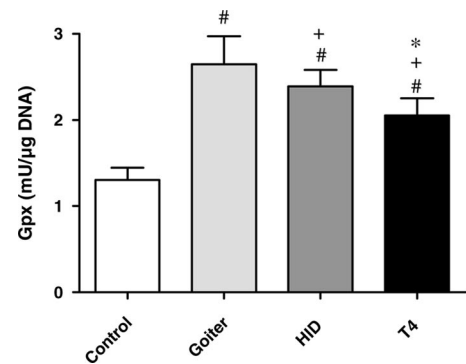


FIG. 5. GPx activity (mU/μg DNA). The results are expressed as mean \pm SEM; $n = 5$. #, $P < 0.05$ vs. control; +, $P < 0.05$ vs. goiter; *, $P < 0.05$ vs. HID.

din (data not shown). 4-HNE expression decreased in 15dPGJ2-treated animals compared with involuting animals without prostaglandin (Fig. 3C'). The number of CD68-positive cells was significantly reduced after 15dPGJ2 treatment (Fig. 4, $P < 0.05$).

These results suggest that 15dPGJ2 reduces both the oxidative stress and the inflammatory reaction in the thyroid of iodine- and T_4 -treated goitrous rats. In contrast, we did not observe 15dPGJ2-induced alteration in PRDX5 expression (Fig. 6).

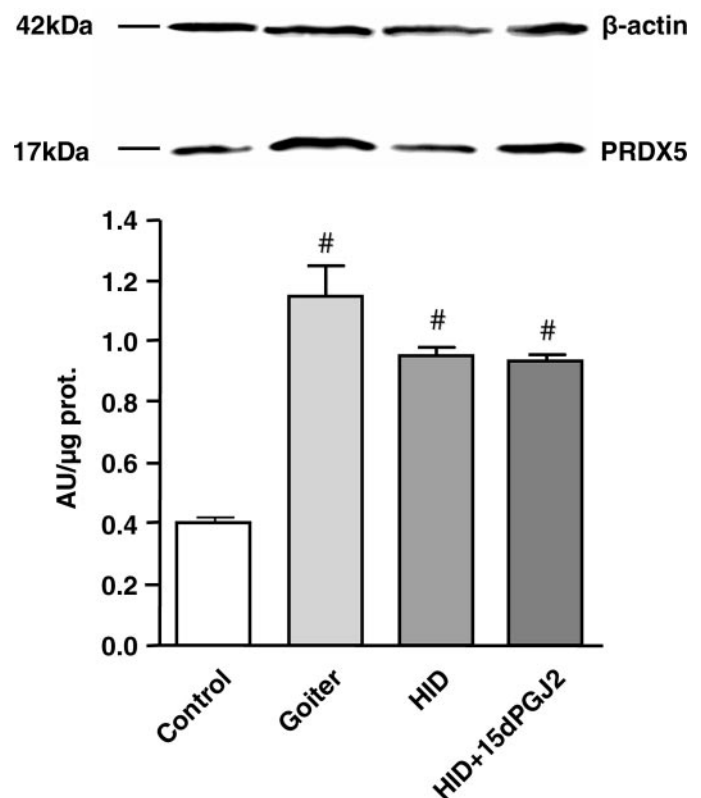


FIG. 6. Western blotting of PRDX5. PRDX5 protein expression showed no significant difference between groups, except for control thyroids where it was the lowest. Densitometric values adjusted to β -actin signal are expressed in arbitrary units (AU) per microgram protein, expressed as mean \pm SEM; $n = 5$. #, $P < 0.05$ vs. control.

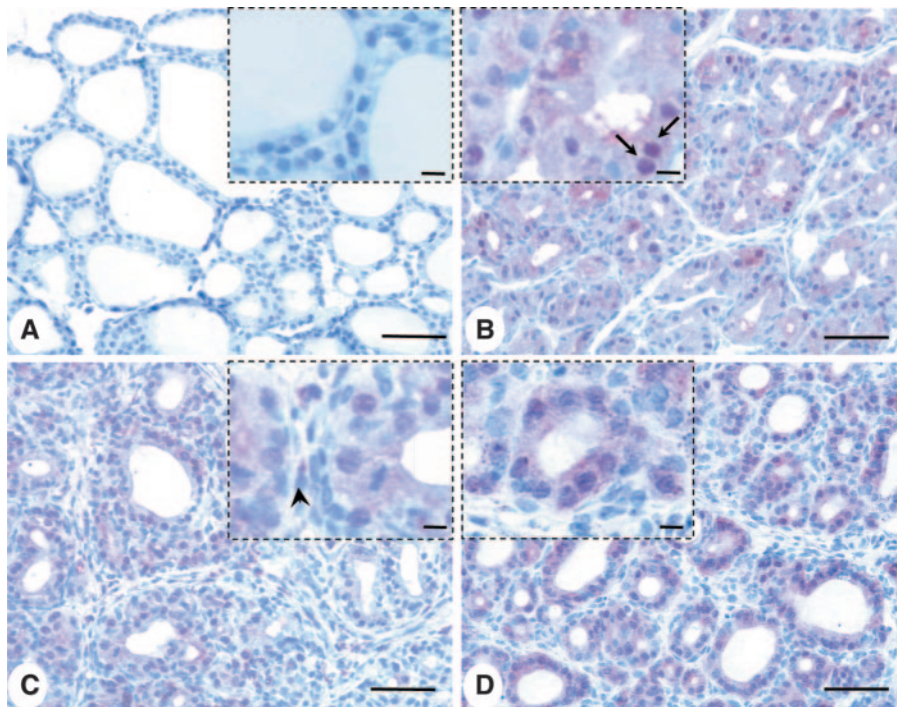


FIG. 7. Immunohistochemical detection of PRDX5. PRDX5 expression in control (A), goiter (B), HID-involution (C), and T_4 -involution (D). PRDX5 expression was increased in thyrocytes from goitrous (B) and involuting (C and D) thyroids compared with control (A). In goiter (B) and HID-involution (C), PRDX5 was mainly located in nuclei (arrows in inset) and sometimes in the cytosol. In HID-involution, some inflammatory cells were labeled (arrowhead in inset). In T_4 -involution, the localization of PRDX5 was predominantly cytosolic and sometimes nuclear (D). Insets are shown with higher magnification to clearly identify the subcellular compartment (nucleus or cytoplasm) that is immunostained (scale bars in insets, 10 μ m). Scale bars, 50 μ m.

15dPGJ2 effects in the thyroid gland are PPAR γ independent

Many cellular events triggered by 15dPGJ2 are due to PPAR γ activation. To verify whether 15dPGJ2 effects were mediated by PPAR γ , we first looked at PPAR γ expression by immunohistochemistry (Fig. 8). A strong signal was observed in the cytoplasm of thyrocytes from control (Fig. 8A), goitrous (Fig. 8B), and involution groups (Fig. 8, C and D) but never in nuclei, suggesting that, in our model, PPAR γ is expressed but not functional at least as a transcription factor. There was no difference in terms of intensity and subcellular localization between the different groups, except for inflammatory cells that were stained in HID-induced involution (Fig. 8C). The cytoplasmic localization of PPAR γ led us to suggest that 15dPGJ2 effects are independent of PPAR γ activation. To verify this hypothesis, an experiment was carried out where 15dPGJ2-treated animals received BADGE, a PPAR γ antagonist. 4-HNE and CD68 immunostaining, which were both influenced by 15dPGJ2 remained unchanged, suggesting that prostaglandin-induced cellular effects are indeed PPAR independent (data not shown).

Discussion

Our laboratory has been committed for many years to elucidate mechanisms underlying acute iodine-induced cytotoxicity in thyroid follicular cells as well as delayed inflammatory processes ending up with chronic autoimmune thyroiditis (1, 5–10, 27–29). Evidence accumulated over the

last decades clearly indicates that the administration of high doses of iodide to goiters provokes thyroid cell necrosis/apoptosis and glandular inflammation (5, 7–10). In the present study, we attempted to figure out what underlies the early inflammatory reaction by looking at the oxidative stress, the antioxidant defenses, and the eventual involvement of PPAR γ (Fig. 9).

We think it makes sense to investigate the involvement of the oxidative stress in this paradigm for the following reason. The synthesis of thyroid hormones crucially depends on H_2O_2 , which works as a donor of oxidative equivalents for thyroperoxidase (17). Because of its great toxicity, H_2O_2 synthesis must always remain in adequation with the hormonal synthesis and strictly contained at the apical pole of the cell. Thyrocytes possess various enzymatic systems, such as GPx, catalase, superoxide dismutases, and peroxiredoxins that contribute to limit cellular injuries when H_2O_2 or other ROS are produced in excess (10, 13–16).

We used 4-HNE as a marker of the oxidative stress (25). We first observed that its level of expression was elevated in goitrous animals. According to previous studies on H_2O_2 production in the thyroid (17, 30–32), it is therefore possible that increased oxidative stress observed in goitrous thyrocytes is due in part to the accumulation of H_2O_2 or, to be more correct, to the fall of consumption in the process of hormone synthesis. Hence, the risk for this toxic substance to accumulate into the cell is high when iodide is lacking and/or when thyroperoxidase is blocked. A previous paper by Mutaku *et al.* (10) showed that the number of necrotic and ap-

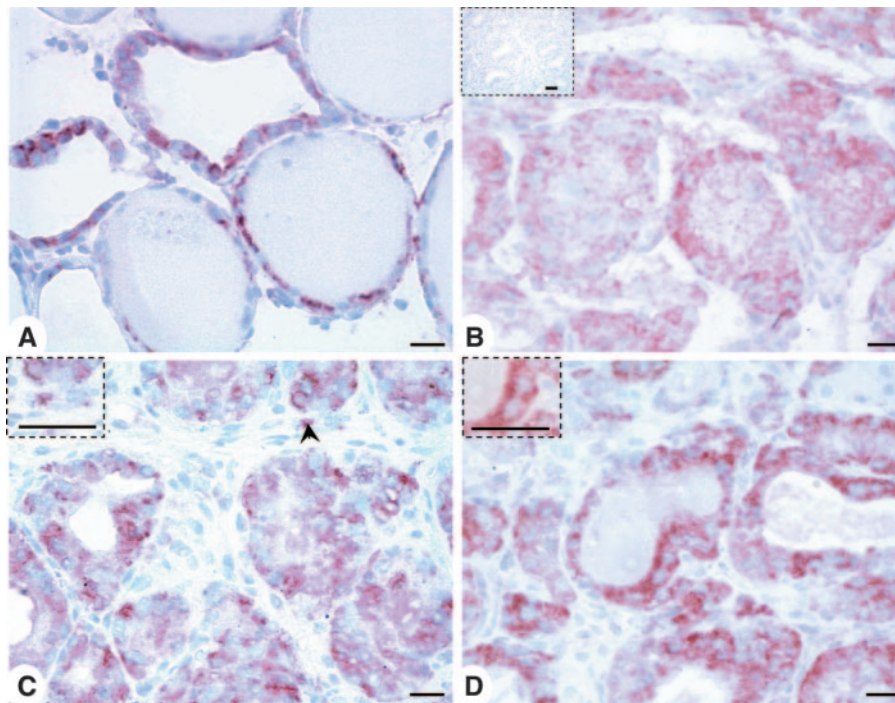


FIG. 8. Immunohistochemical detection of PPAR γ . PPAR γ was present in control (A), goitrous (B) and involuting (C and D) glands. It was expressed in the cytoplasm of follicular cells but never in nuclei. In HID-involution (C), the cytoplasm of some inflammatory cells was labeled (arrowhead and inset; scale bar, 20 μ m). An inset is shown in D at higher magnification to illustrate that nuclei were negative for PPAR γ staining (scale bar, 20 μ m). Negative controls were performed by omitting the primary antibody (inset in B; scale bar, 50 μ m). Scale bars, 20 μ m.

optotic cells somewhat increases in goitrous glands. Noteworthy, despite augmented oxidative stress, cells were barely injured in hyperplastic glands in contrast with iodine-induced involution. Our results suggest that this is likely due to the activation of at least two potent enzymatic antioxidant systems, namely GPx and PRDX5. GPx activity was indeed increased in goitrous glands, suggesting that it may protect thyrocytes when H₂O₂ levels go up (13). PRDX5 expression was also clearly higher in goitrous glands compared with control animals. PRDX5 is a thioredoxin peroxidase that reduces H₂O₂ into H₂O and regulates intracellular levels of peroxides and peroxynitrites (33–36). PRDX5 was expressed in the cytosol of control thyrocytes as previously described in the human (14), suggesting that it could play a role in regulating ROS levels in unstimulated thyrocytes.

In contrast with goitrous glands, iodide-treated hyperplastic thyrocytes were severely injured as shown by the increased number of necrotic/apoptotic cells, the presence of cellular debris, and the massive inflammatory reaction. The acuteness of cellular damage was specifically due to iodine-induced toxicity and not to the involution process *per se*, because the scene was totally different in T₄-induced involution. In this case, involution came along with less acute inflammation, less tissue injury, and less oxidative stress. Of note, T₄-induced involution is known to mainly provoke cell apoptosis but not necrosis, as previously reported (5, 8). In contrast, the oxidative stress was high and accompanied by a strong inflammatory reaction in the HID group. This was not the case in goitrous animals, which means that elevated oxidative stress in the thyroid gland is not systematically associated with inflammation. So, why is iodine toxic when

it is administered in excess to goitrous animals? The answer to this question can be partly found in the paper of Corvilain *et al.* (37), who showed that iodide has a stimulatory effect on H₂O₂ generation, thereby on the level of the oxidative stress. In addition, according to previous studies reporting membrane rupture, formation of endoplasmic reticulum vesicles, mitochondrial swelling, and accumulation of lipofuscin pigment, iodine-induced cytotoxicity may be due to more aggressive free radical attack compared with goiter (2, 5). Our data on altered antioxidant defenses bring an additional clue that helps in sorting out this question. We observed that GPx activity and PRDX5 expression were slightly but significantly lower (at least for GPx activity) in the HID group, suggesting that both systems may fail to detoxify a heavier load of ROS. Of note, although PRDX5 expression was high in the HID group, its subcellular localization was not exactly the same as in goitrous glands. In goitrous thyrocytes, the signal was mainly nuclear. In HID-induced involution, it was expressed in nuclei but also in the cytoplasm, suggesting that the system acts where it is the most needed. Thus, the nuclear localization suggests that the enzyme may exert antioxidant actions to protect the genome of the cell as suggested by recent papers (36, 38, 39). Hence, changes in subcellular location of PRDX5 could indicate that it acts in different cellular compartments as a function of the localization, the nature, and the intensity of the oxidative attack. Thus, whereas thyroid antioxidant systems are likely adapted to detoxify H₂O₂ produced in excess in TSH-stimulated thyrocytes, they may be overwhelmed by a heavier and more broadly toxic free radical attack (lipid peroxides, singlet oxygen, superoxide anions, hydroxyl radicals, peroxynitrites, *etc.*). This hypoth-

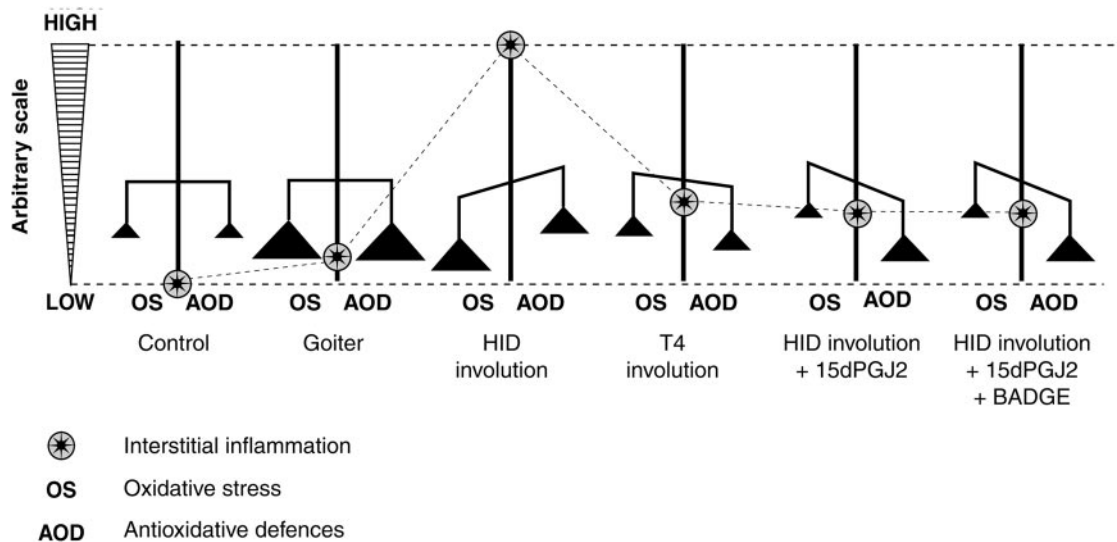


FIG. 9. Oxidative stress and inflammation during goiter formation and involution, a schematic representation. In control conditions, the level of oxidative stress is low as well as the level of antioxidant defenses. There is no inflammation. In goitrous glands, the level of oxidative stress is high but counterbalanced by antioxidant defenses. Inflammation is slightly higher. In HID-induced involution, the oxidative stress strongly increases and is no longer enough counterbalanced by antioxidant defenses. The increase in the oxidative stress could be due to increased quantity and toxicity of ROS. All these events lead to cell necrosis, thereby to stronger inflammation. In T_4 -induced involution, there is a rapid decrease in the level of oxidative stress that is largely blunted by antioxidant defenses. Nevertheless, T_4 involution could induce apoptosis. A moderate inflammatory reaction could thus occur to clear cellular debris. In HID-treated animals receiving 15dPGJ2, there is strong reduction in the level of oxidative stress that is now fully controlled by antioxidant defenses. The ensuing inflammatory reaction is therefore reduced. BADGE does not induce changes, indicating that 15dPGJ2-induced protective effects are not mediated by PPAR γ .

esis is in accordance with previous papers that showed that tissue damage is markedly enhanced when iodide is supplied to goitrous thyrocytes with weakened antioxidant systems. Thus, Contempre *et al.* (40–42) showed that combined iodine and selenium deficiencies make thyrocytes more sensitive to iodine and/or thiocyanate overloads because of altered antioxidant defenses. Data from our laboratory also indicated that iodine-induced necrosis is stronger in vitamin E-deficient goitrous thyrocytes (10).

The acute inflammation occurring after iodide administration can be considered as the early step of an immunological reaction (11) that sometimes evolves toward chronic lymphocytic thyroiditis, especially in genetically susceptible individuals (6, 7). We therefore investigated whether specific pharmacological means may control iodine-induced toxicity. We looked at 15dPGJ2 because this substance is known to control inflammation in various models (23, 43–45). Both 15dPGJ2-induced antioxidant and antiinflammatory actions were indeed observed. It has been lately suggested that 15dPGJ2 acts as a natural ligand of PPAR γ . Besides important metabolic effects on glucose and lipid metabolism, PPAR γ ligands also exert potent antiinflammatory effects by limiting the production of inflammatory mediators (19, 43, 46, 47). To determine the eventual involvement of PPAR γ , we first looked at its expression in the thyroid. Unexpectedly, PPAR γ expression was not found in nuclei but instead in the cytoplasm of the cells. This was also the case in 15dPGJ2-treated animals, suggesting that 15dPGJ2 effects are not mediated by PPAR γ . Although odd, the extranuclear localization of PPAR γ is not totally surprising because it has been already reported in other tissues (48). We do not have a clear explanation for that. This may correspond to a form of cytoplasmic storage before translocation into nuclei, as previ-

ously suggested (48). The amount of proteins in nuclei could be under the threshold of detection by immunohistochemistry. Likewise, results obtained with BADGE, a PPAR γ antagonist, strengthen the idea that 15dPGJ2-induced protective effects are probably not mediated by PPAR γ . One should keep in mind that 15dPGJ2 acts as a relatively weak ligand of PPAR γ , which may also explain the lack of effect of BADGE. Accordingly, a paper published by Kasai *et al.* (49) showed that thyroglobulin production is increased by 15dPGJ2 in human thyrocytes but, again, via a PPAR-independent mechanism. Based on our experiments, it is clear that the PPAR hypothesis cannot yet be definitively ruled out. Other experiments carried out in animal models of autoimmune thyroiditis and/or with specific PPAR ligands such as glitazones are required to sort out this question.

There are alternative explanations for PPAR-independent 15dPGJ2-induced antiinflammatory effects. Thus, 15dPGJ2 inhibits the expression of a variety of proinflammatory factors including cyclooxygenase-2, NOSII, and cytokines (IL-6, IL-12, and TNF- α) (43, 44, 50). It may also modulate (51) or inhibit (44, 52) the nuclear factor- κ B system and activate the MAPK pathway independently of PPAR γ (45). In addition, because our experiments were *in vivo*, a direct effect of 15dPGJ2 on interstitial inflammatory cells including lymphocytes, eosinophils, basophils, and macrophages cannot be ruled out (53). This may explain the significant decrease in the number of macrophages in the thyroid of 15dPGJ2-treated animals. Additional investigations are obviously required to find out which mechanism(s) is (are) working out in our model.

In conclusion, the present study provides evidence that the oxidative status is high in thyroid hyperplasia and iodine-induced involution. In goitrous glands, the oxidative stress

is not associated with an inflammatory reaction likely because antioxidant systems are correctly adapted to detoxify ROS. In involuting gland, inflammation likely occurs because the antioxidant shelter is not strong enough to blunt more robust oxidative attacks. 15dPGJ2 can blunt the oxidative stress and limit the inflammatory reaction, but independently of PPAR γ . The findings open new perspectives of research in models of chronic thyroiditis.

Acknowledgments

Received July 12, 2007. Accepted September 10, 2007.

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Disclosure Statement: S.P., A.-C.G., M.B., M.S., P.B.C., B.L., M.-C.M., and I.M.C. have nothing to declare. B.K. is an inventor on Belgium Patent No. PCT/BE98/00124.

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