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Involvement of the peroxisome proliferator-activated receptor alpha in the immunomodulation caused by peroxisome proliferators in mice

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Abstract

Peroxisome proliferators (PPs) are a large class of structurally diverse chemicals, which includes drugs designed to improve the metabolic abnormalities linking hypertriglyceridemia to diabetes, hyperglycemia, insulin-resistance and atherosclerosis. We have recently demonstrated that exposure of rodents to potent PPs indirectly causes a number of immunomodulating effects, resulting in severe adaptive immunosuppression. Since the peroxisome proliferator-activated receptor alpha (PPARα) plays a central role in mediating the pleiotropic responses exerted by PPs, we have compared here the immunomodulating effects of the PPs perfluorooctanoic acid (PFOA) and Wy-14,643 in wild-type and PPARα-null mice. The reductions in spleen weight and in the number of splenocytes caused by PP treatment in wild-type mice was not observed in PPARα-null mice. Furthermore, the reductions in thymus weight and in the number of thymocytes were potently attenuated in the latter animals. Similarly, the dramatic decreases in the size of the $CD4^+CD8^+$ population of cells in the thymus and in the number of thymocytes in the S and G2/M phases of the cell cycle observed in wild-type mice administered PPs were much less extensive in PPARα-null mice. Finally, in contrast to the case of wild-type animals, the response of splenocytes isolated from the spleen of PP-treated PPARα-null mice to appropriate T- or B-cell activators *in vitro* was not reduced. Altogether, these data indicate that PPARα plays a major role in the immunomodulation caused by PPs. The possible relevance of these changes to the alterations in plasma lipids also caused by PPs is discussed.

Keywords: PPARα; Immunomodulation; Thymus; Spleen; Lymphocyte; Thymocyte; Splenocyte; Peroxisome proliferator; Mice

1. Introduction

Peroxisome proliferators constitute a very large (>1000 at present) and growing family of wide-spread foreign compounds, including numerous industrial chemicals (e.g. plasticizers such as phthalates and surfactants such as perfluoro fatty acids), agrochemicals (e.g. pesticides such as phenoxyacetic acids) and important clinical drugs (e.g. nonsteroidal anti-inflammatory drugs such as acetylsalicylic acid and hypolipidemic agents such as fibrate derivatives) [1,2]. The most extensively characterized effects of PPs on susceptible animal species are increases in the number and size of hepatic peroxisomes, together with potent transcriptional up-regulation of the levels of hepatic fatty acid-metabolizing enzymes and hepatomegaly. Furthermore, prolonged treatment of rodents with peroxisome proliferators results in an increased incidence of liver tumors [2,3].

There is presently an increasing awareness that direct or indirect interactions of xenobiotics (drugs and other foreign chemicals) with the immune system may result in extensive immunomodulation [4,5]. Such changes in the immune system caused by xenobiotics may contribute to an increased incidence and/or severity of infection, increased immunoreactivity towards environmental agents (hypersensitivity) and/or enhanced tumor development [6]. Recently, it has been demonstrated in our laboratory that PPs cause potent immunomodulating effects in mice, involving thymic and splenic atrophy, loss of thymocytes and splenocytes, and potent suppression of adaptive immune responses [7–9]. The mechanism(s) underlying these phenomena are at present unclear.

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Abbreviations: PPARα, peroxisome proliferator-activated receptor alpha; PFOA, perfluorooctanoic acid; ConA, concanavalin A; LPS, lipopolysaccharide; PPs, peroxisome proliferators.

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The peroxisome proliferator-activated receptors (PPARs), form a subfamily of the nuclear receptors superfamily, along with the receptors for thyroid hormone, retinoid acid and Vitamin D. The α isoform of PPAR is well known to be involved in mediating many of the adaptive responses of rodents to exposure to PPs [3,10–12]. In rodents, PPARα is expressed at relatively high levels in the liver, kidney and heart, all of which display peroxisome proliferation in response to PPs and are characterized by high rates of lipid metabolism [13]. Transgenic mice which are homozygous with regards to a functional mutation in the *PPARα* gene do not demonstrate peroxisome proliferation, hepatomegaly or hepatocarcinogenesis, even after chronic exposure to PPs [14,15]. Therefore, the current study was designed to examine the possible involvement of PPARα in the immunomodulation exerted by PPs. For this purpose, PFOA was employed as the model PP, since this compound is not metabolized in mice [16–18] and is one of the most potent PPs presently known. In addition, immunomodulation by PFOA has been previously characterized in some detail in our laboratory [7–9].

2. Materials and methods

2.1. Animals and treatment

All experiments were performed on adult male C57Bl/6 (wild-type; obtained from B&K Universal AB, Sweden) or PPARα-null mice of a pure Sv/129 genetic background (derived from the original colony of mixed background mice [12]; kindly provided by Frank Gonzalez). Animals weighing 22–25 g (about 8–10 weeks old) were randomly divided into groups of four and housed in steel cages with a 12-hr light/dark cycle at 25° and free access to water and laboratory chow (Rat and Mouse Standard Diet, B&K Universal AB, Sweden). The mice were acclimated to these conditions for 1 week prior to commencement of the experiments.

Preparation of the diet containing 0.02% (w/w) PFOA (Aldrich) or 0.125% (w/w) Wy-14,643 (Wyeth Laboratory Inc., Philadelphia, PA, USA) was carried out as described previously [7]. Since mice scatter powdered food, both the normal and PP-containing chow were prepared in the form of pellets in order to allow determination of food intake. The animals were exposed to a normal or PP-containing diet for 7 days, both groups consuming approximately 3.5 g of food per day. The animals were weighed daily, and their final body weights are documented in Table 1.

At the end of the period of treatment, the animals were killed by cervical dislocation and the liver, thymus and spleen removed and weighed. The livers were then homogenized as described earlier [7]. Thymocytes and splenocytes were isolated by teasing the relevant organ apart gently with a forceps in Earle's balanced solution (EBSS). Erythrocytes present in the splenocyte preparations were lysed using a published procedure [7]. The cells were counted in a haemocytometer and cell viability (always >90%) evaluated on the basis of trypan blue exclusion.

2.2. Assay of peroxisomal fatty acid acyl-CoA oxidase

Acyl-CoA oxidase [19] was assayed fluorimetrically with palmitoyl-CoA as substrate employing liver homogenate from both control and PP-treated mice.

2.3. Staining of cellular DNA with propidium iodide

Single-cell suspensions of thymocytes or splenocytes were stained with propidium iodide (PI) according to a reported procedure [20], as described in more detail previously [7].

2.4. Immunofluorescent staining and flow cytometric analysis

Single-cell suspensions of thymocytes or splenocytes were stained with FITC- or Cy-chrome-conjugated monoclonal anti-mouse antibodies (MoAbs) towards CD3, CD4, CD8a or CD19 and subsequently analyzed employing a single-laser FACSCalibur cytometer, according to a previously published procedure [7,8].

Table 1
Effects of dietary treatment of wild-type and PPARα-null mice with the peroxisome proliferator PFOA or Wy-14,643 on body, liver, spleen and thymus weights, and splenocytes and thymocytes numbers

Group and treatment	Body weight (g)	Liver weight (g)	Spleen weight (g)	Splenocyte number ($\times 10^6$)	Thymus weight (g)	Thymocyte number ($\times 10^6$)
Wild-type mice						
None (control)	24.5 ± 1.58	1.38 ± 0.34	0.082 ± 0.006	68.8 ± 16.8	0.061 ± 0.014	81.0 ± 28.2
PFOA	21.0 ± 0.74**	2.57 ± 0.15***	0.050 ± 0.001***	15.3 ± 5.84***	0.013 ± 0.001***	12.8 ± 7.98***
Wy-14,643	20.7 ± 1.42**	3.25 ± 0.31***	0.058 ± 0.005***	20.3 ± 2.12***	0.030 ± 0.000***	19.3 ± 6.80***
PPARα-null mice						
None (control)	23.6 ± 2.9	1.04 ± 0.30	0.064 ± 0.021	84.0 ± 19.3	0.054 ± 0.006	88.3 ± 7.04
PFOA	23.5 ± 1.0†	2.28 ± 0.15***	0.054 ± 0.015	73.8 ± 26.2†††	0.033 ± 0.005***†††	54.0 ± 12.7**†††
Wy-14,643	24.1 ± 1.3†	1.13 ± 0.07†††	0.069 ± 0.011	98.8 ± 10.1†††	0.040 ± 0.004**†	62.0 ± 5.48***†††

All values are means ± SEM for four animals in each group. $^{**}P < 0.01$, $^{***}P < 0.001$ compared to the corresponding control. $^{\dagger}P < 0.05$, $^{\dagger\dagger\dagger}P < 0.001$ compared to the corresponding wild-type group. Other conditions were as described in Section 2.

2.5. Assay of lymphoproliferation

Lymphoproliferation was monitored as described elsewhere [21]. Briefly, splenocytes were suspended in RPMI 1640 medium, buffered with 25 mM HEPE buffer and supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 100 IU penicillin and 100 μg streptomycin per mL and 50 mM 2-mercaptoethanol (all of the above purchased from Life Technologies, Gaitherburg, MD, USA). Cell suspensions with a density of 10^6 cells/mL were seeded onto 96-well flat-bottom culture clusters (Costar, Cambridge, MA), 200 μL being added to each well. Lipopolysaccharide (from *E. coli* 055:B5) and concanavalin A (ConA, AmershamPharmaciaBiotech, Uppsala, Sweden) in RPMI medium were added to the appropriate wells at final concentrations of 25 and 2.5 μg/mL, respectively, and incubation subsequently carried out at 37° under a humidified atmosphere containing 5% CO_2 for the time period indicated.

2.6. ^{3}H-Thymidine incorporation as a measure of DNA synthesis

^{3}H-Thymidine (final level $= 2 \times 10^{-6}$ Ci/mL; Amersham International, UK) was pulsed into the wells described above at different time points. The cells were harvested 12 hr later using a Skatron cell harvester (Lier, Norway) and the radioactivity incorporated was determined utilizing a Rack-Beta Scintillation Counter (LKB Pharmacia, Uppsala, Sweden). The radioactivity expressed as counts per minute (cpm) was used as a measure of proliferation.

2.7. Statistical analysis

Each experimental group contained four animals and data are presented as means ± SEM. The significant differences between groups were tested by one-way ANOVA analysis of variance, followed by planned multiple comparison according to least significant difference methods (Statistica software, Stat Soft Co).

3. Results

3.1. General observations during administration of PFOA or Wy-14,643 to wild-type or PPARα-null mice

In comparison to wild-type animals, PPARα-null mice exhibit normal thymus and spleen weights and normal numbers of thymocytes and splenocytes (Table 1). Dietary administration of PFOA or Wy-14,643 to wild-type mice for 7 days resulted in a significant decrease in body weight; but no such change was observed in the case of PPARα-null mice (Table 1). This is in agreement with observations using another PP, DEHP, which also significantly reduces the body weight of wild-type, but not of PPARα-null mice [15]. These findings indicate that this decrease in body weight is a specific, rather than a toxic effect of PPs. Furthermore, no significant change in food intake by either type of animal upon PP treatment was observed (data not shown). The decrease in body weight occurring in wild-type mice does thus, not reflect reduced food intake.

3.2. Effects of administration of PFOA or Wy-14,643 to wild-type or PPARα-null mice on the weight of the liver and on a marker of hepatic peroxisome proliferation

Significant increases in liver weight were observed in wild-type mice exposed to PFOA or Wy-14,643 in their diet for 7 days. This response was totally absent upon treatment of PPARα-null mice with Wy-14,643; but, surprisingly, virtually unaltered in null mice exposed to PFOA (Table 1). Apparently, the hepatomegaly (reflecting primarily hepatocyte proliferation [3]) induced by PFOA is not a PPARα-dependent process, whereas the same response to Wy-14,643 is mediated by this receptor.

As expected, potent hepatic peroxisome proliferation, as reflected in up-regulation of peroxisomal fatty acid β-oxidation, occurred in wild-type mice administered Wy-14,643 or PFOA; whereas this proliferation was totally lacking in the PPARα-null animals (Fig. 1). This confirms, as shown previously [22], that hepatic peroxisome proliferation in mice in response to PPs is strictly dependent on the presence of functional PPARα.

3.3. Effects of PFOA and Wy-14,643 on the weight of the thymus and spleen and on the numbers of thymocytes and splenocytes in wild-type and PPARα-null mice

In contrast to wild-type mice, PPARα-null mice receiving PFOA or Wy-14,643 at the indicated doses in their diet for 7 days demonstrated no significant decrease in the weight of their spleen and the number of splenocytes (Table 1). At the same time, the decrease in thymus weight and the number of thymocytes caused by PPs in wild-type mice was attenuated, but not eliminated in the PPARα-null animals (Table 1). This clearly indicates that the effect on the spleen is PPARα-dependent, while the decrease in thymus weight is only partially dependent on this same receptor.

3.4. Effects of administration of PFOA or Wy-14,643 on the numbers and phenotypes of thymocytes and splenocytes in wild-type and PPARα-null mice

In order to determine the effects of PFOA and Wy-14,643 on phenotypically distinct subpopulations of thymocytes, we examined CD4 and CD8 expression by these cells employing two-color flow cytometry. In comparison to wild-type animals, PPARα-null mice exhibit a small, but significant increase in the size of the $CD4^+CD8^+$ cell population, with concomitant decreases in other populations.

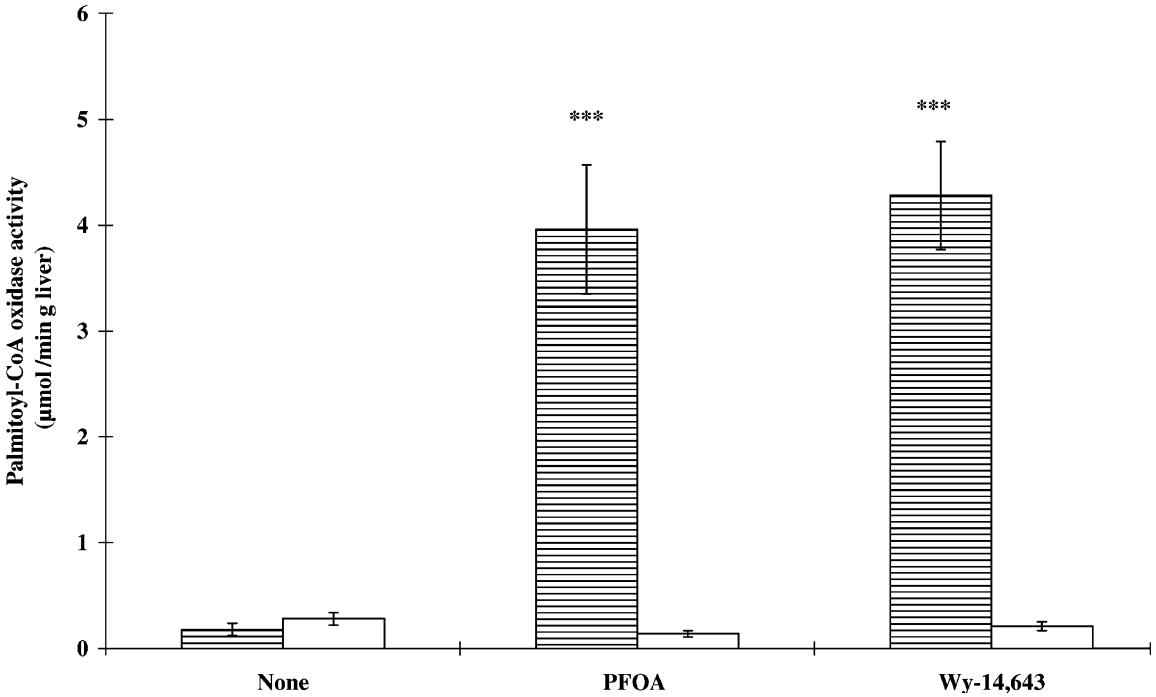

Fig. 1. Effect of the peroxisome proliferators PFOA and Wy-14,643 on hepatic peroxisomal acyl-CoA oxidase activity in wild-type (stripped bars) and PPARα-null (empty bars) mice. The activity was assayed in hepatic homogenates prepared after 7 days of exposure to PFOA or Wy-14,643, employing palmitoyl-CoA as substrates. $^{***}P < 0.001$ compared to the corresponding control value.

Furthermore, these two PPs were found to affect the numbers of phenotypically distinct thymocytes in these animals to different extents. The $CD4^+CD8^+$ cell population was reduced most dramatically in wild-type mice. The observed effects are much weaker in PPARα-null mice, where the response to PFOA is more pronounced than that to Wy-14,643 (Table 2).

In the case of splenocytes, PPARα-null mice also exhibit less potently, but significantly increased numbers of both T-cells ($CD3^+$) and B-cells ($CD19^+$) in their spleen compared to wild-type animals (Table 2). Dramatic decreases in the numbers of both T- and B-cells occurred upon PP treatment of wild-type mice, with the effect on B-cells being more pronounced. In contrast, these decreases were not observed in PPARα-null mice (Table 2). Thus, these immunomodulating effects of PPs on both T- and B-cells are mediated primarily by PPARα.

3.5. Effects of treatment with PFOA or Wy-14,643 on the cell cycle of thymocytes and splenocytes in wild-type and PPARα-null mice

In order to determine whether the decreased number of thymocytes observed after PP treatment reflects a decrease in the percentage of the cells carrying out DNA synthesis (e.g. a reduction in cell proliferation), we examined the cell cycle in these cells using a flow cytometric technique based on binding of the dye propidium iodide to DNA. This

Table 2
Effects of dietary treatment of wild-type and PPARα-null mice with the peroxisome proliferator PFOA or Wy-14,643 on antigenically distinct subpopulations of thymocytes and splenocytes

Group and treatment	Thymocytes				Splenocytes	
	$CD4^+CD8^+$ ($\times 10^6$)	$CD4^+CD8^-$ ($\times 10^6$)	$CD4^-CD8^+$ ($\times 10^6$)	$CD4^-CD8^-$ ($\times 10^6$)	$CD3^+$ ($\times 10^6$)	$CD19^+$ ($\times 10^6$)
Wild-type mice						
None (control)	56.2 ± 1.61	13.8 ± 0.79	5.99 ± 0.43	4.88 ± 0.48	16.9 ± 1.17	32.4 ± 0.21
PFOA	$4.11 \pm 0.37^{***}$	$4.60 \pm 0.42^{***}$	$1.91 \pm 0.14^{***}$	$2.18 \pm 0.53^{**}$	$6.40 \pm 0.15^{**}$	$4.70 \pm 0.18^{***}$
Wy-14,643	$10.9 \pm 0.17^{***}$	$4.77 \pm 0.16^{***}$	$1.90 \pm 0.11^{***}$	$1.79 \pm 0.11^{***}$	$7.50 \pm 0.06^{*}$	$7.00 \pm 0.09^{***}$
PPARα-null mice						
None (control)	$74.1 \pm 0.05^{\dagger\dagger\dagger}$	$7.69 \pm 0.03^{\dagger\dagger\dagger}$	$3.66 \pm 0.02^{\dagger\dagger\dagger}$	$2.67 \pm 0.02^{\dagger\dagger\dagger}$	$21.4 \pm 1.78^{\dagger}$	$38.2 \pm 1.49^{\dagger\dagger\dagger}$
PFOA	$43.5 \pm 0.19^{**\dagger\dagger\dagger}$	$5.89 \pm 0.10^{*\dagger\dagger\dagger}$	$2.85 \pm 0.06^{*\dagger\dagger\dagger}$	$1.72 \pm 0.05^{***}$	$25.6 \pm 0.38^{\dagger\dagger\dagger}$	$35.6 \pm 0.81^{\dagger\dagger\dagger}$
Wy-14,643	$51.2 \pm 0.07^{**\dagger\dagger\dagger}$	$6.04 \pm 0.04^{*\dagger\dagger\dagger}$	$2.83 \pm 0.03^{*\dagger\dagger\dagger}$	$1.92 \pm 0.02^{**}$	$22.3 \pm 0.15^{\dagger\dagger\dagger}$	$41.0 \pm 0.34^{\dagger\dagger\dagger}$

All values are means ± SEM for four animals in each groups. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to the corresponding control. $^{\dagger}P < 0.05$, $^{\dagger\dagger\dagger}P < 0.001$ compared to the corresponding wild-type group. Other conditions were as described in Section 2.

Table 3
Effects of dietary treatment of wild-type and PPARα-null mice with the peroxisome proliferator PFOA or Wy-14,643 on the relative numbers of thymocytes in different phases of the cell cycle

Group and treatment	G1/2 phase (%)	G0/G1 phase (%)	S–G2/M phase (%)
Wild-type mice			
None (control)	6.41 ± 0.67	69.4 ± 1.07	19.5 ± 0.99
PFOA	10.5 ± 2.15*	80.1 ± 4.50**	9.22 ± 3.22***
Wy-14,643	8.51 ± 1.12*	75.1 ± 1.90**	16.4 ± 1.73**
PPARα-null mice			
None (control)	0.98 ± 1.05†††	70.1 ± 1.96	28.5 ± 2.17†††
PFOA	0.69 ± 0.24†††	77.0 ± 1.93**	22.2 ± 2.28**†††
Wy-14,643	0.49 ± 0.06†††	73.2 ± 1.53	26.0 ± 1.76†††

All values (given as percentages of total fluorescence) are means ± SEM for four animals in each group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to the corresponding control. $^{\dagger\dagger\dagger}P < 0.001$ compared to the corresponding wild-type group. Other conditions were as described in Section 2.

approach allowed us to measure the percentage of the cells that were in the G0/G1, S and G2/M stages of the cell cycle.

In comparison to wild-type animals, PPARα-null mice demonstrate a significantly larger number of thymocytes in the S and G2/M phases of the cell cycle and fewer thymocytes in the G1/2 phase (which reflects cells with less than a diploid content of DNA that are undergoing apoptosis) (Table 3). Furthermore, in comparison to the significant decreases in the proportion of thymocytes in the S and G2/M phases and significant increase in the G0/G1 phases in wild-type mice treated with PFOA and Wy-14,643, these effects in PPARα-null mice were strongly reduced (Table 3). Thus, the decreases in the numbers of cells in the S and G2/M phases of the cell cycle (which reflect partial, if not complete, inhibition of thymocyte proliferation) are also mediated largely by PPARα. In addition, a significant reduction in the number of cells in the G1/2 phase, i.e. undergoing apoptosis, was also observed in PPARα-null mice (Table 3). However, more extensive characterization of this apoptosis is required.

3.6. Effects of treatment with PFOA or Wy-14,643 on ex vivo proliferation of splenocytes from wild-type and PPARα-null mice

In order to elucidate whether PPARα is involved in the alteration caused by PPs in the functional response of lymphocytes to proliferative stimulation, we performed an *ex vivo* experiment in which splenocytes isolated from the spleen of wild-type and PPARα-null mice were stimulated with the T- and B-cell activators ConA or LPS, respectively, and their rate of DNA synthesis (determined as incorporation of radioactive thymidine) subsequently measured. Splenocytes from both wild-type and PPARα-null mice exhibit a dramatic *in vitro* proliferation in response to both ConA and LPS following 2–3 days of stimulation (at which time the maximal proliferative response is obtained). In wild-type mice, PP treatment reduced this proliferation of splenocytes in response to both ConA and LPS (Fig. 2A and B). In contrast in the case of PPARα-null mice, PP treatment caused no significant change in these responses (Fig. 2A and B).

4. Discussion

Although PPARα-null mice are unresponsive to PPs, their phenotype is normal in most other respects [14,15]. The normal thymus and spleen weights and cell numbers, as well as the normal *in vitro* responses of splenocytes to T- or B-cell activators indicate that PPAR-null mice have a normal immune system. At the same time, the small, but significant increases in the $CD4^{+}CD8^{+}$ population of thymocytes and the increased number of thymocytes in the S–G/M phase of the cell cycle may indicate that PPARα influences the differentiation of thymocytes. However, in comparison to the dramatic changes in these parameters by PPs, these effects of a functional defect in PPARα are much smaller and, thus, would not be expected to interfere with the present investigation.

We have previously demonstrated that upon treatment of wild-type mice with PPs, the thymus and spleen both exhibit dramatic atrophy, reflecting losses of thymocytes and splenocytes, respectively [7,8]. In contrast, in the present investigation no significant spleen atrophy and loss of splenocytes was observed in PPARα-null mice. Furthermore, although significant thymus atrophy and loss of thymocytes is still observed in PPARα-null mice exposed to PPs, these effects are strongly attenuated. This finding clearly indicates the involvement of both PPARα-dependent and -independent mechanisms in these processes.

In the early stage of thymocyte development, $CD4^{-}CD8^{-}$ cells arising from extrathymic precursors first differentiate into $CD4^{+}CD8^{+}$ thymocytes, after which they undergo dramatic proliferation, followed by a rigorous process of selection and differentiation to produce $CD4^{+}$ and $CD8^{+}$ thymocytes [23]. In our previous investigations, it has been demonstrated that the rapid decreases in the $CD4^{+}CD8^{+}$ population of thymocytes and the number of cells in the S–G2/M phase caused by PP in wild-type mice may be due to inhibition of the proliferation of $CD4^{+}CD8^{+}$ thymocytes, which may also contribute to the thymus atrophy observed [7,8]. The attenuation (in the case of PFOA) or disappearance (Wy-14,643) of these changes in PPARα-null mice observed here further indicates significant involvement of this receptor in the regulation of thymocyte proliferation and differentiation.

In addition, our previous studies indicated that B-cell maturation and/or differentiation in the bone marrow may also be influenced by PP treatment, since the numbers of both T- and B-cells decrease [7,8]. The simplest explanation for this phenomenon is a decrease in the supply of B- and T-cells provided by thymus and bone marrow, respec-

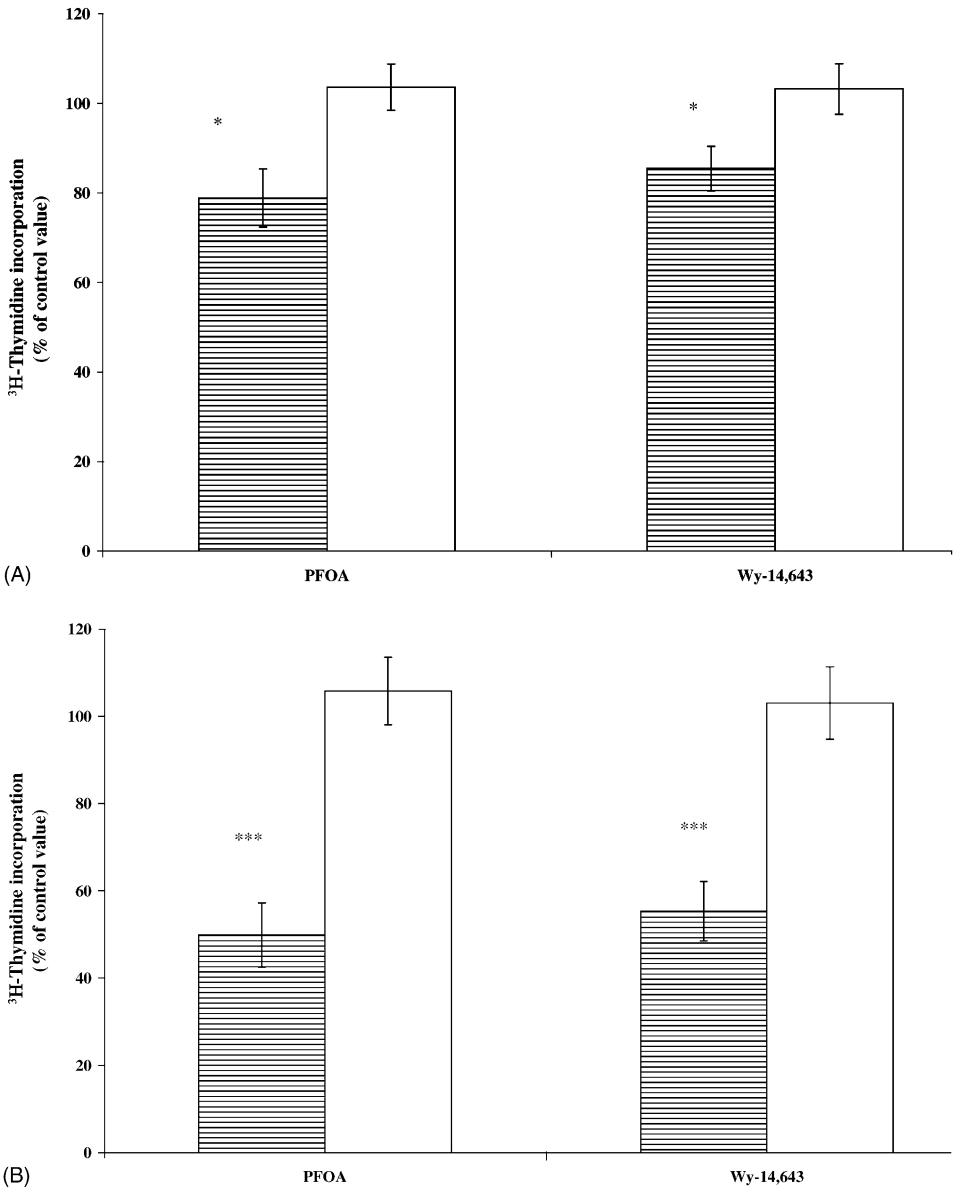

Fig. 2. Effect of *in vitro* treatment with the peroxisome proliferators PFOA and Wy-14,643 on the *in vitro* proliferation of lymphocyte isolated from wild-type (striped bars) and PPARα-null (empty bars) mice. Spleen cells were cultured in medium containing ConA (2.5 μg/mL) (A) or LPS (25 μg/mL) (B), respectively. [^{3}H]-Thymidine was pulsed at proliferation peak time (day 2) and incorporated radioactivity was counted. Mean cpm values for triplicate cultures were thus, determined. The results shown are means ± SEM for four animals. $^{*}P < 0.05$, $^{***}P < 0.001$ compared to the corresponding control group.

tively. The unaltered level of B-cells in the spleen of PPARα-null mice treated with PFOA further suggests that this receptor may be also involved in B-cell maturation and/or differentiation in the bone marrow.

Without stimulation, lymphocytes in the spleen remain in a resting state and, in order to participate in an adaptive immune response, these naive lymphocytes must proliferate and differentiate upon encountering the appropriate antigens [23]. In contrast to our findings with wild-type mice, the *in vitro* response of splenocytes isolated from the spleen of PPARα-null animals to appropriate T- or B-cell activators is not reduced by previous *in vivo* treatment with PFOA or Wy-14,643. This finding indicates the involvement of PPARα in the functional response of lymphocytes to foreign antigens.

At the same time, in contrast to the high levels of expression of PPARα in rodent liver, kidney and heart (all of which exhibit peroxisome proliferation in response to PPARα activators and relatively high rates of lipid metabolism), thymus and spleen exhibit the lowest levels of expression of PPARα and PPARβ of all tissues tested. Furthermore, the spleen expresses rather high levels of PPARγ [13,24]. In agreement with the present observations, our earlier studies indicated that the mechanism(s) underlying the phenomena discussed above is indirect, since *in vitro* exposure to PFOA does not alter the spontaneous proliferation of thymocytes or the proliferation of lymphocytes in response to appropriate antigen stimulation [7–9].

Overall, our data strongly suggest that PPARα mediates many processes connected with the immune system in an indirect fashion. Thus, the question is raised concerning the molecular mechanism(s) *via* which PPARα is involved in these processes. All of the target genes for PPARα identified to date code for proteins involved in lipid transport and metabolism [11]. Therefore, the involvement of lipids in the functions of the immune system is of interest in this connection.

The physiological requirement of the immune system for lipids has been extensively described [25–27]. The immune system requires an external supply of fatty acids for its development and physiological responses, and especially for the spontaneous proliferation of thymocytes and the proliferation of lymphocytes in response to foreign antigens [26–30]. Furthermore, perturbation of the compositions of both external and internal membranes of the lymphocyte can attenuate signal transduction by the T-cell receptor (TCR), which plays a central role in many aspects of immunological defences [31–33].

At the same time, it is well known that most PPs (e.g. the fibrate class of drugs) have hypolipidemic effects on both humans and rodents. It is generally accepted that this action reflects suppression of the expression of apolipoproteins A-I, A-IV and C-III, hepatic lipase and lecithin cholesterol acyltransferase, in addition to the up-regulation of lipid metabolism associated with peroxisome proliferation in the liver of rodents [25,34]. All of these effects are mediated by PPARα and may result in reduced availability of serum lipids to peripheral tissues, including lymphoid tissues. Thus, it is possible that the major mechanism by which PPs exert immunomodulation is by modulating serum levels of lipids.

In this connection, it has been reported that basal fatty acid homeostasis in PPARα-null mice is altered, i.e. they demonstrate abnormal accumulation of lipid in the liver [12] and altered constitutive expression of fatty acid-metabolizing enzymes [22]. Indeed, we have observed here in these transgenic animals without PP treatment small, but significant changes in the size of the $CD4^+CD8^+$ population of thymocytes and in the proportion of thymocytes in the S and G2/M phases of the cell cycle. These alterations may well be related.

At the same time, it is important to note the possible existence of a PPARα-independent mechanism for immunomodulation by PPs, especially in the case of PFOA. In this connection, it has been reported that fibrate derivatives and PFOA can also exert hypolipidemic effects in rodents *via* a PPARα-independent pathway. This pathway involves inhibition of the secretion of very low-density lipoprotein (VLDL) from the liver and a consequent decrease in the supply of lipid to peripheral tissues [35]. In addition, PFOA directly dissociates apolipoprotein B48 from VLDL particles in the rodent [35], which leads to lack of recognition of LDL by peripheral tissue receptors, further reducing the availability of lipid. On the other hand, the interesting finding here that, in contrast to the case of Wy-14,643, the hepatomegaly induced by PFOA is PPARα-independent may also be related to these changes. However, further studies are required to fully explain these observations.

In 1996, it was proposed that PPARα might be involved in inflammatory processes, since PPARα-null mice display a prolonged inflammatory response to stimulation by leukotriene B4, which is both a potent chemotactic agent and an activating ligand for PPARα. PPARα regulates the oxidative degradation of fatty acids and their derivatives, including this lipid mediator. Therefore, the existence of a feedback mechanism by which leukotriene B4 activates PPARα, which in turn regulates the clearance of leukotriene B4 by the liver and thereby the duration of an inflammatory response has been proposed [36]. However, more recent results do not support this hypothesis, since activation of PPARα by LTB(4) or other agonists is not associated with an increase in LTB(4) metabolism [37].

At the same time, inflammatory responses are also regulated by the adaptive immune system. For example, T-lymphocytes release soluble factors (cytokines) which activate the phagocytes to destroy the pathogens they have internalized. In addition, phagocytes use antibodies released by B-lymphocytes to allow them to recognize pathogens more effectively [23,38,39]. Thus, our findings that PPs cause potent suppression of adaptive immune responses [9] suggest an alternative mechanism for the anti-inflammatory effects of these compounds.

Finally, the ability of PPs to suppress adaptive immunity in rodents may contribute to the development of hepatocarcinogenesis in response to these same substances. Further studies along these lines are required and may help elucidate a new mechanism by which non-genotoxic compounds such as PPs cause tumors, as well as improving our assessment of the possible risks posed by PPs to human health.

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