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# Peroxisome proliferator-activated receptor α regulates B lymphocyte development via an indirect pathway in mice

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**Abstract**

Peroxisome proliferator-activator receptor α (PPARα), a member of the nuclear receptor superfamily, has been implicated in the regulation of inflammation and immune response. Adaptive immune responses are suppressed by exposure to PPARα agonists, resulting in severe thymus and spleen atrophy. In addition, the decline in both T and B cells is due in part to the loss of splenocytes upon exposure to PPARα agonists. Thus, the current study was designed to examine the effect of Wy-14,643, a potent PPARα agonist, on B cell development in bone marrow from wild-type and PPARα-null mice. Significantly decrease in pro/pre-B cell and total $B220^+$ cell was observed in wild-type mice in bone marrow upon Wy-14,643 treatment, but not in PPARα-null mice. Immature and mature B cell populations are not affected. This suggests that PPARα is involved in the development of B cell during lymphoid lineage. However, surprisingly, PPARα mRNA was absent in bone marrow as revealed by RT-PCR. Therefore, the effect of PPARα on B cell development is by an indirect mechanism.


*Keywords:* PPARα; B lymphocyte; Bone marrow

## 1. Introduction

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily. Three isoforms of PPARs (α, β/δ, and γ) have been identified in a variety of species including rodents, non-human primates, and humans [1–3]. PPARs function as transcription factors by the classic ligand-dependent nuclear hormone receptor mechanism. After ligand binding, PPARs heterodimerize with RXR and selectively bind to degenerate direct repeats of the hexameric nucleotide sequence, AGGTCA, separated by 1 base pair (DR1) called peroxisome proliferator responsive elements (PPREs) on PPAR response target genes [2]. PPREs have been identified in the promoters of several PPAR target genes which are mainly involved in lipid storage, transport and metabolism [4–6].

PPARα expressed at relatively high levels in hepatocytes, heart, muscle, and kidney mediates the response in rodents to a diverse group of chemicals called peroxisome proliferators (PPs). PPs constitute a very large and growing group of foreign compounds that include clinically important drugs (e.g., hypolipidemic agents such as fibrates derivatives), industrial chemicals (e.g., plasticizers, such as phthalate esters), and agrochemicals (e.g., pesticides such as phenoxyacetic acid) [7–9]. PPs increase the number and size of peroxisomes and cause significant hepatomegaly in susceptible rodent species. PPARα target genes encode proteins involved in peroxisomal and mitochondrial fatty acid β-oxidation, fatty acid transport, fatty acid synthesis, fatty acid binding, and as components apolipoproteins, thus demonstrating a central role for this receptor in lipid metabolism and transport [7,10–12]. The fibrate class of hypolipidemic drugs were shown to lower serum triglycerides, while elevating high-density lipoprotein (HDL) cholesterol in human and mice, effects that require a functional PPARα in mice [13]. PPARα-null mice are refractory to all the above changes when administered PPARα agonists, indicating a PPARα-dependent pathway [13,14].

Generally, PPARs were believed to regulate genes predominantly associated with lipid metabolism. However, understanding the role played by PPARs in the regulation

*Abbreviations:* PPAR, peroxisome proliferator-activator receptor; PPs, peroxisome proliferators; BM, bone marrow; CBC, complete blood cell count

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of inflammation and immunity is rapidly evolving. Recently, it was demonstrated that PPs cause potent immunomodulation effects in mice, involving thymic and splenic atrophy, loss of thymocytes and splenocytes, and potent suppression of adaptive immune response [15–17]. All of these effects occur in a PPARα-dependent manner [18]. In addition, the decline of both T and B cells contributes to the loss of splenocytes after PPs exposure. Thus, the current study was designed to examine the possible effects of PPARα on B cell development in bone marrow (BM). The mechanism(s) that could account for the observed alteration in B cell development, were also investigated. In summary, the present work demonstrates that in addition to exerting profound effects on the immune response, PPARα also influences development of the immune system.

## 2. Materials and methods

### 2.1. Animals and treatment

4-Chloro-6-(2,3-xylidino)-2-pyrimidinylthioacetic acid (WY-14,643) was purchased commercially (ChemSyn Science Laboratories, Lenexa, KS). Pelleted mouse chow containing either 0.0 (control) or 0.1% WY-14643 (Bioserv, Frenchtown, NJ) was prepared and provided to mice ad libitum.

Male wild-type or PPARα-null mice (6–8-week-old) on an Sv/129 background, were housed four to five animals per cage in a pathogen-free animal facility in a temperature- and light-controlled environment ($T = 25$ °C, 12 h light/12 h dark cycle). This study was approved by the National Cancer Institute Animal Care and Use Committee. Mice were killed by overexposure to carbon dioxide. Blood was collected from orbital sinus for complete blood cell count.

### 2.2. Bone marrow cell isolation

Femurs and tibiae were removed from each mouse, cleaned of muscle and connective tissue, and ∼1 mm of the end of each bone was removed. The marrow cavity was flushed with 10 ml of PBS and the marrow cells were placed in suspension by successive passage through 25 gauge needles and filtered through 40-μm nylon cell strainer (BD Falcon, Bedford, MA) to remove any remaining debris. The filtered cells were soaked in 1 ml of lysis buffer (0.17 M $NH_4Cl$, 10 mM $KHCO_3$, 1 mM EDTA) for 5 min to remove red blood cells. The remaining cells were washed once, and the pellet was resuspended. The cells were counted using a hemocytometer. Cell viability (evaluated on the basis of trypan blue exclusion) was always >95%.

### 2.3. Preparation of RNA and RT-PCR

Total RNA from tissues was isolated using Trizol reagent and the manufacturer's recommended procedures (GIBCO-BRL, Grand Island, NY). The following mouse oligonucleotides were used for RT-PCR: PPARα forward, 5′-GGGCAAGAGAATCCACGAAG-3′; reverse, 5′GTTGTTGTTGCTGGTCTTTCCCG-3′; β-actin forward, 5′-CCTAAGGCCAACCGTGAAAAG-3′; reverse, 5′-TCTTCATGGTGCTAGGAGCCA-3′. Reverse transcription was performed using a kit (Invitrogen, CA) and the manufacturer's instruction. The reactions were incubated for 5 min at 65 °C, 2 min at 42 °C and after adding reverse transcriptase, incubated for 50 min at 42 °C. PCRs were conducted in 40 μl volumes and reaction cycles consisted of 45 s at 94 °C, 45 s at 60 °C, and 2 min at 72 °C using a PTC-200 Peltier Thermal Cycler (MJ, Research, MT). Aliquots (10 μl) were removed at 5 cycle intervals between cycles 20–35 and examined on 1% agarose gels stained with ethidium bromide.

### 2.4. Antibodies

The following monoclonal antibodies were used at predetermined saturating levels for detection of B lymphocytes: fluorescein isothiocyanate (FITC)-conjugated anti-mouse CD45R/220 (B220) and PerCP anti-mouse IgM (Pharmingen, San Diego, CA).

### 2.5. Immunofluorescent staining

Single-cell suspensions of bone marrow cells ($1 \times 10^6$) in 100 μL cold (4 °C) PBS containing 1% albumin were incubated in the dark on ice for 20 min with FITC- or PerCP-conjugated monoclonal anti-mouse antibodies (MoAbs). In the case of dual-parameter analysis, two of these MoAbs were present in same incubation. After two washes with same PBS, all samples were fixed in 2% paraformaldehyde (in PBS) for 20 min on ice. After two additional washes in cold PBS, the stained cells were analyzed by flow cytometry.

### 2.6. Flow cytometric analysis

Cells were analyzed using a single laser FACSCalibur cytometer (Becton Dickinson) with excitation at 488 nm. The data were collected and analyzed employing CellQuest™ Software. For analysis of cell phenotype markers, the lymphocyte populations were gated on the basis of the forward scatter (FSC) and the side-scatter (SSC) signals collected in the linear mode, i.e. aggregates of cells were gated out. The two fluorescent signals were analyzed on a logarithmic scale and data presented as the percentage of the total cell population exhibiting the fluorescent signal of interest. For each sample, 10,000 cells were analyzed.

### 2.7. Complete blood cell count (CBC)

Peripheral blood specimens were obtained as above and kept in Microtainer tube (with $K_2$EDTA) on room tem-

perature. Cells were stained using the whole blood lysis technique and were analyzed on flow cytometry as previously described [19].

### 2.8. Statistical analysis

Each experimental group contained four to five animals. Data are presented as means ± S.D. and the results of statistical analysis using the Student's *t*-test two-way ANOVAs for pairwise comparisons are given where appropriate.

## 3. Results

A representative dot plot from both control and Wy-14,643-treated wild-type or PPARα-null mice showing bone marrow B lymphocyte subpopulations in the viable cells gate, using two-color staining with B220 and IgM monoclonal antibodies is shown in Fig. 1. Three subpopulations of B lymphocytes were identified, $B220^{lo}/IgM^{-}$, $B220^{lo}/IgM^{+}$, and $B220^{hi}/IgM^{+}$, which correspond, respectively, to pro/pre-B, immature B, and mature B lymphocytes [20,21]. The cells in these three subpopulations represent 95% of all cells in the viable cell gate staining for B220 in all groups (data not shown).

### 3.1. B lymphocyte profiles for control and Wy-14,643-treated wild-type and PPARα-null mice

Counting the total $B220^{+}$ cells within the viable cell gate indicated that Wy-14,643-treated wild-type mice had significantly less cells. However, this difference was not observed in PPARα-null mice upon same treatment (Table 1). Furthermore, it is also clear that the major alterations accounting for the decrease in $B220^{+}$ cell number occurred in the pro/pre-B cell without a significant change in the immature and mature B cell stages (Table 1).

It was particularly interesting to note that activation of the PPARα by Wy-14,643 produced an effect on the B cell subpopulations that was opposite to that seen after targeted loss of PPARα function. The number of pro/pre-B cells in the PPARα-null mice significantly increased versus the wild-type mice, although the total number of $B220^{+}$ cells in

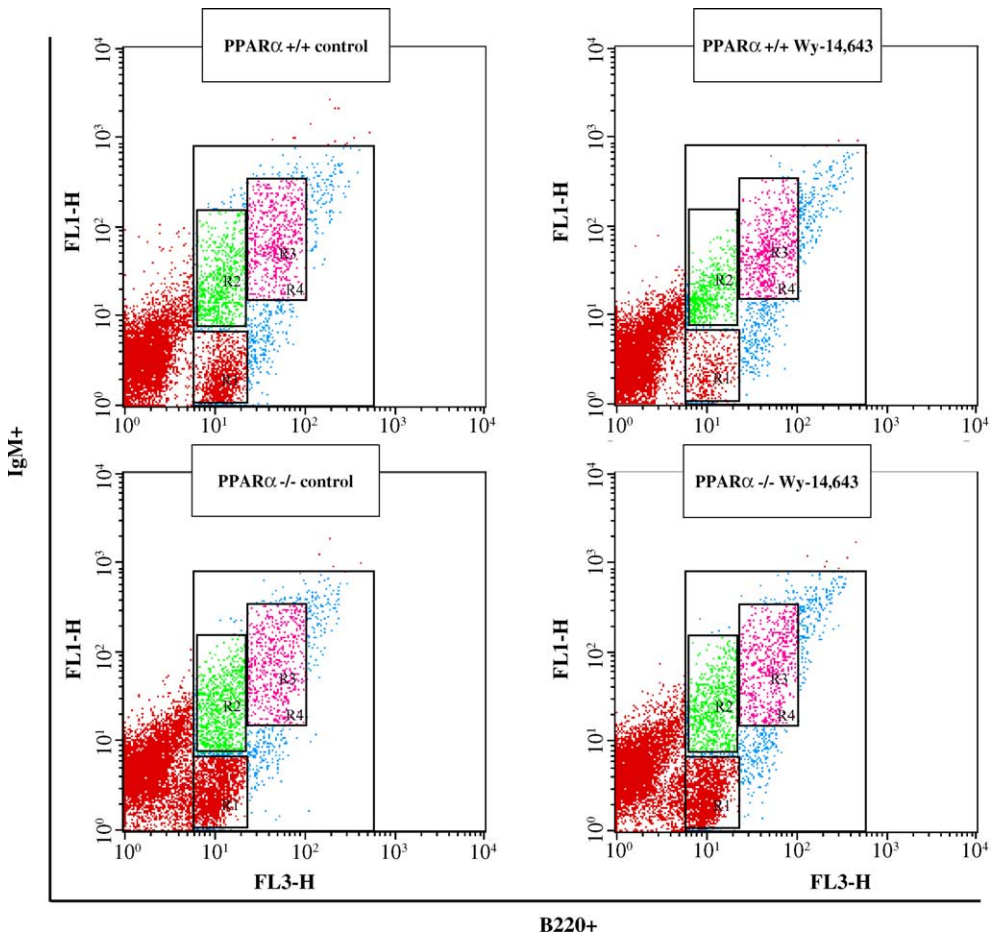


Fig. 1. Representive dot plot for murine bone marrow cells from: (A) PPARα+/+ mice; (B) Wy-14,643-treated PPARα+/+ mice; (C) PPARα−/− mice; (D) Wy-14,643-treated PPARα−/− mice. Dot plot of FL1 (B220) vs. FL3 (IgM) of lymphocyte subpopulation gate showing the three bone marrow B lymphocyte populations: $B220^{lo}/IgM^{-}$, $B220^{lo}/IgM^{+}$, and $B220^{hi}/IgM^{+}$, which correspond to pro/pre-B, immature B, and mature B lymphocytes, respectively. The cells in these three subpopulations represent between 95% of all cells in the viable cell gate staining for B220 in all groups.

Table 1
Analysis of antigenically distinct subpopulations of B cells in bone marrow

| Treatment | Percentage of total $B220^+$ cells | Percentage of pro/pre-B cells | Percentage of immature B cells | Percentage of mature B cells |
|---|---|---|---|---|
| Wild-type mice (control) | 29.3 ± 0.62 | 11.1 ± 0.69 | 6.00 ± 0.47 | 5.52 ± 0.53 |
| Wild-type mice + Wy-14,643 | 23.4 ± 2.17$^{*}$ | 4.92 ± 1.25$^{***}$ | 5.23 ± 0.38 | 6.97 ± 0.62 |
| PPARα-null mice (control) | 32.1 ± 2.81 | 15.2 ± 1.41$^{‡‡}$ | 7.27 ± 0.64 | 5.42 ± 0.60 |
| PPARα-null mice + Wy-14,643 | 33.3 ± 1.65 | 16.0 ± 1.17$^{‡‡‡}$ | 6.94 ± 0.44 | 5.62 ± 0.62 |

All values are means ± S.E.M. for four to five animals in each group. $^{*}P < 0.05$, $^{***}P < 0.001$ vs. the corresponding non-treated control group. $^{‡‡}P < 0.01$, $^{‡‡‡}P < 0.001$ vs. the corresponding wild-type group. Other conditions are as described in Section 2.

Table 2
The effects of Wy-14,643 on RBC and related parameters

| Treatment | RBC (M/μl) | MCV (fL) | HGB (g/dl) | HCT (%) | PLT (K/μl) |
|---|---|---|---|---|---|
| Wild-type mice | 10.6 ± 0.55 | 51.6 ± 0.75 | 16.7 ± 0.78 | 54.5 ± 2.22 | 724 ± 48.4 |
| Wild-type mice + Wy-14,643 | 11.3 ± 0.23 | 48.0 ± 0.10 | 16.6 ± 0.44 | 54.4 ± 1.27 | 687 ± 22.5 |
| PPARα-null mice | 11.0 ± 0.35 | 48.1 ± 0.86 | 16.5 ± 0.50 | 52.9 ± 0.96 | 611 ± 12.7 |
| PPARα-null mice + Wy-14,643 | 10.5 ± 0.15 | 49.0 ± 0.47 | 16.1 ± 0.17 | 51.3 ± 0.44 | 575 ± 88.4 |

RBC: red blood cell; MCV: mean corpuscular volume; HGB: hemoglobin; HCT: hematocrit; PLT: platelets. All values are means ± S.E.M. for four to five animals in each group. Other conditions are as described in Section 2.

the PPARα-null mice was not significantly changed versus wild-type mice. Even the extent of alteration in the number of pro/pre-B cells by loss of PPARα function is not as potent as activation of PPARα. Taken together, these data are consistent with the interpretation that either the loss of PPARα function or its activation plays a significant role in regulation of the B cell maturation process and, in particular, the absolute number of the pro/pre-B cell subpopulation.

### 3.2. Wy-14,643 did not alter development of the myeloid lineage

Since the hemopioetic process includes differentiation events that give rise to all blood cell lineages from hemopoietic stem cells in BM, it is of interest to know whether PPARα has an effect on other linkage and/or on hemopoietic stem cells. Therefore, the complete blood cell count (CBC) was determined revealing that red blood cell (RBC) numbers, hemoglobin levels, and related parameters, e.g., mean corpuscular volume (MCV), mean corpuscular hemoglobin (HCT), mean corpuscular hemoglobin (MCHC), and red cell distribution width (RDW) did not change upon activation or loss of PPARα (Table 2). However, white blood cells (WBC) were significantly changed (Table 3). Absence of PPARα resulted in a dramatic increase in WBC. Treatment with Wy-14,643 significantly decreased WBC in wild-type mice; however, this effect was also observed in PPARα-null mice, although the extent of this decrease in wild-type mice is greater than in PPARα-null mice. Furthermore, the changes in WBC caused by activation of PPARα were mainly due to changes in lymphocytes (Table 3). This result may reflect that PPARα only influences a specific differentiation pathway within the BM, e.g., lymphoid lineage, but not other lineage, e.g., myeloid lineage.

### 3.3. PPARα is not expressed in bone marrow

In order to determine if PPARα has a direct effect on BM, the expression of PPARα was examined by RT-PCR of RNA from BM, thymus and spleen, liver, and adipose tissue. As previously reported, the expression of PPARα was very high in liver and was low in adipose tissue (Fig. 2). In selected lymphoid tissues, the expression of PPARα was high in thymus and low in spleen; however, we were not able to detect a PCR product for PPARα in BM (Fig. 2). Consistence with this conclusion, the expression

Table 3
The effects of Wy-14,643 on WBC

| Treatment | WBC (K/μl) | LYM (K/μl) | MONO (K/μl) | NEU (K/μl) | EOS (K/μl) | BASO (K/μl) |
|---|---|---|---|---|---|---|
| Wild-type mice | 5.21 ± 1.11 | 4.32 ± 0.92 | 0.19 ± 0.08 | 0.60 ± 0.19 | 0.09 ± 0.02 | 0.01 ± 0.007 |
| Wild-type mice + Wy-14,643 | 2.58 ± 0.44$^{*}$ | 2.07 ± 0.35$^{*}$ | 0.09 ± 0.09 | 0.40 ± 0.02 | 0.03 ± 0.01 | 0.01 ± 0.004 |
| PPARα-null mice | 10.9 ± 2.65$^{‡‡‡}$ | 8.67 ± 1.72$^{‡‡‡}$ | 0.34 ± 0.31 | 1.53 ± 0.65$^{*‡‡}$ | 0.32 ± 0.25 | 0.01 ± 0.004 |
| PPARα-null mice + Wy-14,643 | 6.83 ± 0.93$^{*‡‡‡}$ | 5.59 ± 0.47$^{*‡‡}$ | 0.12 ± 0.11 | 1.00 ± 0.17$^{‡‡‡}$ | 0.12 ± 0.02 | 0.00 ± 0.004 |

WBC: white blood cell; LYM: lymphocyte; MONO: monocyte; NEU: neutrophil; EOS: eosophil; BASO: basophil. All values are means ± S.E.M. for four to five animals in each group. $^{*}P < 0.05$ vs. the corresponding non-treated control value. $^{‡‡}P < 0.01$, $^{‡‡‡}P < 0.001$ vs. the corresponding wild-type group. Other conditions are as described in Section 2.

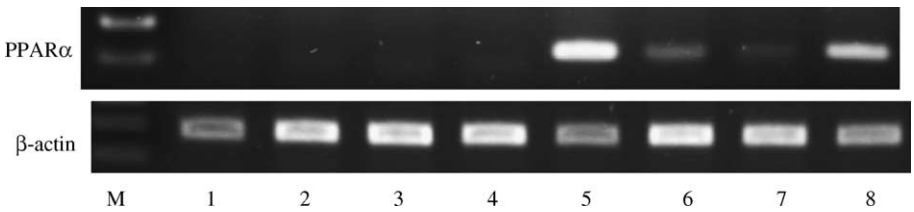


Fig. 2. Ethidium bromide stained 1% agarose gel for RT-PCR amplification of PPARα in bone marrow, thymus, spleen, liver, and adipose tissues. Liver was used as positive control for PPARα expression. Marker (M); (1) bone marrow of wild-type mice; (2) bone marrow of wild-type mice treated with Wy-14,643; (3) bone marrow of PPARα-null mice; (4) bone marrow of PPARα-null mice treated with Wy-14,643; (5) liver of wild-type mice; (6) adipose tissue of wild-type mice; (7) spleen of wild-type mice; (8) thymus of wild-type mice.

of several genes, e.g., recombinase-activating genes RAG-1 and RAG-2, and terminal deoxynucleotidyltransferase (TdT) that are involved in regulating B cell development in BM was not changed by the PPARα agonist Wy-14,643 (data not shown). This indicates that PPARα has an indirect effect on B cell development in the BM.

## 4. Discussion

The cells involved in immune responses are organized into tissues and organs in order to perform their functions most effectively. These structures are collectively referred to as the lymphoid system and are illustrated and described in Fig. 3. Hematopoietic stem cells (HSCs) are defined by their ability to proliferate and generate new HSCs as well as more differentiated progeny from which myeloid and lymphoid cells develop in BM (Fig. 3). Our results show that only the lymphoid lineage and not the myeloid lineage is affected by PPARα agonist. This finding indicates that PPARα is associated with alterations in the development of the lymphoid lineage, but not stem cells.

B cells develop and mature from pluripotent stem cells in the BM. Within the BM, under the influence of the hematopoietic microenvironment provided by stromal cells, differentiation of B lymphocytes occurs in the sequences shown in Fig. 3. The progenitor B cells (termed pro-B) proliferate and differentiate into precursor B cell (termed pre-B) by undergoing immunoglobulin gene rearrangements, concurrent with the appearance of the IL-7 receptor signals. Continued maturation of the pre-B cell to an immature B cell requires light chain gene rearrangement. Once a functional light chain is produced, the immature B cell is now committed to a particular antigenic specificity. Further development of immature B cells, which survive negative selection, lead to the co-expression of IgD and IgM on the membrane, which is characteristic of mature B cells. Thus, the influence of PPARα on pro/pre-B cell stage indicates that this receptor may interfere with the process of pro- or pre-B cell proliferation.

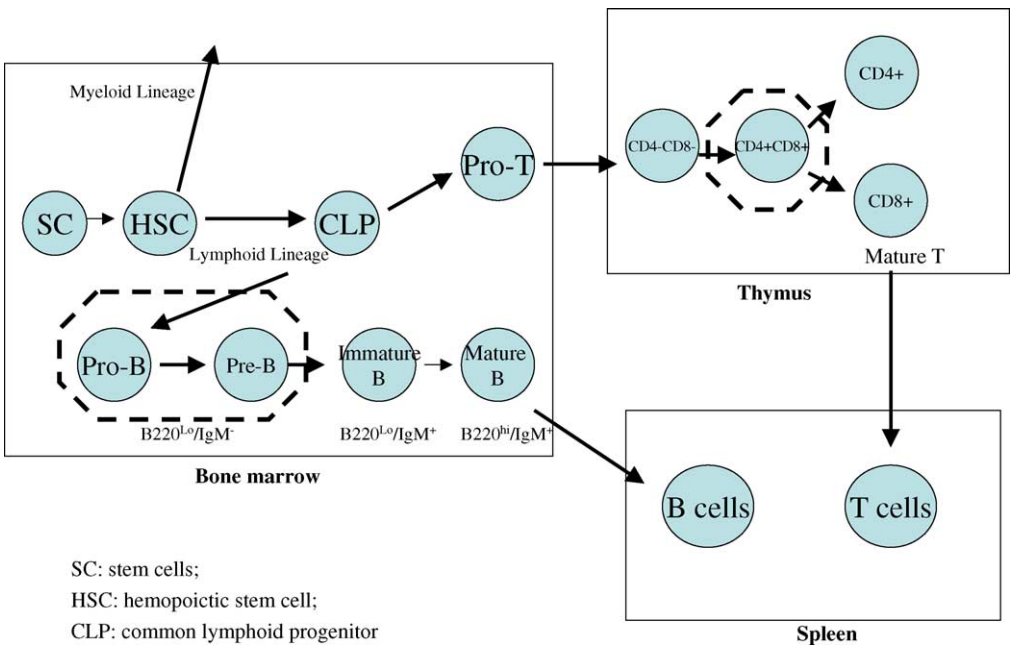


Fig. 3. A simplified schematic of lymphopoiesis from HSC to mature T and B cells in adult mice [46]. Steps in differentiation are delineated by additional surface markers but are not shown.

Although PPARα-null mice are unresponsive to PPs, their phenotype is normal except for some mild hepatosteatosis [22,23]. Previous studies revealed that PPARα-null mice have a normal thymus and spleen weight, and normal cell numbers, as well as normal in vitro responses of splenocytes to T- and B-cell activators, thus suggesting that these mice have a normal immune system [18]. At the same time, the small but significant increase in the $CD4^+CD8^+$ thymocyte population and increase in the number of thymocytes in the S-G/M phase of the cell cycle suggest that loss of PPARα influences the differentiation and development of T and B cell in thymus [18]. In this study, a small but significant increase in the pro/pre-B cells and also the higher numbers of WBC were observed in PPARα-null mice (Tables 1 and 2). Thus, PPARα is indeed involved in the normal development of immune system with influence on both T and B cells.

In comparison to the dramatic changes in the above parameters caused by PPs, the effect in PPARα-null mice is much smaller. Upon Wy-14,643 treatment, the number of total $B220^+$ cells was significant decreased in wild-type mice in a PPARα-dependent manner. The loss of $B220^+$ cells was due to a dramatic decrease in pro/pre-B cells, while immature and mature B cells were unchanged (Fig. 1 and Table 1). This finding may account for the previous observation [15] of dramatic loss of B cells in the spleen upon the same treatment.

Although PPARα has been identified in mature T, B cells [24] and also thymus and spleen, we were unable to detect mRNA for this receptor in BM. Based on this finding, we hypothesized that PPARα mediates many processes connected with the B cell development through an indirect mechanism.

Secondary effects on immune system as a consequence of primary effects on other organs are well known [25,26]. It is well established that glucocorticoid hormone, produced by the adrenal gland, induces the apoptosis of thymocytes in thymus and B cells in BM [27–29]. However, corticosterone-induced apoptosis was observed in all B lymphocytes subsets at both physiological and pharmacological concentrations [29]. Previous studies have shown that other PPs (e.g., nafenopin and clofibrate) do not increase the concentration of corticosterone in the blood of rodents [30,31]. Thus, it appears unlikely that the effects of PPs on immune system development in mice is due to effects on circulating levels of cortisone.

Previous studies indicate that the mechanism(s) underlying decrease in thymocytes upon Wy-14,643 treatment is indirect [15] although PPARα is present in thymus. Thus, a question is raised concerning the molecular mechanism by which PPARα is involved in these processes. All of the target genes for PPARα identified to date encode proteins mainly involved in lipid transport and metabolism [32]. Therefore, the involvement of lipids in the functions of the immune system is of interest.

Among other factors, the immune system must be generally supplied with fatty acids for its development and physiological response, because the cells of immune tissues do not carry out de novo synthesis of fatty acids [33–35]. The physiological significance of lipids, and especially of plasma lipoproteins and cholesterol, for the immune system has been described [33,35–39]. Furthermore, perturbation of the compositions of both external and internal membranes of the lymphocyte can attenuate signal transduction by the T-cell receptor (TCR) and B-cell receptor (BCR), which plays a central role in many aspects of immunological defenses [40,41].

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 apolipoprotein C-III, hepatic lipase and lecithin-cholesterol acyltransferase, in addition to the up-regulation of lipid metabolism associated with peroxisomal and mitochondrial fatty acid β-oxidation [42,43]. All of these effects are mediated by PPARα and may result in reduced availability of serum lipids to peripheral tissues, including lymphoid tissues. Indeed, it was reported that significantly low serum lipid levels influences hematoipoietic activity within BM [44]. Thus, the major mechanism by which PPs exert immunomodulation may be through modulating serum lipid levels.

In this connection, it was reported that basal fatty acid homeostasis in PPARα-null mice is altered, i.e. abnormal accumulation of lipid in the liver, lower serum triglycerides and altered constitutive expression of fatty acid-metabolizing enzymes [11,14]. Indeed, it was previously observed that these animals have 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 [18]. Similarily in this study, the small, but significant increase in the pro/pre-B cells was observed in PPARα-null mice (Fig. 1 and Table 1). 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 Wy-14,643. For example, it was reported that Wy-14,643 can exert in vitro apoptotic effects on splenocytes in rodents via a PPARα-independent pathway [16]. This may contribute the loss of WBC in PPARα-null mice upon Wy-14,643 treatment as observed in this study (Table 3). Thus, further experimentation using other PPs is required to determine whether different PPARα ligands produce the same effects as observed with Wy-14,643.

In 1996, it was proposed that PPARα might be involved in inflammatory processes, since PPARα-null mice display a prolonged inflammatory response [45]. The inflammatory responses are also regulated by the adaptive immune system. For example, T lymphocytes release cytokines which activate the phagocytes to destroy internalized

pathogens. In addition, phagocytes use antibodies released by B lymphocytes to allow them to more effectively recognize pathogens. PPARα-null mice have much higher WBC as observed in this study and the potent immunosuppression by PPARα agonists may suggest an alternative mechanism for the anti-inflammatory effects.

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

In conclusion, these studies revealed that PPARα has a regulatory effect on B cell development within the BM in mice. This regulatory control appears to be very early pro/pre B stage during B cell development. Additional studies using specific markers to more precisely define B cell development is also needed to determine the specific maturation stage at which the PPARα-mediated response occurs.

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