REVIEW

Sex-specific regulation of immune responses by PPARs

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The prevalence of autoimmune, infectious and metabolic diseases is different for men and women owing to the respective ability of their immune systems to respond to self and foreign antigens. Although several factors, including hormones and the X-chromosome, have been suggested to contribute to such sex-specific immune responses, the underlying factors remain poorly defined. Recent studies using peroxisome proliferator-activated receptor (PPAR) ligands and knockout mice have identified sex-dimorphic expression of PPARs, and have shown that the inhibitory functions of PPAR in T cells are substantially affected by the sex hormones. In this review, we consider the sex-specific differences in PPARs and summarize the diverse PPAR-mediated, sex-specific properties of effector T-cell responses, such as T-cell activation, survival and differentiation, as well as their involvement in T-cell-related autoimmune diseases, including colitis, graft-versus-host disease and experimental autoimmune encephalomyelitis. Understanding PPAR-mediated sex differences in immune responses will provide more precise insights into the roles of PPARs in effector T cells.

SEX DIFFERENCES IN THE INCIDENCE OF DISEASES

Sex is a biological factor that contributes to physiological and anatomical differences. Immunological sex differences also exist and cause disparate responses to both self and foreign antigens.[1] In general, males have a greater prevalence and severity of bacterial, fungal and parasitic infectious diseases than females.[2] In addition, the RNA viral load in females with an acute HIV infection is 40% less than that in males,[3] and the risk of death from malignant cancer is twofold higher in males than in females.[1] Moreover, antibody production from vaccination against influenza virus is stronger in females than in males, demonstrating that females generate stronger immune responses than males.[4] Although the heightened immune response of females is highly effective with respect to clearing pathogens and enhancing the vaccination efficiency, it also contributes to an increased susceptibility to inflammatory and autoimmune diseases compared with males.[5,6] Indeed, there is a higher proportion of females with autoimmune disease than males,[7–10] with females accounting for more than 60% of the incidence of Sjögren's syndrome, systemic lupus erythematosus (SLE), thyroid disease, rheumatoid arthritis, multiple sclerosis (MS) and Grave's disease.[11–13]

In addition to immune disease, there are also sex-specific differences in the prevalence of metabolic diseases. The risk of cardiovascular diseases is increased in females compared with that in males, whereas more males have type II diabetes mellitus.[14,15] Insulin-sensitizing drugs also have sex-specific differences and are more effective in females as evidenced by reduced fasting plasma glucose levels.[16] The genetic factors related to the sex chromosomes,[17–19] microRNAs,[20–22] hormonal mediators estrogen,[23–26] progesterone,[27–29] and androgens,[30–33] and environmental mediators of nutrition[34,35] and microbiota[36–38] have all been suggested to affect sex-based differences in various diseases.

SEX-SPECIFIC DIFFERENCES IN IMMUNE RESPONSES
Innate immunity

Sex affects multiple aspects of innate immunity, which has an essential role in the regulation of non-specific and immediate defense against pathogens.[39] Innate immune cells such as macrophages and dendritic cells (DCs) express several pattern-recognition receptors (PRRs) that recognize and respond to various antigens. Male peritoneal macrophages express higher levels of cell surface TLR4 protein than female cells, and lipopolysaccharide-challenged male mice have higher levels of pro-inflammatory cytokine and chemokine production, including IL-6, IL-1β and IL-10.[40,41] Male neutrophils also show higher expression of TLR4 and produce greater amounts of TNF-α than female cells following lipopolysaccharide stimulation.[42] These inflammatory responses in males are

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believed to be responsible for the greater susceptibility of males to bacterial septic shock. In addition, viral ligand-induced TLR9 activation of male peripheral blood mononuclear cells (PBMCs) results in a greater production of IL-10 than that of female cells, a finding that is highly correlated with the plasma level of sex hormones as IL-10 cytokine production between males and post-menopausal women does not show any differences compared with males and females at reproductive age.[43]

The promoter regions of genes in innate immune cells contain putative androgen, glucocorticoid and estrogen elements[44] suggesting that hormones have profound effects on the function of innate immunity. Compared to males, females have greater TLR7 gene expression. As TLR7 is encoded on the X-chromosome, the increased expression in females may be due to incomplete X inactivation, whereas males have a potentially lower expression level of TLR7.[45–47] Female PBMCs and plasmacytoid DCs (pDCs) also have increased IFN-α production compared to male cells following TLR7 ligand stimulation,[48,49] which is associated with reduced viral RNA following viral infection.[44] Interestingly, castrated male mice have anti-viral responses that are comparable with those of females, including the induction of PRRs and anti-viral genes,[19] providing further evidence of the role of sex in the innate immune response.

Adaptive immunity
Sex influences the adaptive immune system, and several studies have shown that females generate a stronger immune response than males. For example, the proportion of CD4$^+$ T cells and Treg cells within the total CD4$^+$ population is higher in females than in males, whereas males have a higher number of CD8$^+$ T cells.[50–53] Owing to the higher number of CD4$^+$ T cells, females generate a higher number of activated CD4$^+$ T cells than males following T-cell receptor (TCR) stimulation.[54] Interestingly, anti-viral and pro-inflammatory genes are upregulated to a greater degree in activated female cytotoxic T cells than in male cells, and half of these genes have estrogen-response elements (EREs) in their promoter region,[55] suggesting that sex hormones may influence the regulation of cytotoxic T-cell activity.

Females have robust Th1 responses, and female CD4$^+$ T cells produce higher levels of IFN-γ than male T cells. IFN-γ serves as an important factor in the onset of experimental autoimmune encephalomyelitis (EAE).[56] The enhanced production of IFN-γ by CD4$^+$ T cells in females is responsible for their superior protection against infectious diseases such as *Leishmania*.[2] In addition, female CD4$^+$ T cells produce less IL-17 than male CD4$^+$ T cells,[33] further demonstrating sex-specific differences in CD4$^+$ T-cell-mediated cytokine production in males and females. Females also display greater antibody responses, including higher B-cell numbers and basal immunoglobulin levels, than males,[57,58] possibly leading to faster viral clearance in females. Finally, antibody responses to viral and bacterial vaccines are higher in females than in males, suggesting that vaccination efficacy is better in females than

in males. For example, a half dose of vaccination against influenza virus in females results in similar antibody production to that of a full dose of vaccine in males.[59]

THE ROLE OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS IN THE IMMUNE RESPONSE

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that regulate lipid and glucose metabolism,[60–63] as well as cell survival[64] and immunity.[65–67] Tissues that require high amounts of energy to maintain their own function, such as liver, adipose, muscle and heart tissues, have high expression of PPARs,[68] implying that PPARs act as regulators of metabolism. PPARs are also expressed in various immune cells such as monocytes, macrophages, T cells and B cells,[69,70] where they may also regulate immune function.

PPARα is expressed in macrophages, granulocytes and lymphocytes.[70] Ligand-activated PPARα represses NF-κB activity and IL-2 production in lymphocytes,[70] and regulates IL-4- and IL-5-induced expression of target genes in T cells.[71] PPARα-deficient T cells produce higher amounts of IFN-γ and TNF-α with increased NF-κB and c-jun activity compared to wild-type T cells. PPARα-deficient mice are also susceptible to EAE, indicating that PPARα has an anti-inflammatory function as a negative regulator of T cells.[56] The PPARα ligand fenofibrate effectively reduces the severity of both EAE and colitis by inhibiting IFN-γ and IL-17 production,[71–73] indicating that PPARα ligands may be useful for the treatment of autoimmune diseases. In addition, PPARα has an important role in the suppression of allergic contact dermatitis by Treg cells[74] as the proportion and suppressive function of Treg cells are decreased in PPARα-deficient mice.

The PPARβ/δ agonist GW-0742 has protective, immune-modulatory functions against EAE.[75] On the other hand, mice lacking PPARβ/δ expression display severe EAE clinical signs with significant accumulation of IFN-γ$^+$IL-17A$^-$ and IFN-γ$^+$IL-17A$^+$ T cells in the spinal cord.[76] Additional anti-inflammatory functions of PPARβ/δ have been identified, including the fact that they are essential for the clearance of apoptotic cells.[77] Specifically, treatment with PPARβ/δ ligands augments the clearance of apoptotic cells, whereas delayed uptake of the cells found in the absence of PPARβ/δ results in a SLE-like autoimmune disease. These findings suggest that PPARβ/δ also has a significant role in inhibiting T-cell activation and preventing autoimmune disease.

PPARγ has two isoforms, with PPARγ1 distributed in most tissues and PPARγ2 dominantly expressed in adipose tissue.[68,78] PPARγ ligands have inhibitory effects on monocyte TNF-α, IL-1β and IL-6 cytokine production.[79,80] PPARγ ligands and knockout mouse studies have demonstrated the importance of PPARγ in T-cell activation, survival, differentiation and autoimmune disease. The PPARγ ligands 15d-PGJ$_2$ and ciglitazone inhibit IL-2 cytokine production and proliferation of T cells,[81] demonstrating the importance of functional PPARγ expression for the ligand-mediated regulation of immune responses. PPARγ expression is decreased in the

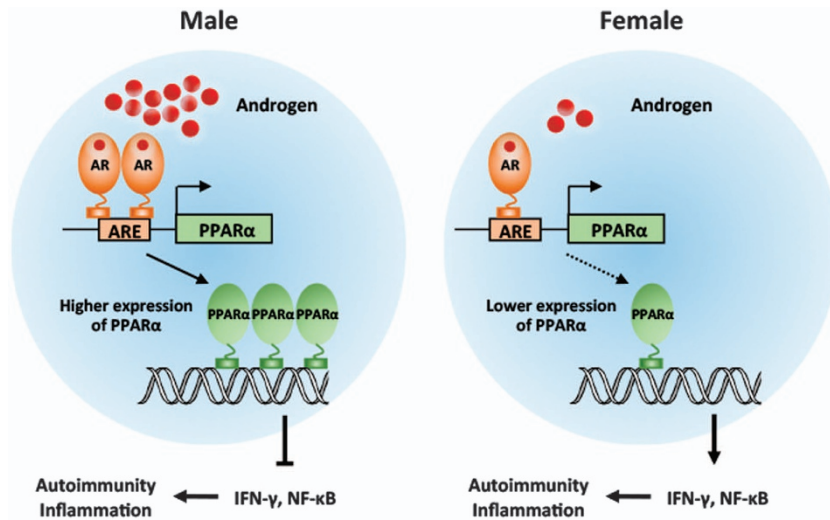Figure 1 An androgen-sufficient environment enhances PPARα activity to regulate autoimmunity by suppressing IFN-γ production. Males have higher androgen hormone levels than females, and this androgen-sufficient environment contributes to enhanced PPARα expression in male T cells by bringing the androgen receptor (AR) to the ARE present in the promoter region of the PPARα gene. Increased expression of PPARα by androgen hormone inhibits NF-κB activity and IFN-γ production in male T cells following TCR stimulation to control inflammation and autoimmunity, such as in experimental autoimmune encephalomyelitis (EAE). In contrast to males, limited amounts of androgen hormone in females do not lead to increased expression of PPARα, which, in turn, fails to suppress NF-κB activity and IFN-γ production in female T cells.

PBMCs of MS patients compared with that in healthy controls,[82] and pioglitazone treatment suppresses the human allogenic T-cell response in an arterial graft model,[83] altogether indicating that PPARγ expression and activation have an important role in preventing the onset of autoimmune disease and graft rejection.

PPARγ has been reported to be indispensable for the accumulation of Treg cells in visceral adipose tissue (VAT). Treg cells do not accumulate in the VAT of mice with Foxp3-specifically deleted of PPARγ,[84] indicating that PPARγ is a crucial mediator of Treg cell accumulation, phenotype and function by regulating the expression of chemokine receptors necessary for the migration of Treg cells. Recent studies have also revealed an important role for PPARγ in the loss of the VAT Treg cell phenotype resulting from the phosphorylation of the serine residue at the PPARγ position 273.[85] Specifically, this PPARγ serine residue acts as a checkpoint for whether VAT Tregs will retain their characteristic transcriptional signature.

PPARγ expression in regulatory T (Treg) cells has an essential role in preventing colitis[86] and graft-versus-host disease (GVHD).[87] PPARγ-deficient Treg cells have an impaired ability to downregulate effector T-cell-mediated colitis and GVHD, suggesting that the expression of PPARγ in Treg cells is essential for the inhibitory function of these cells in the immune response, and points to the immune-therapeutic potential of targeting PPARγ. However, this finding is contradicted by a report that PPARγ is required for the development of autoimmune diseases such as colitis under lymphopenic conditions due to the apoptotic characteristics of PPARγ-deficient T cells with decreased levels of IL-7Rα.[88]

SEX-SPECIFIC PPARα-DEPENDENT DIFFERENCES IN IMMUNE RESPONSES

PPARα expression is more abundant in naïve and activated male T cells than in female cells,[56] suggesting that PPARα has a more substantial role in male T cells than in female T cells. The production of IFN-γ and TNF-α, but not that of IL-17, is significantly increased in PPARα-deleted male T cells with augmented NF-κB and c-jun activity,[56] implying that PPARα effectively inhibits the production of inflammatory cytokines in males. Moreover, male PPARα-deficient mice are more susceptible to developing EAE with increased level of cytokines, including IFN-γ, TNF-α and IL-2, whereas female PPARα-deficient mice have comparable cytokine production and EAE pathogenesis compared to sex-matched littermate controls.[56] These findings suggest that PPARα can control the immune responses in males due to its increased expression.[56]

The male hormone androgen has been suggested to influence the expression of PPARα in male T cells. Chip analysis has shown that the androgen receptor can interact with the promoter region of PPARα.[33] Furthermore, castrated male mice exhibit increased Th1-cell infiltration into the CNS compared with the weak Th1 infiltration seen in sham male mice, as well as increased IFN-γ and decreased IL-17 cytokine production. Altogether, these data suggest that the androgen hormone is essential for maintaining PPARα expression in males to inhibit Th1 responses in the mouse EAE model.[33,89] In human T cells, PPARα-mediated suppression of IFN-γ production is more sensitive in males than in females.[33] Treatment of male T cells with PPARα siRNA results in increased IFN-γ production, whereas PPARγ siRNA-transfected female T cells have augmented IL-17 expression, suggesting that the different regulatory roles of PPARα and
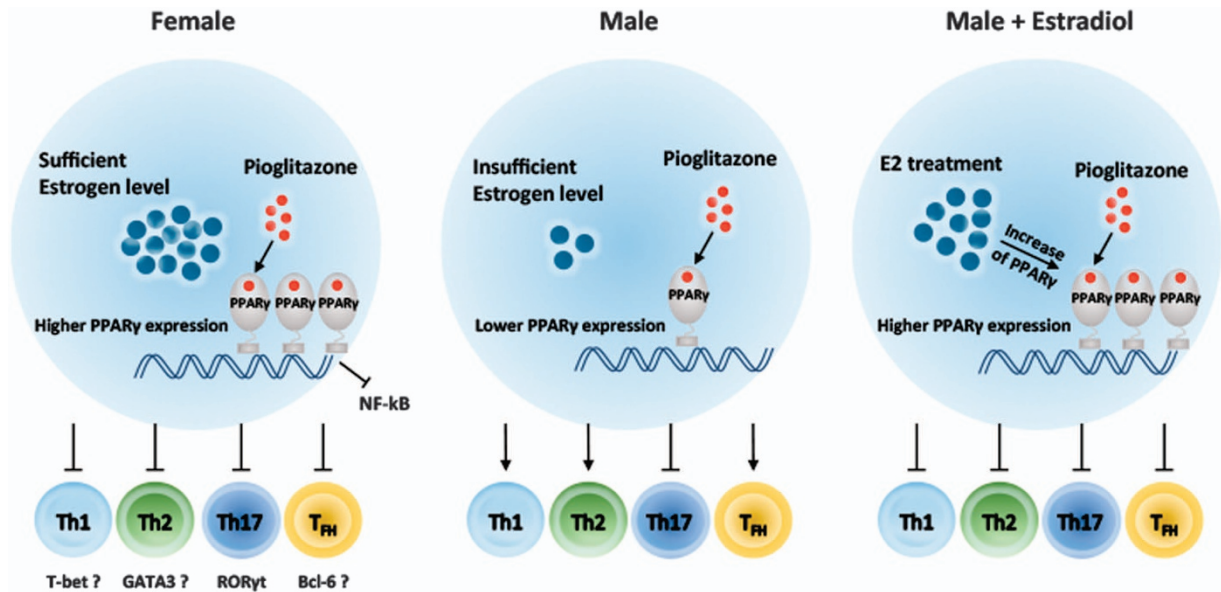
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Figure 2 An estrogen-sufficient environment promotes PPARγ activity to regulate effector T-cell responses. The estrogen-sufficient environment in females contributes to increased PPARγ expression levels in female T cells. A higher expression level of PPARγ enables female T cells to respond more effectively than male T cells to treatment with the PPARγ ligand pioglitazone to regulate Th1, Th2, Th17 and T_{FH} cells. In contrast, males have lower expression of PPARγ due to limited available estrogen hormone. Thus, they fail to suppress Th1, Th2 and T_{FH} cells in the presence of the PPARγ ligand pioglitazone, but instead exhibit the selective inhibition of Th17-cell differentiation. Estradiol administration in males enhances PPARγ expression and rescues pioglitazone-mediated repression of Th1, Th2 and T_{FH} cells as seen in females. Thus, sufficient levels of estrogen are advantageous for promoting PPARγ activity, and a synergistic effect between pioglitazone and estrogen is critical for effector T-cell responses.

PPARγ are dependent on sex. The sex-specific role of PPARα in T cells is summarized in Figure 1.

SEX-SPECIFIC PPARγ-DEPENDENT DIFFERENCES IN IMMUNE RESPONSES

The basal expression of PPARγ in CD4^{+} T cells is higher than that in CD8^{+} T cells and B cells.[90] The expression of PPARγ is also increased in CD4^{+} T cells following TCR stimulation.[56,90] PPARγ expression is also higher in female T cells than in males,[33,56,91] and the treatment of male T cells with estradiol enhances the expression of PPARγ, suggesting that the female sex hormone estrogen profoundly influences the expression of PPARγ in T cells. These data may also suggest that PPARγ may be more sensitively regulated by PPARγ ligand treatment in female T cells than in male T cells. In addition, PPARγ expression is higher in the estrus phase of the menstrual cycle than in the diestrus phase,[91] further demonstrating the importance of sex hormones in the regulation of PPARγ in females.

Recently, a study on the sex-specific differences regarding the role of PPARγ in T-cell survival showed that male PPARγ-deficient T cells undergo increased apoptosis with decreased levels of Bcl-2 and IL-7Rα, and comprise a larger proportion of apoptotic cells than female PPARγ-deficient T cells.[91] Another report suggested that PPARγ is required for the development of colitis in lymphopenic conditions, and that a lack of PPARγ results in decreased IL-7Rα, suggesting an important role of PPARγ in T-cell survival.[88] Although more convincing data are required to solve this

discrepancy, PPARγ may function as a survival factor in female T cells.

PPARγ ligand studies have shown that PPARγ acts as a negative regulator of T-cell activation by inhibiting NF-κB and NFAT transcription factors[92] to suppress cytokine production and proliferation. PPARγ-deficient T cells also have increased levels of cytokines and NF-κB activity following TCR stimulation.[90] This inhibitory role of PPARγ in T-cell activation is observed in female PPARγ-deficient T cells, but not in male T cells, suggesting that PPARγ is more important in females for NF-κB regulation. In addition, female PPARγ-deficient T cells produce enhanced lineage-specific cytokines in Th1, Th2, Th17 and Th9 cells under T-cell-differentiation-skewing conditions.[90] Recently, the sex-specific regulatory functions of PPARγ have been investigated in the differentiation of Th1, Th2 and Th17 cells between males and females. The PPARγ ligand pioglitazone inhibits the differentiation of female Th1, Th2 and Th17 cells, whereas it specifically reduces only Th17-cell differentiation in males.[93] These findings suggest that PPARγ profoundly and non-specifically influences the differentiation of female T cells but selectively inhibits the formation of male Th17 cells. Although pioglitazone single treatment does not affect the differentiation of Th1 and Th2 cells in male T cells, estradiol enhances PPARγ expression and suppresses Th1 and Th2-cell differentiation,[93] suggesting that PPARγ may have stronger effects on inflammatory and allergic diseases in an estrogen-repleted environment. These data are supported by

another recent study that investigated the sex-specific regulatory functions of PPARγ, showing that PPARγ selectively inhibits the differentiation of Th17 cells, but not that of Th1, Th2 and Treg cells, by suppressing the RORγt transcription factor without affecting T-bet, GATA3 or Foxp3.[94]

The sex-specific functional activity of PPARγ has been proposed for T_{FH} cells and germinal center (GC) responses.[90] Spontaneous autoantibody production, glomerular inflammation and increased T_{FH} cells identified as $CD4^+CD44^{high}$ $PSGL1^{low}CXCR5^+PD-1^+$,[95] and GC reactions are present in female CD4-PPARγ^{KO} mice, but not in male mice, suggesting that PPARγ regulates T_{FH} responses more sensitively in females than in males. T_{FH} responses are suppressed by PPARγ in SRBC- or NP-OVA-immunized mouse models.[90,91] Interestingly, this regulatory function of PPARγ in T_{FH} responses and autoimmune phenotypes is sex-specific.[91] Specifically, the PPARγ ligand pioglitazone reduces $CD4^+CD44^{high}Bcl-6^+$ $CXCR5^+$ T_{FH} cells and GC formation only in females dependent on estrogen, and this response is well-correlated with PPARγ expression.[91] Estradiol administration in males increases the expression of PPARγ, and pioglitazone also inhibits T_{FH}-cell and GC responses in males. This result implies that estrogen sufficiency is advantageous for promoting PPARγ activity, and that the synergistic effect of pioglitazone and estrogen is critical for controlling T_{FH} responses. The sex-specific regulatory roles of PPARγ in effector T cells are summarized in Figure 2.

SEX-SPECIFIC PPARβ/δ-DEPENDENT DIFFERENCES IN IMMUNE RESPONSES

The role of PPARβ/δ was investigated in the EAE animal model, and PPARβ/δ was shown to suppress the production of IFN-γ, IL-17, IL-12p35 and IL-12p40 in the brain and spleen, and ameliorate EAE independent of sex.[96] This result may be due to the comparable level of PPARβ/δ expression in male and female naive and activated T cells.[56] On the other hand, female skeletal muscle has higher PPARβ/δ mRNA expression than males but comparable levels of protein.[97] Although the regulatory role of PPARβ/δ in T cells has not been well studied, sex-specific differences in the regulation of PPARβ/δ should be considered in future studies.

CONCLUSIONS

In general, the prevalence of autoimmune, infectious and metabolic diseases are distinctly different for males and females. Sex-specific differences in immune responses contribute to the differences in both the prevalence and severity of these diseases. More specifically, the recent literature has suggested that the sex-specific functional regulation of PPARs in T cells is mediated by sex hormones, including estrogen and androgen, which may provide an explanation for the observed differences in disease outcomes. Therapeutic strategies using PPAR ligands in T-cell-mediated diseases such as autoimmune disease should consider co-treatment with sex hormones. Further studies will be required to more precisely elucidate the molecular mechanisms of PPARs and other transcription

factors that regulate effector T-cell functions and hormonal responses.

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