A Functional Relay from Progesterone to Vitamin D in the Immune System

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Progesterone is a steroid hormone that promotes and maintains pregnancy. Vitamin D (vit. D), another steroid hormone, regulates calcium levels and bone health among many of its functions. The two hormones play important roles also in regulating the immune system. Recently, we discovered that the vitamin D receptor (VDR) is induced in T cells by progesterone. This finding connects the function of progesterone to that of vit. D and suggests that the two steroid hormones cooperate with each other for sequential and effective regulation of the immune system. Potential implications of the regulation in health and disease are discussed.

Introduction

STEROID HORMONES, such as progesterone (P4), vitamin D (vit. D), estrogen, androgen, and mineralocorticoids, play important roles in regulating a number of different biological processes. Steroid hormones each have their own specific biological functions mediated by one or more ligand-specific receptors, but they can work together at the same time for combinatorial effects on a cell type. Also, one steroid hormone can enhance the function of another hormone for sequential regulation over time. Individual actions of steroid hormones are important, but without their cooperative and sequential actions, it is difficult to achieve highly complex regulations of a number of biological processes. A good example of such functional relays is the induction of progesterone receptor expression in breast cells and uterine stromal cells by estrogen (Kastner *et al.*, 1990; Graham and Clarke, 1997; Kurita *et al.*, 2001). This process is important for the menstrual cycle, mammary gland development, and pregnancy maintenance. Recently, we discovered that P4 induces vitamin D receptor (VDR) expression (Thangamani *et al.*, 2015). We observed this regulation in T cells, and therefore, the immediate impact of our finding is on immune regulation (Fig. 1).

P4 Is a Primary Suppressor of T Cells During Pregnancy

Successful pregnancy requires suppression of inflammatory T cells that are reactive to pregnancy. Some of these T cells can recognize and mount inflammatory responses to fetal antigens. Failed suppression of these T cells during pregnancy can lead to pregnancy complication and miscarriage. Major T-cell subsets that are reactive in pregnancy are effector T cells that produce inflammatory cytokines such as

IFN γ or IL-17 (Hill *et al.*, 1995; Raghupathy, 1997; Nakashima *et al.*, 2010; Toldi *et al.*, 2011). IFN γ is a key cytokine that activates tissue cells, macrophages, and other cell types to create inflammatory conditions. IL-17 is another cytokine that activates tissue cells to express chemokines to recruit neutrophils and cytokines to activate the recruited neutrophils. A major mechanism to suppress the T-cell response during pregnancy is mediated by P4. P4 effectively suppresses the generation of T cells producing IFN γ (Th1 cells) or IL-17 (Th17 cells) (Piccinni *et al.*, 1995; Miyaura and Iwata, 2002; Lee *et al.*, 2011, 2012). In females, P4 is produced by the corpus luteum of the ovaries after ovulation and in the placenta during pregnancy. The concentration can reach from a few nanograms per milliliter at the peak of the menstrual cycle to a few micrograms per milliliter during the third trimester of pregnancy. This high concentration may be important to regulate many cell types that are relatively less sensitive to P4 than uterine or mammary gland cells, which highly express nuclear P4 receptors and thus are sensitive to P4. T cells are regulated at relatively high P4 concentrations (e.g., greater than a few hundred nanograms per milliliter). This level of P4 can effectively suppress the generation of Th1 and Th17 cells (Lee *et al.*, 2011, 2012). At the expense of the effector T cells, P4 induces a subset of T cells called “regulatory T cells.” P4-induced regulatory T cells effectively suppress the activation and expansion of effector T cells (Lee *et al.*, 2011, 2012). T cells that are activated in the presence of P4 are low in their proliferative potential and display a feature similar to that of tolerized T cells, which are unable to produce inflammatory cytokines. P4 also regulates the development of myeloid cells and dendritic cells to promote immune tolerance (Butts *et al.*, 2007). The impact of P4 can reach beyond female pregnancy because both males and females begin to produce P4,

FIG. 1. P4 induces vitamin D receptor (VDR) in T cells for enhanced regulation by vitamin D (vit. D) metabolites. T cells undergo activation in lymphoid tissues by antigen-presenting cells such as dendritic cells. This activation is antigen specific and induced mainly by foreign protein antigens, which can be from pathogens or even the fetus during pregnancy. In high P4-producing conditions or tissue sites such as the placenta or fetal/maternal blood circulation during pregnancy, P4 induces VDR induction in T cells undergoing TCR-mediated activation. This induction is mediated by P4 receptor-binding sites in the regulatory region of the *VDR* gene. Enhanced expression of VDR in T cells confers the ability to detect suboptimal concentrations of vit. D metabolites. Alternatively, the induction allows T cells to increase their response to vit. D in a normal physiological range. A consequence of this regulatory process would be effective suppression of inflammatory T cells (e.g., IFN γ -producing Th1 cells and IL-17-producing Th17 cells) that can induce tissue inflammation and pregnancy termination. It is expected that a similar collaboration between the two hormones may occur beyond pregnancy as P4 is produced in nonpregnant conditions although at relatively low concentrations.

although at low levels from the adrenal glands after the onset of adrenarche during puberty. In this case, P4 may play a homeostatic role in regulating the immune system and other biological processes in a relatively more limited manner in space and magnitude compared to that of pregnancy.

Vitamin D Is Another Immune-Regulatory Steroid Hormone

A cholesterol precursor (7-dehydrocholesterol) undergoes a series of metabolic processes in multiple organs to make calcitriol (also called 1,25-dihydroxyvitamin D3), the most active form of vit. D. This involves an ultraviolet-dependent process in the skin and sequential enzymatic processes in the liver and kidneys. Calcitriol is also made locally in myeloid cells and some cells in the lungs, skin, breast, and other organs because these cells express 1α -hydroxylase/CYP27B1 (Hewison *et al.*, 2004), the last enzyme in the calcitriol synthesis pathway. Vit. D has been extensively studied for its effect on immune cells. Active forms of vit. D such as calcitriol regulate T-cell maturation into effector and regulatory T cells (Boonstra *et al.*, 2001; Gorman *et al.*, 2007; Thangamani *et al.*, 2015). Calcitriol suppresses naive T-cell differentiation into Th1 and Th17 cells, but reciprocally enhances T-cell differentiation into regulatory T cells. Calcitriol at a few nanomolar concentrations is sufficient to induce the regulatory effects.

The regulatory T cells induced by calcitriol are a mixture of FoxP3⁺ and FoxP3⁻ regulatory T cells and express regulatory cell-associated CD25, FoxP3, CD38, and/or LAP-TGF- β 1 (Stoeckler *et al.*, 1996; Kang *et al.*, 2012; Thangamani *et al.*, 2015). In a manner somewhat similar to P4, vit. D also regulates the maturation of myeloid dendritic cells to indirectly generate regulatory T cells (Penna *et al.*, 2007). Overall, vitamin D is an important regulator to prevent T-cell-dependent inflammatory responses.

Vitamin D Receptor Induction by P4 and Its Impact on the Immune System

We found that calcitriol becomes highly effective in regulating T cells when P4 is present at the same time (Thangamani *et al.*, 2015). P4 enhances the T-cell response to calcitriol by three to five times. Thus, there is a clear synergistic collaboration between vit. D and calcitriol in regulating T cells. We found that P4 induces *VDR* gene expression (Fig. 1). This induction of VDR by P4 is mediated directly through the binding of nuclear P4 receptors to the 5' upstream regulatory region of the *VDR* gene. Since P4 can work through additional receptors, including the glucocorticoid receptor and progesterone receptor membrane component 1 (PGRMC1), we do not exclude the possibility that additional receptors may regulate VDR expression. Blockade of VDR expression abolished the synergistic regulation of T cells by P4 and calcitriol (Thangamani *et al.*, 2015). Thus, VDR induction by P4 is to more effectively regulate T-cell differentiation by vit. D. There are a number of potential benefits with this type of sequential regulation by the two steroid hormones. First, it allows cell type-specific coregulation by the two hormones, provided that VDR is induced by P4 only in certain cell types such as T cells. Second, this mechanism allows vit. D-dependent regulation in a tissue-specific manner. For example, the induction of VDR in T cells can occur specifically in the female reproductive tract where the P4 concentration is high. Third, it allows time-dependent regulation. For example, P4 acts first and then vit. D acts later after a time gap. Fourth, it allows counter-regulation or modification of some of the functions of the first hormone (i.e., P4) by the second hormone (i.e., vit. D). P4 and vit. D are not identical to each other in their regulatory functions, and therefore, the sequential regulation by the two hormones can induce more than additive effects.

Potential Implications in Other Biological Processes

While the finding of VDR induction by P4 is primarily important for the immune system, it has far-reaching ramifications in other systems. VDR is upregulated in multiple tissues, including the uterus, placenta, and spleen of pregnant females (Walters, 1981; Thangamani *et al.*, 2015). The collaborative regulation between the two hormones may occur in more cell types than T cells. For example, VDR is expressed by intestinal epithelial cells and kidney cells, and vit. D is a central regulator of calcium absorption and bone mineralization (Favus *et al.*, 1988; Xue and Fleet, 2009; Wang *et al.*, 2012). It is a question of interest if these tissue cells are also regulated by P4. This may shed light on the potential interaction between P4 and vit. D during skeletal development and menopause. Another function of vit. D is

to protect the central nervous system (CNS). Low Vit. D levels are linked to cognitive impairment, Alzheimer's disease, and multiple sclerosis (Kesby *et al.*, 2011). Interestingly, P4 is another neuroprotective hormone, protecting the CNS from injuries and tissue inflammation (Djebaili *et al.*, 2004; Wright *et al.*, 2007). Vit. D also has a similar protective effect on the CNS (Garcion *et al.*, 2002). The two hormones when used in combination had synergistic effects in protecting CNS cells from cell death (Atif *et al.*, 2009; Cekic *et al.*, 2009). There is a report that the protective efficacy of P4 was significantly decreased in vit. D deficiency (Cekic *et al.*, 2011), indicating that the neuroprotective function of P4 depends on vit. D availability. The mechanism for this synergism is yet to be established, but this may be explained by the sequential interplay of the two steroid hormones. At least in an animal model of multiple sclerosis, it has been shown that the vit. D effect is mediated through T cells (Chang *et al.*, 2010; Mayne *et al.*, 2011).

Concluding Remarks

The two nuclear hormones, P4 and vit. D, each play important roles in regulating a number of biological processes, including the immune system. T cells are directly and indirectly regulated by these two hormones. The connection between P4 and VDR expression in T cells may explain some of their overlapping regulatory functions in the immune system and other tissue systems such as the CNS. T cells are perhaps behind in many of the cooperative interactions between the two hormones, but it is possible that other cell types may be similarly regulated. More work is required to fully establish the functional interaction between the two steroid hormones in the regulation of health and disease in the body.

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Disclosure Statement

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