Progesterone-based compounds affect immune responses and susceptibility to infections at diverse mucosal sites

Olivia J Hall¹ and Sabra L Klein¹

Over 100 million women worldwide are currently on progesterone-based contraceptives to improve their health outcomes through reduced maternal mortality and family planning. In addition to their role in reproduction, progesterone-based compounds modulate immune responses throughout the body, particularly at mucosal sites. By binding to receptors located in immune cells, including natural killer cells, macrophages, dendritic cells, and T cells, as well in non-immune cells, such as epithelial and endothelial cells, progesterone-based compounds alter cellular signaling and activity to affect the outcome of infections at diverse mucosal sites, including the genital, gastrointestinal, and respiratory tracts. As the use of progesterone-based compounds, in the form of contraceptives and hormone-based therapies, continue to increase worldwide, greater consideration should be given to how the immunomodulatory effects these compounds alter the outcome of diseases at mucosal sites beyond the reproductive tract, which has profound implications for women's health.

INTRODUCTION

Females and males experience changes in the hormonal milieu throughout the life course, which are typically not considered in the context of infectious diseases at mucosal sites. Prior to puberty, circulating concentrations of estrogens and progesterone (P4) in females and androgens in males are low, with concentrations increasing significantly after puberty and through adulthood, with fluctuations in females occurring during the menstrual cycle and to a greater extent during pregnancy (see **Figure 1**).¹ With older age, concentrations of estrogens and progestins decline rapidly in females and concentrations of androgens decline gradually in males. Only recently, have we begun to appreciate that these changes in sex steroids over the life course impact immune responses² and the outcome of infectious diseases within but also outside of the reproductive tract.³ Mucosal sites, including the genital, respiratory, and gastrointestinal tracts, are particularly vulnerable to infections as they come in contact with the exterior of the body and form the main route of entry for infections.⁴ Despite these mucosal sites being anatomically distinct compartments, immune responses and the impact of sex steroids, in particular

P4 and related compounds, can be similar and suggest that P4 can affect the outcome infectious diseases beyond the reproductive tract.

Progestins and receptor signaling

Natural P4 is produced by the corpus luteum during the menstrual cycle in non-pregnant females and its production is sustained at high levels by the placenta during pregnancy. Serum levels of P4 fluctuate during the menstrual cycle with a peak of 20 ng ml⁻¹ during the luteal phase and the nadir (<1 ng ml⁻¹) during the follicular phase⁵ (**Figure 1**). During pregnancy, the production of P4 is maintained by the placenta with increases from 12 to 90 ng ml⁻¹ during first trimester and up to 300 ng ml⁻¹ during the third trimester of pregnancy⁵ (**Figure 1**). Synthetic analogs of P4, termed progestins, are used as hormonal contraceptives by over 20 million young adult women in the United States alone.⁶

Progestins and P4 can freely diffuse through the cell membrane and signal through the progesterone receptor (PR), located in the cytoplasm, which when bound, translocates to the nucleus, and binds progesterone response elements to

¹W. Harry Feinstone Department of Molecular Microbiology and Immunology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland USA. Correspondence: SL Klein (sklein2@jhu.edu)

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Figure 1 Serum progesterone concentrations vary between men and women and over the life course. Concentrations of progesterone fluctuate over the life course in females, with increased concentrations at puberty, cyclical changes during the menstrual cycle, and a steady rise during pregnancy followed by a sharp decline post-partum. At menopause, concentrations steadily decline to levels that are similar to those prior to puberty. In men, levels of progesterone remain steady throughout the life course. Based on values from.¹

Figure 2 Progesterone receptor signaling in immune cells. Pathogens and pathogen associated molecular patterns (PAMPs) are sensed through pattern recognition receptors (PRRs) either on the surface of cells or intracellularly. PRRs signal through the NF-κB pathway to induce the production of pro-inflammatory cytokines and inflammation. Progesterone (P4), being lipophilic, diffuses across the lipid bilayer of the cell membrane, binds to the progesterone receptor (PR) in the cytosol, and the complex dimerizes and translocates to the nucleus of the cell to alter cellular transcription. This interaction can directly interfere with the NF-κB pathway to lower inflammatory responses. Progesterone when bound to its receptor can also induce the transcription of proteins and growth factors that dampen inflammation and promote repair.

alter transcription and downstream signaling (**Figure 2**). PR signaling can also occur in a non-genomic signaling manner via the MAPK or PI3K/Akt pathway, for example, or through

membrane-bound PRs that signal by either increasing cAMP or through the JNK pathway and bypass the classical PR pathway all together.^{7–10} PRs have two isoforms, A and B, which are

derived from the same gene.¹¹ Progestins bind the PR with higher affinity than P4; with levonorgestrel (LNG), for example, binding the PR with a 300% affinity relative to P4.¹² These PR are present in a wide range of tissues, in a variety of different cell types, including immune cells such as natural killer (NK) cells, macrophages, dendritic cells (DCs), T cells and non-immune cells, such as epithelial cells, endothelial cells, and neuronal cells.^{13–15} Natural and synthetic forms of P4 can also signal through other steroid receptors including the androgen, glucocorticoid, and mineralocorticoid receptors.^{16,17} For example, depot medroxyprogesterone acetate (DMPA) signals through both the PR and glucocorticoid receptor, LNG signals only through the PR, and P4 signals preferentially through the PR but can also signal through the glucocorticoid receptor.¹² As each of these receptors are widely distributed throughout the body, the effects of natural and synthetic forms of P4 are not limited to the reproductive tract, but can occur at other mucosal sites, including the respiratory and gastrointestinal tracts.

Bringing together diverse bodies of literature, we seek to illustrate that progestins affect immune responses to microbes at diverse mucosal sites. Because women are exposed to both natural and synthetic forms of P4 throughout their lifetime, we will use this hormone to make the broader point that the hormonal milieu can drastically alter the outcome of infectious diseases at mucosal sites. Whether the immunomodulatory effects of P4-based compounds confer protection or increase susceptibility to infectious diseases can be cell- and site-specific, which will be addressed. Although pregnancy and the associated hormonal changes, including the rise in P4, can have profound effects on immune responses and the outcome of infectious diseases at diverse sites, this goes beyond the goals of this review and are discussed in detail elsewhere.¹⁸

Progesterone-based contraceptives. The “pill” which was approved by the Food and Drug Agency in 1960 is typically composed of a combination of progestins (Table 1) and estrogen, but many hormonal contraceptives, such as intrauterine devices, contain progestin only formulations.¹⁹ Progestins exert their reproductive effects by thickening the mucus, suppressing ovulation by altering the luteinizing hormone and follicle stimulating hormone surges, and making the endometrium inhospitable for implantation.²⁰ Many different contraceptive formulations exist, with new generations of progestins being developed that have higher affinity to the PR thereby reducing the dose required to be efficacious as well as reducing potential side-effects. Progestins can be used in conjunction with estrogen in combined oral contraceptives, or alone in the form of implants, injections (usually with DMPA), intrauterine devices, rings, or as an emergency contraceptive (Table 1).¹⁷ In the United States alone, 35% of adult women are currently using hormonal methods of contraception, and 88% of all adult women in the United States have been exposed to progestins in some form of contraceptives.²¹ Male hormonal contraception is currently being evaluated, with administration of either LNG or etonorgestrel reducing pituitary luteinizing hormone and

follicle stimulating hormone production, testicular testosterone production, and spermatogenesis without affecting secondary sexual characteristics.²²

Progesterone-based therapies. In addition to their use in birth control, P4 and progestins are also used in combination with estrogen in hormone replacement therapy in perimenopausal women to prevent the proliferative effects of estrogen on endometrial cells, which can lead to cancer and increased bone density.²³ The potential side-effects of progestin-containing hormone replacement therapy, including increased breast cancer risks and heart disease, raised concerns following the Women’s Health Initiative trial, but the study was conducted with medroxyprogesterone acetate (MPA), which can transactivate the androgen and glucocorticoid receptor and thus lead to increased undesired side-effects.²⁴ Newer generations of progestins are now used to prevent these side-effects and reduce the risk of breast cancer.²⁵

Progesterone-based oral contraceptives also have been used for their “off-label” beneficial effects on conditions such as acne, polycystic ovary syndrome, and dysmenorrhea. Progestins can increase bone density and prevent osteoporosis.^{26,27} More recently, P4 was shown to have a neuroprotective role following injury in the central nervous system by dampening inflammation and promoting repair of myelin fibers in the context of traumatic brain injury and multiple sclerosis.^{28,29} As such, P4 is currently being used in clinical trials for treatment of traumatic brain injury and strokes in both men and women.^{29–31}

Effects of progestins on immune responses

The presence of PRs on a wide variety of immune cells, suggests that these cells can respond to P4-based treatments (Figure 2). *In vitro* studies demonstrate that exposure of cells to P4 alters the immune environment in various tissues and cell culture systems by promoting an anti-inflammatory state (Table 2). Progesterone generally inhibits inflammatory innate immune responses. *In vitro* studies show that P4 can suppress activation of macrophages and DCs.^{32,33} When P4 is bound to its receptor, it directly interferes with the transcription factor NF- κ B through transrepression and inhibits gene transcription downstream of the NF- κ B pathway, including cyclooxygenase-2 to decrease inflammation^{32,34–36} (Figure 2). In human primary myometrial cells, P4 inhibits the MAPK pathway and downstream cyclooxygenase-2 and IL-1 β expression via the glucocorticoid receptor.³⁷ Progesterone can also decrease inflammation by inhibiting the production of proinflammatory cytokines (e.g., TNF- α , IFN- γ , and IL-12) and increasing production of anti-inflammatory cytokines, including IL-10.^{33,38–40} Progestin treatment of endometrial stem cell cultures, isolated from female patients with endometriosis reduces the expression of *Il6*, *Mcp1*, and *Tnf α* mRNA.⁴¹ In addition, exposure of endometrial stem cell cultures to either TNF- α or IL-1 β decreases intracellular expression of PRs.³⁸ Not all studies demonstrate anti-inflammatory effects of progestins. For example, concentrations of MIP-1, IL-6, and IL-8 in

Table 1 List of commonly used progestins

Progestin	Uses (reproductive system)	Brand name ^a
Progesterone (P4)	POC, HRT, dysmenorrhea treatment, ART	Prometrium
Norethindrone	COC, POC, endometriosis treatment, dysmenorrhea treatment	Micronor, Camila
Depot medroxyprogesterone acetate (DMPA)	Injectable contraceptive, HRT	Depo-Provera
Levonorgestrel (LNG)	COC, IUD, emergency contraception, implants	Mirena, Plan B
Etonogestrel	Vaginal ring, implants	NuvaRing, Implanon
Drospirenone	COC, HRT	Yasmin

Abbreviations: ART, assisted reproductive technology; COC, combined oral contraceptives; HRT, hormonal replacement therapy; IUD, intrauterine device; POC, P4 only contraceptive.

^aBrand names may differ depending on the country.

Table 2 The effects of progestins on immune responses

Immune response	Progestin	Species	Outcome	References
TLR activity	P4, LNG	H, M	↓ TLR3, ↓ TLR4, ↓ TLR7, ↓ TLR9	33,35,44,108
Type I IFNs	P4, MPA	H, R	↓ IFN-β	36,39
NF-κB signaling pathway	P4	H	Pathway inhibited ↓ COX-2	32,34,37
Macrophages	P4, LNG	M	↓ iNOS, ↓ NO ↑ Fizz-1, ↑ Ym-1	44–46
Dendritic cells	P4	H, M, R	↓ CD40, ↓ CD80, ↓ CD86 ↓ TNF-α, ↓ IL-6, ↓ IL-12, ↓ IL-1β ↑ IL-10, ↑ IL-8 ↑ CD11c	33,39,40,107
NK cells	P4	H	↓ IFN-γ ↑ Apoptosis (caspase-dependent)	47
Eosinophils	MPA	M	↑ Numbers	109
Pro-inflammatory cytokines	P4, MPA, DNG, NETA, LNG	H, M, R	↓ TNF-α, ↓ IFN-γ, ↓ IL-12, ↓ IL-6	35,38,39,46,50,56
Anti-inflammatory cytokines	P4, MPA	H, M, P	↑ IL-4, ↑ IL-5, ↑ TGF-β, ↑ IL-13, = IL-10	40,49,51,52,54,109
Chemokines	P4	M	↓ MIP-2, ↓ MCP-1	110
CD4 + Th1	P4	M, R	↓ Development	39,50
CD4 + Th2	P4	H, M	↑ Development ↑ IL-4, ↑ IL-5, ↑ IL-13, ↑ IL-27 ↓ IFN-γ	40,49,50
Tregs	P4	H, M	↑ Percentages	53,54
B cells	P4	M	↓ CD80 and CD86 Expression ↓ Proliferation ↓ AID and class switching ↓ Antibody responses	55–57,111

Abbreviations: AID, activation-induced cytidine deaminase; COX-2, cyclooxygenase 2; DNG, dienogest; H, human; iNOS, inducible nitric oxide synthase; M, mouse; MPA, medroxyprogesterone acetate; NETA, norethisterone acetate; NO, nitric oxide; P, primate; P4, progesterone; R, rat; TLR, toll-like receptor.

endocervical swabs collected from women taking DMPA are increased,^{42,43} which may be mediated by signaling through the glucocorticoid receptor rather than the PR in the genital tract (Table 2).

In rodent bone-marrow derived DCs (BMDCs), *in vitro* administration of either P4 or LNG downregulates the activation of the toll-like receptors (TLR) 3 and 4 and the production of IL-6, IL-12p40, TNF-α, and IL-1β as well as the expression of CD80 and CD86 following stimulation with TLR ligands.^{33,39} When BMDCs are co-cultured with T cells, the presence of P4 reduces T-cell proliferation in response to

lipopolysaccharide (LPS).³⁹ Similarly, P4 treatment of the macrophage cell line RAW264.7 inhibits TLR3, TLR4, and TLR9 signaling in response to Poly I:C, LPS, or CpG DNA, respectively, and downstream IL-6 and nitric oxide production.^{35,44} *In vitro* treatment with P4 alters the phenotype of murine macrophages by inducing the expression of Fizz-1 and YM-1, two markers of alternatively activated macrophages, and decreasing the production of nitric oxide following LPS stimulation.⁴⁵ In murine bone marrow-derived macrophages treated with LPS, P4 but not LNG decreases nitric oxide production and the ability to lyse *Leishmania* intracellular

parasites.⁴⁶ Natural killer cells isolated from human peripheral blood mononuclear cells (PBMCs) are susceptible to P4-induced cell death, which can be blocked by treatment with the P4 antagonist, RU-486.⁴⁷ Eosinophil numbers in ovariectomized rats treated with P4 are increased as compared with ovariectomized rats treated with placebo.⁴⁸

Progesterone also alters the distribution and activity of T cells. Treatment of either murine or human T cells *in vitro* with P4 can skew naive T cells away from Th1 responses and toward a Th2 type response, with increased production of IL-4, IL-5, and IL-10.^{40,49,50} In human monocyte-derived DCs, treatment with P4 induces a Th2 environment with increased concentrations of IL-10, IL-13, and IL-27.⁴⁰ In T-cell lines, the addition of P4 to the culture media induces a Th2 environment, with greater secretion of IL-4 and IL-5 as compared with non-P4-treated cultures.⁴⁹ Murine T cells cultured to differentiate into either Th1 or Th2 cells in the presence of P4, show a strong bias toward Th2 with decreased IFN- γ production and enhanced production of IL-4.⁵⁰ *In vivo*, the presence of P4 induces the production of TGF- β in the endometrium.^{51,52} During pregnancy, high levels of P4 can shift the immune response towards a CD4 + Treg (T regulatory) phenotype.⁵³ In human cord blood cells, P4 induces the differentiation of Tregs and suppresses Th17 cells through the modulation of transcription factors, such as STAT5 and STAT3.⁵⁴

In B cells, the effects of P4 treatment are less well characterized. *In vitro* studies with B cells and endometrial cell co-cultures from mice show that in the presence of P4, B cells have reduced expression of CD80 and CD86 and a limited ability to present antigen.⁵⁵ B-cell hybridomas treated with P4 show lower cellular proliferation and antibody production.⁵⁶ In murine splenic B cells, P4 treatment decreases activation-induced deaminase mRNA and the ability of these cells to undergo somatic hypermutation and class-switch recombination.⁵⁷ Acute administration of P4 in combination with low dose estradiol to female mice significantly reduces B lymphopoiesis, suggesting that these hormones are negative regulators of B-cell development in bone marrow.⁵⁸ During pulmonary infection, females treated with time-release capsules of either P4 and LNG exhibit lower antibody production systemically in serum and locally in bronchoalveolar lavage fluid, than do non-hormone treated female mice.⁵⁹ Taken together, these data illustrate that P4 and related compounds alter the functioning of immune cells, which could have profound effects on the pathogenesis of diseases at mucosal sites.

THE EFFECTS OF PROGESTINS ON DISEASES AT MUCOSAL SITES

Infections of the genital tract

Epidemiological studies show that when compared with males, females have higher rates of sexually transmitted infections (STIs), including human immunodeficiency virus (HIV), herpes simplex virus (HSV), gonorrhea, and chlamydia. The different stages of the menstrual cycle along with the use of hormonal contraceptives have been shown in both

human and animal models to increase susceptibility to STIs,⁶⁰ with progestins implicated as a factor that contributes to this heightened susceptibility^{61–64} (Table 3). Many reasons have been proposed for the detrimental effects of progestins on STIs including altered thickness of the vaginal epithelium,^{65,66} increased expression of virus entry co-receptors,^{67–69} and induction of an anti-inflammatory cytokine milieu lacking essential anti-microbial, pro-inflammatory cytokines, such as IFN- γ and TNF- α .^{64,68} In some cases, these effects can be species-specific. For example, although progestins thin the vaginal epithelium in non-human primates,⁶⁶ they can cause a thickening of the vaginal epithelium in humans.⁶⁵

HIV. The effects of progestins have been extensively studied in the context of HIV, where multiple epidemiological studies, *in vivo* studies in non-human primates, and *in vitro* studies have assessed the effects of progestins on the outcome of HIV acquisition, transmission, and virus replication. In both humans and non-human primates, the risk of HIV acquisition is increased in females during the luteal phase of the menstrual cycle, when P4 is at its highest levels, and following use of hormonal contraceptives containing progestins.^{70–75} In non-human primates, there is an increased risk of simian immunodeficiency virus and higher viral loads during the luteal phase of the menstrual cycle⁷⁶ and following P4 treatment.^{64,77} In macaques treated with DMPA, acute viremia and transmission of multiple viral variants is increased concomitantly with a decrease in virus-specific IFN- γ production.⁶⁴ In women taking DMPA, levels of the chemokine CCL5 are significantly increased in cervical swabs, but concentrations of pro-inflammatory cytokines such as IL-1 β or IL-6 remain similar to those in women not taking contraceptives.⁴³ *In vitro* data show that treatment of human PBMCs with P4 leads to increased expression of the HIV receptor CXCR4 but not CCR5 in both HIV infected and non-infected women.⁶⁹ Conversely, in endometrial biopsies from HIV-negative females, LNG reduced the expression of CCR5 on both CD4 + and CD8 + T cells.⁷⁸ Genital epithelial cells also have a higher uptake of HIV by endocytosis when exposed to P4 and to a greater extent with MPA.⁷⁹ Although controversial, these data suggest that progestins alter the risk of HIV infection by modulating the expression of chemokine receptors used for virus entry.

In addition to affecting the expression of chemokine receptors, progestins alter the cytokine responses of immune cells during HIV infection. In human PBMCs, upon activation with cytokine-coated activating beads, PBMCs treated with MPA have reduced IFN- γ , IL-6, IL-12, IL-17, and TNF- α production, and both MPA and P4 reduce IL-2 and IL-4 production.⁶⁸ When these PBMCs were infected with HIV, MPA but not P4 increased viral titers.⁶⁸ In addition, cervicovaginal fluid from women taking DMPA has lower concentrations of IFN- α as compared with concentrations in women not taking any form of contraceptive.⁶⁸ Conversely, higher concentrations of P4 in women are associated with increased production of type I IFNs by plasmacytoid DCs following stimulation with HIV encoded TLR7 ligands.⁸⁰

Table 3 The effects of progestins on viral, bacterial, and parasitic infections at mucosal sites.

Pathogen	Site	Progestin	Outcome of disease	Immunomodulatory effects	References
Human immunodeficiency virus (HIV)	Human female reproductive tract	DMPA	<ul style="list-style-type: none"> ↑ Risk acquisition and transmission ↑ Virus uptake by genital epithelial cells ↓ Risk of acquisition in some human studies 	<ul style="list-style-type: none"> ↓ Innate and adaptive immunity ↓ IFN-γ, TNF-α, IL-2, IL-4, IL-6 IL-12, MIP-1α, TNF-α, ↓IFN-α ↓ IFN-α and TNF-α production in pDCs ↓ CCR5 and CXCR4 ↑ Presence of CXCR4 or ↓CCR5 ↓ TNF-α, IL-2 and IL-4 N/A ↓ Epithelium lining 	68,73,79,82,112
Simian immunodeficiency virus (SIV)	Primate female reproductive tract	Net-EN P4	<ul style="list-style-type: none"> No significant risk increase ↑ Transmission ↑ Viral Loads 	<ul style="list-style-type: none"> ↓ HIV replication in human trophoblasts 	67,69,113
Herpes Simplex Virus type 1 (HSV-1)	Rodent female reproductive tract	DMPA	<ul style="list-style-type: none"> ↑ Susceptibility ↑ Acquisition and shedding ↑ Virus reactivation 	<ul style="list-style-type: none"> ↓ IFN-γ secreting CD8 T cells 	64
Herpes simplex virus type 2 (HSV-2)	Rodent female reproductive tract	DMPA	<ul style="list-style-type: none"> ↑ Susceptibility ↑ Virus shedding ↑ Pathogenesis ↓ Protection of HSV-2 vaccine ↓ Protection of vaccine against challenge ↓ Virus shedding 	<ul style="list-style-type: none"> ↓ Virus-specific CD8 T cells ↓ IFN-γ and TNF-α ↓ Granzyme B and lytic granules ↓ Macrophages, NK and NKT cells ↓ CD40 and CD80 expression on DCs ↓ IgG and IgA levels ↓ IL-1β, CXCL7, CXCR1 ↑ IL-10 ↑ Mucosal permeability 	62,89
<i>Chlamydia trichomatis</i> (chlamydia)	Rodent female reproductive tract	LNG P4	<ul style="list-style-type: none"> ↑ Susceptibility ↑ Susceptibility ↑ Pathogen load 	<ul style="list-style-type: none"> ↑ Neutrophil infiltration ↑ CCL5, CXCL2, CCL2, CXCL10 ↑ IL-1β 	85,86,92
<i>Neisseria gonorrhoeae</i> (gonorrhea)	Mammalian female reproductive tract	P4	<ul style="list-style-type: none"> ↑ Bacterial load in primary human cervical epithelial cells ↑ Length of infection in mice 	<ul style="list-style-type: none"> ↑ TSLP ↑ Regulatory T cells ↑ Th17 cells ↑ Production of nitric oxide 	88 61,93 95,96
<i>Gardnerella vaginalis</i> (vaginitis)	Human female reproductive tract	DMPA DMPA or Net-EN	<ul style="list-style-type: none"> ↓ Rates infection in women ↓ Prevalence and incidence 	<ul style="list-style-type: none"> N/A N/A 	94 97
<i>Candida albicans</i> (candidiasis)	Human female reproductive tract	P4	<ul style="list-style-type: none"> ↓ Biofilm formation, colonization and invasion 	<ul style="list-style-type: none"> N/A 	98
<i>Salmonella typhimurium</i> (typhoid)	Rodent gastrointestinal tract	P4	<ul style="list-style-type: none"> ↑ Survival of the host ↑ Resistance ↑ Clearance of bacteria 	<ul style="list-style-type: none"> ↑ Cellular infiltration 	99
<i>Helicobacter pylori</i> (gastritis)	Rodent gastrointestinal tract	P4	<ul style="list-style-type: none"> ↓ Incidence of gastritis 	<ul style="list-style-type: none"> N/A 	101
<i>Taenia solium</i> (taeniosis)	Rodent gastrointestinal tract	P4	<ul style="list-style-type: none"> ↑ Host survival by increasing inflammation ↓ Parasite load 	<ul style="list-style-type: none"> ↑ Proliferation of parasite-specific leukocytes ↑ IL-4, IL-6 and TNF-α 	100
<i>Mycobacterium tuberculosis</i>	Rodent respiratory tract	DMPA	<ul style="list-style-type: none"> ↑ Bacterial load 	<ul style="list-style-type: none"> ↓ TNF-α, G-CSF, IL-10 and IFN-γ 	104
Influenza A viruses	Rodent respiratory tract	P4, LNG	<ul style="list-style-type: none"> ↓ Pathogenesis and severe outcome ↑ Host survival ↑ Repair and pulmonary function 	<ul style="list-style-type: none"> ↑ TGF-β, IL-6, IL-22 ↑ Amphiregulin ↑ Th17 cells 	15

Abbreviations: DMPA, depot medroxyprogesterone acetate; LNG, levonorgestrel; N/A, not available; Net-EN, norethisterone oenanthate; P4, progesterone.

In conclusion, many of the epidemiological studies of progestins and HIV focus on women using DMPA, which may increase the risk of HIV acquisition; whereas neither Net-EN nor LNG increase the risk of HIV acquisition.^{73,81–83}

DMPA may promote HIV acquisition by modulating immune responses through glucocorticoid receptor signaling, whereas LNG reduces HIV acquisition through PR signaling.

HSV. Progestins can increase susceptibility to HSV and decrease protection during HSV vaccination in humans and mice.^{62,63,84–88} Studies in ovariectomized mice show that DMPA leads to HSV-1 reactivation, with reduced numbers, lytic activity, and cytokine production (e.g., IFN- γ and TNF- α) in virus-specific CD8 + T cells.⁶² DMPA impairs the CD8 + T-cell response by decreasing the expression of co-stimulatory molecules on DCs.⁸⁹ In the genital mucosa of mice, treatment with DMPA, P4, or LNG increases susceptibility to HSV-2 by stimulating the production of IL-1 β , mucosal permeability, and entry of inflammatory cells through reduced expression of cadherin desmoglein-1a.^{88,90,91} Human primary genital epithelial cells are highly susceptible to HSV-2 infection, but unlike murine models, P4 treatment is protective and limits HSV-2 shedding as compared with control-treated cultures.⁹² In the context of HSV-2 vaccination in mouse models, both DMPA and P4 reduce anti-HSV-2 IgG and IgA in vaginal washes, increase viral shedding, and fail to protect mice following HSV-2 challenge as compared with estradiol-treated mice.^{85,90,91} Taken together, progestins increase susceptibility to HSV at population and organismal levels of analysis, but the precise cell types mediating these effects remain elusive.

Other STIs. In rats, P4 increases susceptibility to the bacterium *Chlamydia trichomatis* by increasing inflammatory responses, as measured by MHC class II expression in uterine epithelial cells, production of IFN- γ and IL-10, and pathogen load.^{61,93} In women using DMPA, susceptibility to infection with *Neisseria gonorrhea* is significantly decreased,⁹⁴ but treatment of primary human cervical cells with P4 increases bacterial survival within human cervical epithelial cells, which is associated with increased AKT activity and inducible nitric oxide synthase production.⁹⁵ When P4-treated female mice are infected with *N. gonorrhea*, bacterial loads and the duration of infection increase, which are associated with greater levels of thymic stromal lymphopoietin and Tregs and lower Th17 cell responses.⁹⁶ Vaginosis can be caused by many pathogens, the most common being *Candida albicans*; P4 and progestin-based hormonal contraceptives have all been shown to be beneficial by reducing prevalence and incidence of vaginitis.⁹⁷ *In vitro* studies show that P4 can decrease *C. albicans* biofilm formation thus preventing colonization and invasion of the vaginal mucosa.⁹⁸ Taken together these data show that the anti-inflammatory properties of progestins may lead to increased susceptibility to STIs, yet the type of progestin may affect the risk of acquisition or disease progression differentially in part through differences in hormone receptor signaling pathways.

Infections of the gastrointestinal tract

A limited number of studies have examined the role of P4-based compounds in the gastrointestinal tract, with focus on either bacteria or parasitic infections (Table 3). In the context of murine infection with *Salmonella typhimurium*, the causative agent of typhoid, P4 treatment increases host survival by increasing the infiltration of peritoneal cells and promoting clearance of the bacteria.⁹⁹ Similarly, following infection of hamsters with the parasite, *Taenia solium*, P4 increases host survival and decreases tapeworm length by recruiting mast cells and increasing levels of the pro-inflammatory IL-4, IL-6, and TNF- α in the intestinal mucosa which can promote parasite elimination.¹⁰⁰ In the context of gastritis due to *Helicobacter pylori* infections, P4 reduces gastrointestinal inflammation in ovariectomized gerbils and reduces the effects of gastritis.¹⁰¹ In addition to pathogenic bacteria, commensal bacteria in the gastrointestinal tract are responsive to and can even alter concentrations of sex steroids.^{102,103} For example, adoptive transfer of gut commensals from male to female mice increases concentrations of androgens in females and alters the pathogenesis of autoimmune diseases.^{102,103} Whether P4-based compounds, including the use of contraceptives alter the composition of the microbiome requires consideration. Based on available data, it can be concluded that progestins protect against gastrointestinal infections by increasing host survival through their immunomodulatory effects.

Infections of the respiratory tract

To date, few studies have assessed the role of P4-based compounds on infectious diseases in the respiratory tract (Table 3). DMPA alters the outcome of *Mycobacterium tuberculosis* infection in mice by inducing an anti-inflammatory environment, including reduced production of TNF- α , IFN- γ , and G-CSF, which contributes greater bacterial burden.¹⁰⁴ In mice infected with influenza A virus, treatment with either P4 or LNG prevents severe outcome by decreasing pulmonary inflammation and promoting faster recovery during a primary infection.^{15,59} P4-based treatments promote pulmonary repair following clearance of influenza A viruses by elevating levels of TGF- β , IL-6, IL-22, and increasing numbers of regulatory CD39 + Th17 cells in the lungs. Production of the epidermal growth factor amphiregulin is also increased following P4 treatment, which promotes proliferation and repair of respiratory epithelial cells during influenza A virus infection¹⁵ (Figure 3). Treatment with either P4 or LNG also reduces virus-specific antibody titers as well as virus-specific memory CD8 + T cells numbers, which results in worse outcome following secondary influenza A virus challenge in female mice.⁵⁹ Although the anti-inflammatory effects of P4-based compounds protect against a primary virus infection, the reduction in memory T-cell responses increase susceptibility to secondary influenza A virus challenge. Because P4-based compounds reduce inflammation and expedite repair of lung tissue following primary influenza A virus infection, females may be less susceptible to secondary bacterial

Figure 3 Progesterone (P4) reduces inflammation and promotes repair of the respiratory epithelium to protect against influenza A virus (IAV) infection. Influenza A viruses (IAV) infect airway epithelial cells which can produce pro-inflammatory cytokines and activate immune cells, such as alveolar macrophages (brown cells), DCs (blue cells), and neutrophils (purple cells), to cause inflammation and, along with CD8 + T cells (not depicted), lead to virus clearance. In the presence of P4, pulmonary production of IL-6 and TGF- β and recruitment of regulatory CD39 + Th17 cells (green cells) is increased and creates an anti-inflammatory milieu. Th17 cells can also produce IL-22, which like TGF- β , promotes proliferation of epithelial cells (orange cells) and repair of the damaged epithelium. Amphiregulin (AREG) is a growth factor that is induced by P4 signaling through the progesterone receptor (PR). AREG signals through the epidermal growth factor receptor (EGFR) to induce the proliferation of epithelial cells, which leads to repair of the damaged epithelium.

infections, which are the primary cause of mortality following influenza A virus infection in humans.

CONCLUSIONS AND FUTURE DIRECTIONS

The use of P4-based compounds is growing and despite strong evidence for a role in modulating immune responses, their effects outside of reproductive health are rarely considered. Not only have hormonal contraceptives been used by over 88% of adult women in the United States,²¹ but they are further recommended for use in situations where there are extreme concerns about congenital infection, such as during the current Zika virus epidemic in the Americas.¹⁰⁵

The impact of progestins on infectious disease outcome depends on the type of immune response induced to control the infection and repair damaged tissue. For example, during influenza A virus infection of the respiratory tract, excessive inflammation and tissue damage lead to severe symptoms, whereas the presence of P4 or LNG dampens inflammation and leads to faster recovery.^{15,59} Conversely, during HSV-1 infection of the reproductive tract, dampened inflammation, including reduced numbers CD8 + T cells and lower secretion of pro-inflammatory cytokines, caused by DMPA leads to increased virus reactivation.^{62,89} Similarly, following infection of the reproductive tract with *Neisseria gonorrhoeae*, treatment with progestins decreases the number of Th17 cells and prevents proper control of the bacterial infection.^{95,96,106} As illustrated in these examples, progestins alter the number and function of multiple immune cell types, which likely

contributes to differences in the impact of progestins on the outcome of infections at diverse mucosal sites.

One common observation across diverse infections at mucosal sites is that treatment with progestins leads to reduced inflammation. In antigen-presenting cells, such as DCs, progestins inhibit activation and inflammatory pathways.^{33,39,40,107} In T cells, progestins promote anti-inflammatory phenotypes^{40,49,50,53,54} and in epithelial cells^{14,15} at mucosal sites, progestins promote repair and proliferation. We interpret these observations to illustrate the importance of progestins in mucosal immunity. Future studies must evaluate the mechanistic effects of progestins on receptor signaling pathways in diverse cell types, including immune cells, epithelial cells, and endothelial cells, at mucosal sites. We must also systematically evaluate the possible therapeutic as well as the detrimental effects of progestin-based compounds in the context of infectious as well as non-infectious diseases at mucosal sites. In summary, the data reviewed provide significant evidence that P4 and related compounds have profound effects on mucosal immunity and the pathogenesis of diverse diseases in females.

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AUTHOR CONTRIBUTIONS

O.J.H. and S.L.K. conceived the idea and drafted the outline, O.J.H. and S.L.K. wrote the manuscript, S.L.K. edited the manuscript, O.J.H. drafted the figures and tables.

DISCLOSURE

The authors declared no conflict of interest.

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