Sex Steroid Hormones, Hormonal Contraception, and the Immunobiology of Human Immunodeficiency Virus-1 Infection

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Worldwide, an increasing number of women use oral or injectable hormonal contraceptives. However, inadequate information is available to aid women and health care professionals in weighing the potential risks of hormonal contraceptive use in individuals living with HIV-1 or at high risk of infection. Numerous epidemiological studies and challenge studies in a rhesus macaque model suggest that progesterone-based contraceptives increase the risk of HIV-1 infection in humans and simian immunodeficiency virus (SIV) infection in macaques, accelerate disease progression, and increase viral shedding in the genital tract. However, because several other studies in humans have not observed any effect of exogenously administered progesterone on HIV-1 acquisition and disease progression, the issue continues to be a topic of intense research and ongoing discussion.

In contrast to progesterone, systemic or intravaginal treatment with estrogen efficiently protects female rhesus macaques against the transmission of SIV, likely by enhancing the natural protective properties of the lower genital tract mucosal tissue. Although the molecular and cellular mechanisms underlying the effect of sex steroid hormones on HIV-1 and SIV acquisition and disease progression are not well understood, progesterone and estrogen are known to regulate a number of immune mechanisms that may exert an effect on retroviral infection. This review summarizes current knowledge of the effects of various types of sex steroid hormones on immune processes involved in the biology of HIV-1 infection. (Endocrine Reviews 31: 79–97, 2010)

I. Introduction

Safe and effective methods of contraception represent a critical component of preventive health care reducing maternal and infant mortality, especially in women living in resource-limited settings. Although the number of women using oral or injectable hormonal contraception is rapidly increasing in areas of high HIV seroprevalence such as Sub-Saharan Africa, a clear understanding of the impact of this birth control method on HIV-1 infection is lacking (1–4). The effect of hormonal contraceptives on the acquisition and immune control of HIV-1 and other coinfecting pathogens represents an important and under-investigated women’s health-specific issue with potentially large implications for public health policy. The World Health Organization has identified the availability and use of effective contraception by HIV-1-infected

Abbreviations: CTL, Cytotoxic T lymphocyte(s); DC, dendritic cell; DMPA, depot medroxyprogesterone acetate; GR, glucocorticoid receptor; HSV-2, herpes simplex virus-2; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; LC, Langerhans cell; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; NK, natural killer; OC, oral contraceptives; PIBF, progesterone-induced blocking factor; pIgR, polymeric Ig receptor; SC, secretory component; SIV, simian immunodeficiency virus; VCAM-1, vascular cell adhesion molecule-1.
TABLE 1. Impact of hormonal contraception on HIV-1 infection and disease progression

<table>
<thead>
<tr>
<th>Hormonal contraception</th>
<th>Effect</th>
<th>Population size</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC</td>
<td>Increased risk of HIV-1 acquisition</td>
<td>+/- + + + b</td>
<td>5, 8, 10, 11, 13–15</td>
</tr>
<tr>
<td>DMPA</td>
<td>Increased risk of HIV-1 acquisition</td>
<td>+ + +</td>
<td>7, 16</td>
</tr>
<tr>
<td>DMPA, OC</td>
<td>Increased risk of HIV-1 acquisition</td>
<td>+ + +</td>
<td>6</td>
</tr>
<tr>
<td>DMPA, OC</td>
<td>Increased HIV-1 viral load</td>
<td>+ + +</td>
<td>17, 31</td>
</tr>
<tr>
<td>OC</td>
<td>Increased risk of HIV-1 acquisition, meta-analysis of 28 studies</td>
<td>+ + +</td>
<td>9</td>
</tr>
<tr>
<td>DMPA, OC</td>
<td>No effect on HIV-1 acquisition</td>
<td>+/- +/- +/-</td>
<td>19–24</td>
</tr>
<tr>
<td>DMPA, OC</td>
<td>No effect on HIV-1 viral load or disease progression</td>
<td>+ + +</td>
<td>33, 34</td>
</tr>
<tr>
<td>DMPA, OC</td>
<td>Increased risk of Chlamydia, Gonococcus, and Candida infections in the genital tract</td>
<td>+ + +</td>
<td>39–41</td>
</tr>
<tr>
<td>DMPA, OC</td>
<td>Accelerated CD4 T cell depletion and disease progression</td>
<td>+/- +/- +/-</td>
<td>32</td>
</tr>
<tr>
<td>DMPA</td>
<td>Increased risk of SIV infection in the rhesus macaque model; higher viral load during acute phase of infection</td>
<td>+ + +</td>
<td>42–44, 46</td>
</tr>
</tbody>
</table>

* OC preparations differ among various studies.

b Approximate population size: +, small; ++, medium; +++, large.

women wishing to avoid pregnancy as one of the primary strategies for preventing mother-to-child transmission of HIV-1. Any contraceptive intervention that increases the chance of HIV-1 infection or the shedding of HIV-1 in the female genital tract, or hastens the progression to AIDS has important implications for patients’ health, the spread of the virus in the population, and overall economic and social impacts of the epidemic.

II. Impact of Progestosterone-Based Hormonal Contraception on HIV-1 Acquisition and Disease Progression

Whether or not hormonal contraceptives significantly affect the probability of acquiring HIV-1 remains a highly controversial issue and a topic of an intense discussion. To date, more than 10 studies have demonstrated a correlation between the use of hormonal contraception and increased risk of HIV-1 infection (5–16) (Table 1). Several studies have focused on injectable contraceptive DMPA (depot medroxyprogesterone acetate; Depo-Provera), a highly effective progesterone-based contraceptive currently used by more than 90 million users worldwide. DMPA, typically administered as a 3-monthly injection, is gaining increasing popularity in Sub-Saharan Africa. One of the best designed studies was a 10-yr prospective study involving more than 1500 sex workers in Mombasa, Kenya. Women who used DMPA were twice as likely to acquire HIV-1 compared with women without contraception (6, 17, 18). A recent report using data from the Demographic and Health Surveys of 4549 young women in four African countries confirmed higher HIV-1 seroprevalence in DMPA users and estimated that 6% of new HIV-1 cases are attributable to DMPA use (16). In contrast, several other studies have failed to observe an overall effect of DMPA or other forms of hormonal contraception on the incidence of HIV-1 infection (19–24) or reported increased risk of infection only in subgroups of subjects differing in age and herpes simplex virus (HSV-2) status (25–27). It is important to note that the quality of the epidemiological studies addressing the issue varies considerably, and great care should be taken in analyzing their results. Interpretation of these studies is complicated by multiple factors including the type, dose, and method of administration of hormonal contraceptives; method of selection of study subjects; and population sizes. Furthermore, unsafe sharing and reuse of needles and syringes used for delivering DMPA could be a confounding factor for the association between DMPA use and HIV-1 incidence, and future studies should take this into consideration (28). Supporting this point are the results of a study in Tanzania demonstrating a correlation between DMPA use and increased hepatitis C infection rate (29).

Another important issue is whether the use of hormonal contraception during chronic HIV-1 infection affects the rate of viral replication and overall disease progression. A multivariate analysis of the Mombasa study demonstrated a significantly higher HIV-1 viral load at the set-point and acquisition of more diverse viral genotypes in women using hormonal contraception at the time of HIV-1 infection compared with women without contraception (6, 17, 18). In accordance with the notion that viral load is highly predictive of HIV-1 disease progression (30), Lavreys et al. (31) reported a correlation between higher viral set-point and mortality in the Mombassa cohort. The results of our recent randomized trial suggest that the use of hormonal contraceptives [DMPA or oral contraceptives (OC)] is associated with more rapid disease progression characterized by accelerated loss of CD4+ T cells and increased death rate in HIV-1-infected women (32). In contrast, other studies failed to confirm a relationship between the use of hormonal contraceptives and acceleration of the disease (33, 34). Several reports have shown an associa-
tion between the use of hormonal contraception and increased cervicovaginal shedding of HIV-1 with a significant dose dependency on progesterone levels (35–37). Progesterone-based contraceptives also appear to increase the number of inflammatory cells in cervicovaginal fluid (38). Importantly, both of these factors may potentially promote HIV-1 transmission. The effects of hormonal contraception do not seem to be limited to HIV. Application of hormonal contraception, particularly DMPA, was also shown to be associated with an increased acquisition of cervical chlamydial and gonococcal infections and candidiasis, which may increase the susceptibility to HIV-1 or further deteriorate the condition of HIV-1-infected individuals (39–41).

In summary, although multiple studies suggest a significant effect of progesterone-based hormonal contraception on the risk of HIV-1 infection, viral load, frequency of opportunistic infections, and the rate of progression to AIDS, the epidemiological data obtained so far are inconclusive, and more comprehensive clinical trials are urgently needed to verify these observations. However, extreme caution must be exercised in the design and analysis of these studies. Research data that would encourage stopping the use of progesterone-based contraceptives in areas with high HIV-1 prevalence could create a multitude of problems including lack of available birth control methods and increased incidence of mother-to-child HIV-1 transmission.

III. Nonhuman Primate Studies: Progesterone Increases the Susceptibility Whereas Estrogen Protects against SIV Transmission

Well-controlled nonhuman primate experiments addressing the effect of exogenous hormones on the acquisition of SIV have yielded more significant results than studies in humans. Rhesus macaque studies showed that administration of DMPA enhances the risk of SIV acquisition by more than 7-fold and significantly increases viral levels in the acute phase of infection (42–44). Trunova et al. (43) showed that the genetic complexity of the replicating virus was greater, replication of the CXCR4-using virus was favored, and cellular immune response rate was slower in DMPA-treated animals. Progesterone treatment is routinely used in some laboratories to increase the susceptibility to vaginal SIV infection (44, 45). In addition, Christopher Miller and colleagues (46, 47) have shown that DMPA abrogates the protective effect of an attenuated lentivirus-induced protection against intravaginal challenge with pathogenic SIV\textsubscript{MAC239}. Although it is unknown why macaque studies demonstrate a more profound effect of progesterone than the epidemiological studies in humans, multiple factors can play a role in the observed difference, including the kinetic of progesterone concentra-

IV. Potential Defense Mechanisms Modulated by Sex Steroid Hormones

What are the mechanisms responsible for the progesterone-mediated sensitization vs. estrogen-mediated protection against SIV infection in macaques? It is plausible that the main mechanism is the effect of sex steroid hormones on the physiological properties of the vaginal wall. Epithelial and subepithelial layers of the vaginal wall represent a natural barrier protecting the host against pathogenic infections. Indeed, the low probability of women becoming infected with HIV-1 via heterosexual intercourse (1:200–1:2000) is a testimony to the effectiveness of the vaginal and cervical mucosal barrier (50). Although HIV-1 can infect via the single layer of polarized columnar epithelial cells of the endocervical mucosa, most heterosexual transmissions likely occur through the pluristratified epithelial layer of the vaginal and ectocervical mucosa. The combined surfaces of vaginal wall and ec-
tocervix exceed 15 times the surface area of the endocervix. HIV-1 infection occurs in women who lack a uterus at birth and in female macaques after surgical removal of the uterus (50–52). In a recent large randomized trial in African women, no significant reduction of HIV-1 infection occurred in women using a diaphragm (53). It has been argued that most transmissions occur through damaged or atrophied vaginal epithelium (50).

Sex steroid hormones exert a significant effect on the vaginal mucosal tissue. Estrogen induces thickening of the vaginal stratified epithelium in women and female macaques (48, 49, 54–58) (Fig. 1). A thick epithelium might block the access of the virus to target Langerhans cells (LCs), CD4+ T cells, and macrophages in the epithelial and subepithelial layers. An inverse correlation between epithelial layer thickness and susceptibility to SIV in estrogen and progesterone-treated macaques was observed (42, 48, 49). Smith et al. (48) reported that whereas the average thickness of the epithelial layer was about <10 μm in untreated ovariectomized female macaques, it expanded to about 240 μm in the estrogen-treated animals. The estrogen-induced expansion of the epithelial layer is less pronounced in women compared with female macaques (54, 55, 58). Cornification of epithelial cells appears as soon as 24 h after estrogen treatment, and the response persists for at least 1 wk after the cessation of treatment in macaque vagina and human foreskin (49, 59). Macaques intravaginally exposed to SIV appear to be less susceptible to infection when in the follicular ovarian phase (high estrogen) compared with the luteal phase (high progesterone) (60). This susceptibility pattern correlates with the cycling of the thickness of the epithelial layer, which is highest at the peak of the follicular phase around ovulation and lowest during menses (45). Because females are more likely to have intercourse during ovulation, increased thickness of epithelium likely represent a physiological mechanism to prevent traumatic effect of intercourse and infection with pathogens introduced during the intercourse. Estrogen treatment also decreases cervicovaginal pH in women and female macaques, making it hostile to the virus (48, 54, 61, 62). In postmenopausal women, low estrogen is associated with thinning of the vaginal epithelium and atrophy (63). This condition is associated with a dry, friable, thin vaginal epithelium prone to bleeding after minimal trauma. Postmenopausal women are 4- to 8-fold more susceptible to HIV-1 transmission (64, 65). Atrophic vaginitis is successfully treated with local or systemic estrogen therapy resulting in thickening of the vaginal wall.

In contrast to estrogen, administration of progesterone causes significant thinning of the cervicovaginal epithelium in rhesus macaques, possibly explaining the increased susceptibility to SIV in DMPA-treated animals (42, 66). However, the effect of DMPA on the vaginal wall thickness appears to be much less profound in humans, with several studies actually reporting increased epithelial thickness caused by hyperplasia (67–70). An alternative mechanism of DMPA action has been proposed by Miller et al. (70) who described how DMPA induces changes in the human vaginal microbiota, namely decreased colonization with H2O2-positive Lactobacillus. H2O2-produc-
ing Lactobacillus in the vagina may directly kill free virus (71). In addition, the production of H$_2$O$_2$ by Lactobacillus represents an important mechanism by which it maintains dominance over other vaginal microbiota. The acquisition of HIV-1 was found to be significantly higher in women without Lactobacillus or with abnormal vaginal microbiota compared with those with H$_2$O$_2$-producing Lactobacillus (72). Colonization with Lactobacillus might prevent bacterial vaginosis that is associated with increased risk of HIV-1 transmission (7, 73, 74).

Steroid hormones might also modulate the susceptibility to SIV and HIV-1 by altering the availability of target cells in the vaginal epithelium and stroma. Of particular interest is the population of LCs that is abundant in the epithelial layer of the vaginal and ectocervical mucosa of the female lower reproductive tract (50, 75). They acquire the virus by multiple mechanisms involving CD4, CCR5, and langerin-dependent binding as well as endocytosis (75). Although only a few LCs become productively infected, they might play a key role in viral dissemination by effectively transporting the membrane-associated virus to CD4$^+$ T cells and macrophages in the lamina propria and draining lymph nodes (the Trojan horse theory) (50, 75). Importantly, exogenous estrogen decreases (76), whereas progesterone increases (77) the frequency of LCs in the vaginal epithelial and stromal tissue. In contrast, the frequency of LCs appears constant throughout the menstrual cycle in women and female macaques (78, 79). Furthermore, peak estrogen levels decrease the recruitment of inflammatory T cells and macrophages through down-regulation of intercellular adhesion molecule-1 (ICAM-1), E-selectins, and vascular cell adhesion molecule-1 (VCAM-1) (80). In contrast, progesterone was reported to increase the expression of CCR5 on human cervical CD4$^+$ T cells, making them possibly more susceptible to infection (81, 82). Thus, estrogen might decrease and progesterone might increase the frequency of HIV-1 target cells in the vaginal epithelium and lamina propria. Importantly, steroid hormones might also act by regulating the early response of the innate and adaptive immune systems in the female lower reproductive tract, as discussed in Sections VI and VII.

If the results of macaque studies are reproduced in future studies in humans, vaginally applied estrogen derivatives may represent a new, inexpensive, and safe method of HIV-1 prevention. Ideally, estrogen treatment will enhance the natural protective properties of the genital tract tissue, resulting in a “natural female condom” effectively interfering with HIV-1 transmission. In contrast to microbicides, which have to be applied around the time of exposure, the effect of vaginal estrogen therapy is long lasting (49, 59). Estrogen cream has been used by millions of postmenopausal women to treat vaginal atrophy and has not been associated with any negative systemic effects. According to some reports, long-term hormonal replacement therapy has been linked to an increased chance of breast cancer (83). However, estrogen can be replaced by selective estrogen receptor modulators to avoid any potential carcinogenic effect. Vaginal estrogen cream is likely to be affordable because plant-derived phytoestrogens can be produced at a low cost. A potential protective effect of natural products such as soybean, black cohosh, or red clover phytoestrogens that have been used by women worldwide to treat vaginal dryness and atrophy should be investigated in future studies. Topical estrogen therapy is likely to be highly effective in sexually active postmenopausal women, a largely unrecognized subset of population with high susceptibility to HIV-1 infection (64, 65). Vaginal estrogen cream can be combined with microbicides and antiretrovirals for additional protection and with contraceptives in women wishing to avoid pregnancy.

V. Modulation of the Immune System by Hormonal Cycle

Tightly regulated production of ovarian hormones estrogen and progesterone modulates the menstrual cycle and exerts a dramatic effect on immune processes in the female reproductive tract, other mucosal tissues, as well as the systemic compartment (76, 84–86) (Fig. 2). Although the effect of hormonal cycle is generally more pronounced in the tissues of the upper reproductive tract (ovaries, fallopian tubes, uterus), a significant effect on immune mechanisms in the lower reproductive tract (endocervix, ectocervix, and vagina) is well documented. We and others have previously demonstrated that the levels of antibodies in genital secretions significantly fluctuate throughout the menstrual cycle (87–92). This fluctuation is associated with shifts in the relative frequency of B cell subsets in tissues as well as with the changes in the rate of antibody production and transepithelial transport (78, 90). IgA and IgG levels in cervical mucus increase approximately 3 d before ovulation and decrease during the luteal phase of the menstrual cycle, paralleling the increase in the concentration of progesterone (87–95). Total and antigen-specific IgG- and IgA-secreting cells in the cervix and vagina of women and female macaques are significantly higher during the periovulatory stage (d 11–15) and decreased before menstruation, coinciding with highest progesterone levels (90, 96). Importantly, the cycle-associated effect of sex hormones on antibody-secreting cell frequen-
cies is also observed in nonreproductive tract immune tissues such as the spleen and lymph nodes (90). Gockel et al. (97) have demonstrated that animals immunized orally with tetanus toxoid at the peak of progesterone concentrations during the postestrous stage exhibited 10- to 100-fold lower IgA and IgG responses in fecal extracts and vaginal washes compared with animals immunized at other phases of the menstrual cycle. These data strongly suggest that sex hormones regulate IgA and IgG responses not only in the genital tract but also, importantly, in the gut-associated lymphoid tissue. This may be attributed to the common draining lymphatics, namely the caudal and lumbar lymph nodes, shared by the colon and female genital tract.

Cytotoxic T lymphocytes (CTLs) in the female genital tract exhibit high cytotoxic activity during the follicular phase of hormonal cycle and almost complete loss of this activity during the luteal phase (98). This may be caused by the fact that estrogen inhibits antigen presentation and CTL activity in the female reproductive tract, possibly by inducing TGF-β (77, 98–100). There is a lack of correlation between genital tract mucosa and peripheral blood CTL activity in HIV-1-infected women, suggesting discrete hormonal regulation (77, 101). However, the observed difference may be caused by local inflammation or variations in viral replication in the systemic vs. mucosal compartments. An increased level of estrogen in the luteal phase is associated with increased concentration of TNF-α, IL-4, and sIL-6R in plasma (102–104). Natural killer (NK) cell cytotoxicity is higher in the follicular than in the luteal phase, and the number of NK cells per milliliter of blood is highest during the periovulatory phase (105, 106).

It remains to be established whether and to what degree the upper female reproductive tract serves as a portal of HIV-1 infection (107, 108). Human endometrial epithelial cells express CD4, CCR5, and CXCR4 receptors, and CXCR4 levels gradually increase during the proliferative phase and remain elevated throughout the secretory stage of the menstrual cycle, possibly invoking sex hormones-mediated mechanisms (109–111). Endometrial epithelial cells bind and transcytose R5 and X4 HIV-1 viruses to permissive submucosal cells (107, 112, 113). The endometrial mucosal epithelium expresses innate immune factors, including α- and β-defensins, secretory leukocyte protease inhibitor, and RNA-dependent protein kinase, in a hormonal cycle-dependent fashion (76, 108, 110, 114). Sex hormones regulate the recruitment of lymphocytes into large lymphoid aggregates accumulating in the endometrium of human uterus and consisting of a central core of B lymphocytes surrounded by a large number of CD8+ and a lower number of CD4+ T cells (115). The size of these aggregates peaks at the secretory phase with about 3000 cells/aggregate and decreases at the proliferative stage. Analysis of T cell receptor subtypes within endometrium suggests that these aggregates develop largely by trafficking of cells into the endometrium rather than by local proliferation (116). Progesterone and estradiol decrease the expression of chemokine monocyte chemoattractant protein-1 (MCP-1) by human endometrial stromal cells and decrease the infiltration of leukocytes, neutrophils, and macrophages to the female upper and lower genital tract (117–119).

Several studies suggest that plasma HIV-1 load and HIV-1 shedding at the lower reproductive tract are lowest during the follicular ovarian stage and increase during the luteal stage, corresponding to high progesterone levels (120–123). However, because other studies found no association (124, 125), it remains to be established whether endogenously produced sex hormones affect the rate of proliferation of HIV-1 in genital mucosal tissue. Interestingly, women who are pregnant or recently postpartum (conditions characterized by increased progesterone production) have been shown to be more susceptible to HIV-1 infection (126, 127). Furthermore, virus excretion in cervicovaginal secretions appears to be elevated in HIV-1-infected pregnant women (128).
TABLE 2. Reported effects of progesterone and its derivatives on the immune system and immunobiology of HIV-1 infection

<table>
<thead>
<tr>
<th>Reported effect of progesterone or its derivatives</th>
<th>Refs.</th>
</tr>
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<tbody>
<tr>
<td>Inhibition of IgG and IgA production and transepithelial transport</td>
<td>78, 87–96, 129–134</td>
</tr>
<tr>
<td>Decreased frequency of antibody-secreting cells in women and female macaques</td>
<td>90, 96</td>
</tr>
<tr>
<td>Decreased specific IgG and IgA responses after mucosal immunization with attenuated HSV-2; induction of permissive conditions for intravaginal infection of mice with HSV-2 and Chlamydia trachomatis</td>
<td>132–134</td>
</tr>
<tr>
<td>Inhibition of T cell responses and cytotoxic activity</td>
<td>139–143, 147</td>
</tr>
<tr>
<td>Inhibition of perforin expression in T cells</td>
<td>140–142, 144–146</td>
</tr>
<tr>
<td>Decreased proliferation and Th1-type cytokine production by VZV-specific CD4&lt;sup&gt;+&lt;/sup&gt; T cells in HIV-1 patients</td>
<td>148</td>
</tr>
<tr>
<td>Altered migration and decreased activity of NK cells</td>
<td>105, 106, 106, 135, 159, 251, 252</td>
</tr>
<tr>
<td>PIBF-mediated shift toward Th2 cytokine expression profile</td>
<td>133, 149–154</td>
</tr>
<tr>
<td>Altered migration and infiltration of lymphocytes, macrophages, and NK cells into the female genital tract tissues</td>
<td>117, 118, 157, 158, 183, 191, 253</td>
</tr>
<tr>
<td>Increased expression of CCR5 on cervical CD4&lt;sup&gt;+&lt;/sup&gt; lymphocytes</td>
<td>81, 82</td>
</tr>
<tr>
<td>Thinning of cervicovaginal epithelium in rhesus macaques</td>
<td>42, 66</td>
</tr>
<tr>
<td>Increased frequency of LCs in vaginal epithelium</td>
<td>76, 77</td>
</tr>
<tr>
<td>Regulation of HIV replication and LTR activity</td>
<td>254</td>
</tr>
<tr>
<td>Suppression of IL-1, IL-2, and IL-6 release by human lymphocytes</td>
<td>148, 177</td>
</tr>
<tr>
<td>Inhibition of TLR-9-induced IFN-α production by human and mouse pDCs</td>
<td>162</td>
</tr>
<tr>
<td>Increased shedding of HIV-1 in the genital tract</td>
<td>35–37</td>
</tr>
<tr>
<td>Decreased FcγR expression on monocytes</td>
<td>159, 160</td>
</tr>
<tr>
<td>Decreased vaginal colonization with H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;-producing Lactobacillus</td>
<td>70</td>
</tr>
</tbody>
</table>

VZV, Varicella-zoster virus; TLR, Toll-like receptor; LTR, long terminal repeat; pDC, plasmacytoid dendritic cell.

VI. Immunoregulatory Effects of Progesterone

Progesterone exerts a strong immunosuppressive effect on the production and transepithelial transport of IgG and IgA (78, 87–96, 129–134) (Table 2). Treatment of ovariectomized rats with progesterone results in a significant decline in cervicovaginal content of IgA, IgG, and secretory component (SC), which represents an IgA-associated extracellular segment of polymeric Ig receptor (pIgR) expressed by epithelial cells (129, 130). Progestin-containing contraceptives have been reported to decrease the levels of IgG and IgA in serum but not in secretions in humans, although the results seem to be dependent on the composition and concentration of particular steroid hormones used (93, 131, 135). Treatment of mice with DMPA results in decreased specific IgG and IgA responses after intravaginal or intranasal immunization with attenuated HSV-2 and causes a failure to develop protective responses (132, 133). The inhibitory effect of progesterone on vaginal humoral responses is routinely used to establish permissive conditions for intravaginal infections of mice with HSV-2 and Chlamydia trachomatis (132–134). However, in addition to the effect of progesterone on immune factors, the increased susceptibility to Chlamydia and HSV-2 may be caused by thinning of the vaginal epithelium, allowing easier access of pathogens to basal layer (136). Progesterone derivatives were also reported to alter the pattern of Ig glycosylation, specifically by inducing the synthesis of asymmetric antibodies characterized by the presence of an oligosaccharide group with high mannose content only on one of the two Fab fragments (137, 138). This prosthetic group sterically hinders the interaction with antigen, reducing the affinity by about 100-fold. Asymmetric antibodies are increased during pregnancy, mostly associated with the placenta (138).

It is well established that the levels of progesterone reached as a result of administration of injectable contraceptives such as DMPA exert a number of effects on T cell-mediated immune mechanisms including inhibition of cytolytic activity of T cells and blocking perforin expression in T cells, both directly and indirectly (139–147). Progesterone and its derivatives also alter the expression of cytokines, generally favoring Th2-type responses (133, 148–154). In a recent study, Enomoto et al. (148) demonstrated that progesterone at concentrations achieved during hormonal therapy decreased the proliferation and Th1-type cytokine production of varicella-zoster virus-specific CD8<sup>+</sup> and CD4<sup>+</sup> T cells; interestingly, the effect was significantly stronger on cells obtained from HIV-1-infected patients. The effect of progesterone appears to be mediated at least in part by progesterone-induced blocking factor (PIBF), a 34-kDa protein produced by lymphocytes after activation in the presence of progesterone (144, 149, 150). PIBF binds to IL-4R α-chain and GPI-anchored receptor (155) and blocks CTL-mediated killing by inhibition of perforin expression (156). PIBF induces the production of IL-3, IL-4, and IL-10 and inhibits the production of IL-12 by human peripheral blood mononuclear cells, suggesting its role as a mediator of a progesterone-
induced shift toward Th2 cytokine expression profile and dampening Th1-dependent responses (133, 149–154).

Progestins significantly alter infiltration of female genital tract tissue with NK cells, lymphocytes, and macrophages (115–119, 157, 158). Progesterone inhibits NK cell activity and FcγR expression on monocytes, thus reducing the two arms of antibody-dependent cell cytotoxicity (106, 159, 160). Decreased antibody production, CTL, interferon (IFN)-α, and antibody-dependent cell cytotoxicity activity may contribute to the increased shedding of HIV-1 detected in women using contraception (35–37, 161).

Recently, Hughes et al. (162) demonstrated that medroxyprogesterone, the active compound of DMPA, inhibits Toll-like receptor-9-induced IFN-α production by human and mouse plasmacytoid dendritic cells (DCs), likely through a selective blockade of IFN regulatory factor-7 activation. IFN-α normally induces an antiviral state in nonimmune tissues and primes adaptive antiviral responses by directly activating antigen-presenting cells and T cells. By blocking its production, medroxyprogesterone impairs a critical mechanism of innate antiviral immunity.

Membrane-associated and intracellular progestrone receptors are expressed by a number of cells including B cells, macrophages, NK cells, and γδ-T cells taken from healthy pregnant women (a high progesterone state) (163–165). The expression of progesterone receptors in αβ CD4+ and CD8+ T cells remains controversial and appears to be affected by the activation state and other factors (166–169). Several studies reported the presence of progesterone receptors on T cells isolated from pregnant patients with endometriosis (143, 146, 168, 170–172).

Importantly, in contrast to progesterone, medroxyprogesterone does not exhibit its biological effect exclusively via the progesterone receptors, but also by binding to the glucocorticoid receptor (GR) that is widely expressed by various cells of the immune system (173–176). Medroxyprogesterone displays a higher binding affinity than progesterone for the human GR (Ki 10.8 and 215 nM for progesterone and medroxyprogesterone, respectively) (173). Medroxyprogesterone induces greater phosphorylation at Ser211 and conformational changes in the liganded GR different from those induced by progesterone, and it displays greater glucocorticoid transactivation agonist potency and a higher potency for transrepression than progesterone or norethindrone acetate (an alternative contraceptive) (173). Medroxyprogesterone, but not estrogen or progesterone, suppresses IL-1, IL-2, and IL-6 release by human lymphocytes to a similar extent as classic glucocorticoids dexamethasone and hydrocortisone (148, 177). Liganded GR-mediated repression of IL-2 gene expression is thought to be due to the direct interaction of GR with transcriptional enhancers such as activator protein-1 and nuclear factor-κB (178–180). Thus, by binding to the GR, medroxyprogesterone, the active component of many contraceptive preparations, may exert significantly stronger immunosuppressive activity than progesterone.

**VII. Immunoregulatory Effects of Estrogen**

Depending on its concentration, estrogen can exert either a proinflammatory or antiinflammatory effect (80, 181) (Table 3). At low levels, estrogen induces TNF-α, IL-6, and IL-1β; inhibits Th2-type cytokines; and increases migration of leukocytes to the site of inflammation (80). In sharp contrast, at high levels, estrogen inhibits cell-mediated immunity and decreases the expression of a number of activation markers (148, 182). Estrogen inhibits the production of TNF-α, IL-1β, and IL-6 by T cells, macrophages, and DCs, and induces Th2-type cytokines IL-4, IL-10, and TGF-β, resulting in an antiinflammatory effect (183). Peak estrogen levels reduce the production of Th1-type cytokines such as TNF-α and IFN-γ by T cells, macrophages, and DCs (80, 184–186).

Estrogen enhances antibody production by human peripheral blood mononuclear cells *in vitro* by enhancing IL-10 production by monocytes (187). However, the effect of estrogen on antibody production in the female genital tract appears to be more complex. Although estradiol elevates the levels of IgG, IgA, and SC in the uterus of ovariectomized rats, it decreases their levels in cervicovaginal secretions (129, 130, 188). The action of estradiol on cervicovaginal IgA, IgG, and SC appears to be independent of

<table>
<thead>
<tr>
<th>TABLE 3. Effects of estrogen and its derivatives on the immune system</th>
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<tr>
<td><strong>Reported effect of estrogen and its derivatives</strong></td>
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<tr>
<td>Protection against HIV vaginal transmission in ovariectomized rhesus macaques</td>
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<tr>
<td>Increased thickness of vaginal wall</td>
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<tr>
<td>Decreased IgA, IgG, and SC in female genital tract</td>
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<tr>
<td>Increased expression of plgR by endocervical and endometrial epithelial cells</td>
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<td>Decreased antigen presentation, CTL activity, increased TGF-β in genital tract</td>
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<td>Decreased MCP-1 production, decreased macrophage migration into genital tract tissue</td>
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<td>Decreased NK cell activity</td>
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<tr>
<td>Decreased frequency of LCs in vaginal epithelium</td>
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<tr>
<td>Decreased recruitment of inflammatory T cells and macrophages through down-regulation of ICAM-1, VCAM-1, and E-selectins</td>
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VIII. Possible Mechanisms of the Effect of Progesterone-Based Contraceptives on Disease Progression in HIV-1 Infected Individuals

HIV-1 infection is characterized by rapid and extensive infection and depletion of memory CD4+ T cells in mucosal lymphoid tissues, most prominently in the intestinal lamina propria and genital tract-associated tissues early in the acute phase of infection (193–202). CD4+ T cells play a critical role in the regulation of Ig isotype switching and broadening of antibody diversity by somatic hypermutation in B cells residing in mucosal lymphoid tissues (203–206). The regulation of class switch recombination, somatic hypermutation, and transepithelial transport of IgA is dependent on stimulatory cytokines, such as IL-4, IFN-γ, IL-5, IL-6, IL-10, and TGF-β, normally produced by CD4+ T cells (204, 207–210). A near-complete elimination of the memory CD4+ T cell population in mucosal lymphoid tissue results in an absence of important regulatory and effector functions these cells normally provide in the control of immune responses to environmental antigens and commensal bacteria on one side and infecting pathogens on the other (211, 212). Their depletion, in the long term, compromises mucosal defenses by several mechanisms including reductions in specific IgA responses, decreased barrier function of epithelial cells, and functional alterations of CD8+ cytotoxic T cells (196, 203, 213–215). Extensive evidence suggests that HIV-1 and SIV cause severe damage to gastrointestinal mucosal surface and antimicrobial functions of the mucosal barrier (216–220). Increased mucosal leakiness was observed in SIV-infected macaques (221) and in HIV-1+ patients with low levels of HIV-1-specific IgA antibodies (222, 223). Recently obtained data strongly suggest that the impairment of mucosal barrier function associated with increased absorption of environmental microbial antigens to the systemic compartment may be the major cause of the continuous activation of CD4+ and CD8+ T cells in HIV-1 infection (193, 216, 224–229). Importantly, direct evidence of increased microbial translocation was recently obtained by Brenchley et al. (229), who demonstrated that significantly raised levels of plasma bacterial lipopolysaccharide (LPS) in chronically HIV-1-infected individuals and SIV-infected rhesus macaques, evidence for chronic in vivo stimulation of monocytes by LPS, an association between plasma LPS and the level of T cell activation, and an association between the reduction in plasma LPS and CD4+ T cell reconstitution during highly active antiretroviral therapy. However, the underlying causes of increased microbial translocation across the mucosal barrier in HIV-1-infected patients remain unclear.

Recent studies, including those in our laboratory, indicate that HIV-1-specific IgA antibodies are either absent or unexpectedly low in external secretions and sera of HIV-1-infected individuals (230–232). In contrast, HIV-1-specific IgG antibodies are easily detectable and quantitatively measurable, even in secretions where IgA is an overwhelmingly dominant isotype (223, 230–238). These results are in remarkable agreement with studies performed in HIV-1-infected chimpanzees (239) and SIV-infected rhesus macaques (221, 240). Thus, HIV-1 infection in humans and SIV infection in primates appear to result in a selective and profound suppression of virus-specific IgA but not IgG responses in the mucosal as well as systemic compartments (204, 232, 241). These data suggest that the suppression of antigen-specific IgA responses in mucosal tissues is the cause of increased bacterial translocation across the mucosal barrier in the chronic phase of infection. IgA plays a critical role in the regulation of the immune response to microbial community in the gut and in the reduction of inflammation induced by bacterial products and proinflammatory agents (216, 224, 225). Mucosal IgA antibodies play an essential role in the control of a number of mucosal pathogens, such as Salmonellae spp., Campylobacter jejuni, Shigellae spp., Giardia lamblia, Clostridium difficile, and Cryptospo-
ridium, that cause significant morbidity in HIV-1-infected patients (242–244). Impaired mucosal humoral responses to these pathogens are likely to increase the incidence and prolong the prevalence of mucosal infections (245–250).

As described above, progesterone and its derivatives exert a strong effect on humoral immune responses in the genital tract and other mucosal tissues including decreasing the frequency of antibody-secreting cells, inhibition of IgG and IgA production, and transepithelial transport, and altered Ig glycosylation pattern. Thus, administration of progesterone-based contraceptives to HIV-1 infected women likely results in further inhibition of specific mucosal IgA and IgG responses either directly via its effect on B cells or indirectly via accelerated depletion of CD4⁺ T cells or inhibition of receptor-mediated Ig transcytosis. Due to the physiological role of progesterone in the regulation of menstrual cycle and immune responsiveness in the female genital tract, it is likely that progesterone-mediated inhibition of the cytotoxic activity of T and NK cells and altered Ig production will be more pronounced in the genital tract tissue compared with the systemic compartment. Attenuated immune responses against HIV-1 in combination with increased frequency of CD4⁺CCR5⁺ T cells are likely to result in preferential HIV-1 proliferation in the genital tissue and increased shedding of HIV-1. This is supported by the observed association between hormonal contraception and increased shedding of HIV-1 in cervicovaginal lavage with a significant dose dependency on progesterone levels (35–37).

It has not yet been investigated whether medroxyprogesterone treatment increases the level of HIV-1 proliferation at other mucosal tissues, such as gut-associated lymphoid tissue, the largest lymphoid organ in the body and preferential site of HIV-1 proliferation (193). If this is the case, increased depletion of CD4⁺ T cells at mucosal lymphoid tissue could represent a key mechanism responsible for accelerated CD4⁺ T cell decline in progesterone-treated women. We believe this to be a plausible hypothesis due to the documented immunosuppressive effect of progesterone and its derivatives on specific immune responses in mucosal lymphoid tissues. Redistribution of viral proliferation in women using hormonal contraceptives may result in a CD4⁺ T cell “sink” responsible for the continuous removal of CD4⁺ T cells from the system.

IX. Conclusions

The data summarized here suggest that hormonal contraception may exert a significant effect on the susceptibility to HIV-1 infection as well as on the progression of the ensuing disease. However, the epidemiological data obtained so far are inconclusive. More detailed and comprehensive studies are needed to provide information as to which type of contraception should be used by HIV-1-infected women and women at high risk of infection. Optimally, these studies should involve large numbers of subjects at high risk of HIV-1 exposure; employ randomized, controlled, and safe administration of defined doses of contraceptives; and control for other confounding factors such as genital infections. Although such studies may be financially demanding, their cost is justified by the importance of the question and the potential impact on the spread of HIV-1 epidemic. Importantly, data obtained in the nonhuman primate model strongly suggest that estrogen enhances the natural protective properties of the female genital tract tissue and decreases its susceptibility to virus transmission. If confirmed by future studies, systematically or topically applied estrogen may represent an inexpensive and safe method of HIV-1 prevention. Further studies are needed to elucidate the basic underlying mechanisms of estrogen-induced mucosal protection against SIV and HIV-1 viruses.

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