Viruses in the Mammalian Male Genital Tract and Their Effects on the Reproductive System

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INTRODUCTION

Concern about the sexual transmission of viruses in humans and its health consequences has peaked with the appearance and development of the AIDS pandemic. There is also much concern about the possibility of sexual transmission of viruses in animals, particularly in economically important farm animals. Such sexually transmissible diseases (STDs) may cause epidemics, which may be spread further by the worldwide export of the gametes and embryos of these animals. In light of these major health and economic issues concerning sexually transmitted viral diseases (Table 1), it is paradoxical that, to our knowledge, no general review has been published concerning the presence of viruses in human and animal reproductive tracts and semen and the possible effects of these viruses within infected organs and on reproductive endocrinology. This review aims to address this subject by describing the various viruses identified in the semen and reproductive tracts of mammals, their distribution in tissues and fluids, their possible targets, and the functional consequences of their infectivity on the reproductive and endocrine systems.

VIRUSES AND SEMEN

Human Semen

The viruses present in human semen and their consequences are listed in Table 2 and Fig. 1.
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Abnormalities detected in the presence of virus

Infections of semen and infected mononuclear cells, are used for acute because spermatozoa from HIV-positive men, cleared of about the vertical transmission of HIV. This problem is very of debate and has recently been reviewed (8), raising questions of the presence of HIV-1 in the spermatozoa themselves is now a matter (risk of transmission to subsequent generations)

Infection of ova and embryo, miscarriage, and embryonic and fetal abnormalities

In Table 1, matters of concern relating to viral infection of the reproductive tract or semen

Spreading of diseases
Infection/sterility resulting from:
- Changes in one or more testicular compartments (e.g., germ cells, Sertoli cells, Leydig cells)
- Infection into the reproductive tract or semen of leukocytes causing a T-cell-mediated response to spermatozoa
- Cachexia induced by a drop in testosterone production
- Incorporation of the viral genome into the germ cell genome (risk of transmission to subsequent generations)
- Infection of ova and embryo, miscarriage, and embryonic and fetal abnormalities

Human immunodeficiency virus type 1. Human immunodeficiency virus (HIV) is now the most extensively studied sexually transmitted virus. Its presence in the semen was rapidly established, but the nature of the cells infected remains unclear. One key question is whether the virus strains present in semen arise from the same compartment as those detected in blood cells, since new multidrug therapies are still unable to eradicate the virus completely (71).

The virus was initially isolated from the mononuclear cell fraction of the semen of two men in the process of developing the disease and from one HIV-1-seropositive man (142, 355, 356). It has been shown to be transmitted via the semen of asymptomatic carriers (14, 19, 311). HIV-1 has also been detected in the cell-free seminal fluid of both an AIDS patient and an asymptomatic HIV-positive individual (47). Detection of the virus in this location led to the suggestion that the epididymal epithelial cells may become infected and release HIV into the epididymal fluid. The prostate and seminal vesicles may also act as a virus reservoir and release HIV into the semen. This contamination would occur in addition to that of macrophages and lymphocytes, which are often present in the semen and are natural targets for HIV (9). The possible presence of HIV-1 in the spermatozoa themselves is now a matter of debate and has recently been reviewed (8), raising questions about the vertical transmission of HIV. This problem is very acute because spermatozoa from HIV-positive men, cleared of seminal plasma and infected mononuclear cells, are used for medically assisted reproduction in serodiscordant couples (293–295). Thus, although semen washing on a density gradient before artificial insemination may reduce the risk of HIV transmission from the infected man to an uninfected woman (166, 188), the virus may still be detected in the fraction of motile spermatozoa used for insemination (70, 203, 316). Insemination in such cases is, of course, not performed. These positive results may be false positives due to the use of a single set of primers in the PCR detection of HIV (203) or due to the presence of contaminating cells of nonseminal origin (the mean proportion of such cells is 1/1,000) (316). However, electron microscopy and immunocytochemistry studies have provided evidence that HIV-1 can attach to the surface of spermatozoa and enter these cells through the intact plasma membrane (24–26). An in vitro study has also demonstrated that spermatozoa from healthy donors may carry HIV-1 on their surface and subsequently transmit it to lymphocytes in culture (104). Although still controversial (258), several lines of evidence suggest that HIV is able to bind to spermatozoa. However, it is unclear whether the virus penetrates and replicates in these cells or simply integrates into them. In another study (23), in situ PCR was used to determine HIV-1 provirus levels in seminal cells from 94 HIV-1-infected men at various stages of the clinical disease. Both seminal mononuclear cells and spermatozoa (35 and 33% of samples studied, respectively) were found to harbor HIV-1 proviral sequences. HIV DNA was also detected by PCR in the motile sperm fraction of all three samples tested (288). However, these results are not consistent with those of three other studies using PCR to detect HIV DNA (22, 213, 259). Experimental HIV penetration into the spermatozoa of healthy donors has been reported by Bacetti et al. (22), who used immunocytochemistry and in situ hybridization with electron microscopy to detect antigens and viral RNA. HIV RNA was detected within spermatozoa, but no viral DNA was detected in this study, indicating that if HIV did penetrate spermatozoa, it did not integrate and replicate within these cells. Consistent with this, it has been found that spermatozoa do not represent a significant source of HIV in semen (353). Indeed, a study has shown that vasectomy has little effect on the infectivity of semen, leading to the conclusion that most cell-free HIV in seminal plasma arises distal to

In Table 2, presence of viruses in human semen and its consequences

<table>
<thead>
<tr>
<th>Virus-infected cells and semen fractions (reference)</th>
<th>Abnormalities detected in the presence of virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocytes/macrophages and lymphocytes</td>
<td>Infertility</td>
</tr>
<tr>
<td>HIV (9, 142, 356)</td>
<td>HSV (109, 172)</td>
</tr>
<tr>
<td>CMV (265)</td>
<td>Adenovirus (80)</td>
</tr>
<tr>
<td>HBV (84, 135)</td>
<td>Azoospermia, oligospermia</td>
</tr>
<tr>
<td>HTLV-1 ? (318)</td>
<td>HIV (205)</td>
</tr>
<tr>
<td>Spermatozoa</td>
<td>HSV (172)</td>
</tr>
<tr>
<td>HIV ? (22, 26, 104, 203)</td>
<td>Morphologically abnormal spermatozoa</td>
</tr>
<tr>
<td>HBV (84)</td>
<td>HIV (AIDS) (175)</td>
</tr>
<tr>
<td>HSV (172)</td>
<td>Hematospermia</td>
</tr>
<tr>
<td>Cellular fraction (no specific cell type identified)</td>
<td>CMV (169)</td>
</tr>
<tr>
<td>Papillomavirus (181, 183, 184)</td>
<td>Pyospermia</td>
</tr>
<tr>
<td>Adenovirus (80)</td>
<td>HIV (AIDS) (175)</td>
</tr>
<tr>
<td></td>
<td>Decrease in the number of CD4+ cells</td>
</tr>
<tr>
<td></td>
<td>CMV (190)</td>
</tr>
<tr>
<td></td>
<td>Asthenospermia</td>
</tr>
<tr>
<td></td>
<td>Papillomavirus (183)</td>
</tr>
</tbody>
</table>
FIG. 1. Summary of the viruses found in the genital tract and semen of mammals.

### Prostate

<table>
<thead>
<tr>
<th>Human</th>
<th>HPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-human primate</td>
<td>Oncomavirus</td>
</tr>
<tr>
<td>Bull</td>
<td>BDV</td>
</tr>
<tr>
<td>Mouse</td>
<td>CMV, MMTV</td>
</tr>
<tr>
<td>Rat</td>
<td>C-type RNA virus</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>HSV-2</td>
</tr>
</tbody>
</table>

### Seminal Vesicle

<table>
<thead>
<tr>
<th>Human</th>
<th>CMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-human primate</td>
<td>SIV</td>
</tr>
<tr>
<td>Bull</td>
<td>BDV</td>
</tr>
<tr>
<td>Mouse</td>
<td>MMTV, HPV-18</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>HSV-2</td>
</tr>
</tbody>
</table>

### Epididymis

<table>
<thead>
<tr>
<th>Human</th>
<th>Coxsackie B4 virus ?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-human primate</td>
<td>SIV</td>
</tr>
<tr>
<td>Boar</td>
<td>Rubulavirus</td>
</tr>
<tr>
<td>Mouse</td>
<td>MMTV</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>HSV-2</td>
</tr>
</tbody>
</table>

### Semen

<table>
<thead>
<tr>
<th>Human</th>
<th>HIV, HHV-8, CMV, EBV, HPV, HBV, HCV, HSV, Adenovirus, HTLV-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-human primate</td>
<td>Herpes virus, SIV</td>
</tr>
<tr>
<td>Bull</td>
<td>BDV, BTV, BHV-1, FMDV, BLV, BIV</td>
</tr>
<tr>
<td>Boar</td>
<td>Rubulavirus, Parvovirus, PRRSV</td>
</tr>
<tr>
<td>Mouse</td>
<td>Retroviruses, CMV</td>
</tr>
<tr>
<td>Xenopus laevis</td>
<td>Rous sarcoma virus</td>
</tr>
<tr>
<td>Gibbon</td>
<td>Herpes virus</td>
</tr>
<tr>
<td>Cat</td>
<td>FIV</td>
</tr>
</tbody>
</table>

### Testis

<table>
<thead>
<tr>
<th>Human</th>
<th>Mumps, HIV-1, EBV, Parvovirus B19, HSV-1, HSV-2, Adenovirus, Coxsackievirus A9, Bat-salivary gland-virus ?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-human primate</td>
<td>SIV, CMV-like simian herpes virus</td>
</tr>
<tr>
<td>Mouse</td>
<td>CMV, EMCV, Influenza virus ?</td>
</tr>
<tr>
<td>Belgian Alouatta disease virus, Rubulavirus ?</td>
<td></td>
</tr>
</tbody>
</table>

| Boar | Mouse | Guinea pig | Mule Deer | Cat | Hamster |

- | Belgian Alouatta disease virus |
- | Rubulavirus ? |
- | PRRSV ? |
- | HSV-2 |
- | EHDV-2 ? |
- | BTY |
- | Feline leukemia virus ? |
- | ECMV |
the vas deferens (176). Recently, semen samples from 52 men, 21 of whom were receiving antiviral therapy, were tested for HIV and the amount of virus present was quantified (316). HIV RNA was detected in 86% of semen plasma samples and in 14% of spermatozoa fractions (as stated above, the authors suggested possible contamination by non-spermatozoa cells at a mean frequency of 1/1,000), whereas HIV DNA was present in only 57% of non-spermatozoa cell fractions.

It is unknown what prevents HIV replication in spermatozoa and which receptors are used by the virus to enter these cells. The CD4 receptor is present on semen lymphocytes and monoocytes (125, 126), but it is unclear whether it is present on spermatozoa. Ashida and Scofield (21) were the first to describe a sperm ligand that reacts with CD4 antibodies and interactions between sperm and HLA-DR-positive cells, providing strong evidence that CD4 is expressed on spermatozoa. CD4 molecules were detected on mouse sperm heads by both immunofluorescence and western Blotting (189). Their presence was also suggested, indirectly, in a study using a semen protein (gp17) that binds to CD4+ T cells and soluble recombiant CD4, as well as spermatozoa (38). However, the CD4 antigen has never been detected on the surface of human spermatozoa or on CD4+ ejaculate cells (epithelial and germinal cells) (15, 110, 125, 166, 240, 352). Neither of the two main HIV coreceptors (CXCR4 and CCR5) were detected on the surface of spermatozoa in flow cytometry experiments (166), although the possibility that they are expressed at a very low level cannot be ruled out. It has been suggested that other receptors are responsible for the entry of HIV-1 into spermatozoa. Thus, several studies have described a glycolipid that may function as an HIV receptor on the surface of spermatozoa and that may be involved in transmission of the virus (21, 22, 52, 53, 289). Sperm proteins that bind HLA-DR and are therefore likely to interact with somatic cells have also been described (276, 289). HIV may also infect germ cells early in spermatogenesis, resulting in the clonal transmission of the virus into spermatozoa; this is discussed below (see “Viruses and the testis,” below).

The effect of HIV infection on semen characteristics has been investigated. HIV was isolated from 15 (30%) of 50 specimens from asymptomatic individuals and from 1 of 3 specimens from patients with AIDS (175). The men with AIDS had pyospermia and grossly abnormal spermatozoa. In contrast, the semen specimens from other seropositive men did not differ significantly from those of healthy seronegative donors. No abnormality in sperm count, morphology, number or type of leukocytes in semen, or any other semen characteristic was associated with HIV shedding into semen. In another study, a significant positive correlation was found between blood CD4+ cell number and sperm motility in seropositive men, and a significant inverse correlation was found between CD4+ cell number and sperm abnormalities. The authors suggested that this may be due to a decrease in testosteronemia, resulting in defective epididymal sperm maturation (102).

Several studies have investigated whether the level of HIV-1 in semen varies with the stage of infection. It has been reported that the isolation of HIV from semen does not correlate with CD4+ or CD8+ T-lymphocyte counts and that seropositive men may shed HIV in semen early in the course of infection (175). Other studies have also reported that the presence of HIV DNA in semen is not related to the CD4+ cell count or disease status (198, 213). Indeed, although HIV-1 is more common in the semen of men with advanced HIV-1 infection and seminal leukocytosis, it can also be isolated from the semen of men with neither of these conditions (16, 342). Furthermore, men with HIV-1 infection are already potentially infectious through sexual relations during the first few weeks after infection (329). It has now been established that HIV-1 may be present in semen in both cell-free and cell-associated forms (and that it may be isolated from both asymptomatic individuals and AIDS patients) and that both forms are transmissible (360). Surprisingly, HIV seems to be shed intermittently into semen (50, 174). Concomitant STDs such as cytomegalovirus (CMV) (174), chancroid, syphilis, gonorrhoea, and Chlamydia infections (112) may affect the level of HIV shedding (206, 312). Herpes simplex virus (HSV) increases plasma HIV levels severalfold (221), and this increase may be reflected in seminal fluid. Also, the membrane proteins of CMV and human T-lymphotropic virus type 1 (HTLV-I) have large regions of similarity to CD4 (276), suggesting that cells infected by these viruses may be more susceptible to HIV infection.

Cohen et al. (72) compared two groups of HIV+ patients, consulting for dermatological problems or genital infections (urethritis). The median HIV RNA level in the semen of the group with dermatological infections was one-eighth that in the group with genital infections. In the group with genital infections, the subgroup of patients with gonorrhoea had the highest seminal viral load. Antibiotic treatment of urethritis reduced the viral load in the semen but did not affect the plasma viral load. This study clearly establishes that local infections of the male reproductive tract are important cofactors of HIV load in the semen.

The question of viral compartmentalization was raised in a longitudinal analysis of eight subjects who went on to develop AIDS. The seminal viral load increased in most cases, but the viral load was consistently higher in blood plasma than in semen (132). An absence of correlation between plasma and semen loads was also reported in another study and suggests that the semen and blood are separate viral compartments (198). A comparison of HIV-1 gp120 sequences from five recent seroconverters with those from their corresponding sexual partners (transmitters) revealed that in each couple studied, the variant transmitted corresponded to a minor population in the semen of the transmitter, providing evidence that HIV-1 selection occurs during sexual transmission (360). Protease gene sequences also differ in semen and blood (55, 165), as do viral phenotypes (342) and the ratio of infected to uninfected leukocytes (165). Further evidence of viral compartmentalization is provided by the following observations: the lack of association between the culturability of the virus in semen and viral RNA levels in blood, the discordant distributions of viral phenotypes, the discordant viral RNA levels, the absence of correlation between viral RNA levels in semen and CD4+ cell counts in blood, differences in the biological variability of viral RNA levels, and differences in viral load following antiretroviral treatment (75). HIV-1 comprises two main phenotypic strains: NSI (non-syncytium inducing) strains, which infect macrophages, use CCR5 as a coreceptor for cell entry, and are preferentially transmitted; and SI (syncitium inducing) strains, which use the CXCR4 coreceptor, appear later in the disease,
and are poorly transmitted. It has therefore been suggested that there is selection between NSI and SI strains in the genital tract. However, restriction of SI variants in the male genital tract, such as would account for the observed NSI transmission bias, remains to be established, because SI and NSI strains do not seem to be compartmentalized in semen (90). The precise identification of the viral reservoir in the body is now of the utmost importance for antiretroviral treatment. Thus, although the development of potent treatments raises the hope that HIV-1 eradication might be possible, we do not yet know whether all the compartments in which the virus replicates are accessible to antiretroviral compounds. Several studies have investigated whether drug-resistant strains develop in seminal plasma and whether patients undergoing treatment carry infectious variants in their semen. A study by van’t Wout et al. (340) analyzed the relationship between HIV-1 quasispecies extracted from the blood at various times and from various organs at autopsy. The brain quasispecies were very homogeneous and differed from the peripheral blood mononuclear cell variants, suggesting compartmentalization and the early spread of HIV-1 to the brain. Tissue-specific quasispecies were also observed in the testis, but in this case they were suggestive of a later invasion, possibly secondary to lymphocyte infiltration due to the disease (340). An evaluation of blood and genital secretions from HIV-infected men under treatment showed no genotypic changes consistent with protease inhibitor resistance in semen, despite the presence of these agents in blood plasma. This therefore suggests that protease inhibition may have limited penetration into the male genital tract (111). Replication-competent virus was detected in the semen of HIV-infected men receiving antiretroviral treatment, although there was no detectable virus in the peripheral plasma (358). In light of these studies, it appears that treatment strategies for the complete elimination of HIV-1 from the genital tract should now be a public health priority. It is therefore urgent to study the penetration of antiretroviral compounds into the male genital tract, such as would account for the observed NSI transmission and the progression of HIV-1 disease, a group of 234 asymptomatic HIV-1 antibody-positive homosexual men were previously erroneously reported to be cold labile, whereas it in fact survives in frozen and thawed semen (137). CMV is thought to be a possible causative agent of hematospermia (169). The virus generally remains in a latent form and causes a lifelong infection, but it may be activated either by a primary infection, for example after organ transplantation, or by the impairment of cellular immunity. Prospective studies in the United States have demonstrated that CMV is responsible for more prenatal and perinatal virus infections than is any other transmissible agent identified to date. The impact of these infections on fetal and neonatal health is unclear. However, preliminary data suggest that CMV is probably the most important agent responsible for congenital infection and damage (185). The French government decided some time ago to reject CMV-seropositive donors for artificial insemination. It then pulled back from this decision, deciding instead to reject only donors with recent seroconversion.

**Human T-lymphotropic virus type I.** HTLV-I, like HIV, is a retrovirus that infects T cells. It is epidemiologically linked to adult T-cell leukemia-lymphoma (230). HTLV-I is sexually transmitted by semen (231, 317, 318), most probably via contaminated lymphocytes in the semen.

**Human herpesvirus 8.** Kaposi’s sarcoma (KS) is frequently associated with AIDS and occurs mainly in homosexual men. The epidemiology of KS in HIV-infected patients suggests that it may be caused by a sexually transmitted infectious agent (37, 144). This agent has recently been identified as a new human herpesvirus called human herpesvirus 8 (HHV-8) or KS-associated herpesvirus. According to many studies using extremely sensitive serologic techniques (76, 97, 133, 145, 225, 321, 322), the prevalence of HHV-8 in the general population is low. However, it is generally accepted that the virus can be detected in the semen of KS-positive patients. A recent study detected HHV-8 DNA in 12% of semen samples from KS patients, with a mean copy number per microgram of positive target DNA of 300, versus 9,000 in blood cells (182). Significant amounts of HHV-8 DNA were also detected in semen samples from 28 HIV-1-infected individuals and were shown to infect the mononuclear cell fraction (45). A recent multicenter comparison study concluded that HHV-8 DNA is present in semen but at concentrations that are probably too low to facilitate its consistent detection (251). Thus, the semen viral load should be measured to determine whether it is high enough for sexual transmission to occur. In addition, the prevalence of HHV-8 in the semen of healthy men has not yet been accurately established. It should, however, be borne in mind that the incidence of KS in HIV-negative individuals is much higher in Italy than, for example, in the United Kingdom (30 times higher) or the United States (20 times higher). In Denmark, a recent study performed on 100 healthy donors did not detect any seminal HHV-8 DNA (161). Therefore, the prevalence of HHV-8 in semen may be higher in some geographical areas than in others. The nature of the infected cells in semen and the origin of the virus also remain to be determined.

**Cytomegalovirus.** CMV also belongs to the *Herpesviridae* family. It is very common, with 50% of the otherwise healthy population being infected (349). Infection is spread by intimate contact with infected body fluids including semen (185), and 40% of the semen from healthy donors is infected. This virus was previously erroneously reported to be cold labile, whereas it in fact survives in frozen and thawed semen (137). CMV is thought to be a possible causative agent of hematospermia (169). The virus generally remains in a latent form and causes a lifelong infection, but it may be activated either by a primary infection, for example after organ transplantation, or by the impairment of cellular immunity. Prospective studies in the United States have demonstrated that CMV is responsible for more prenatal and perinatal virus infections than is any other transmissible agent identified to date. The impact of these infections on fetal and neonatal health is unclear. However, preliminary data suggest that CMV is probably the most important agent responsible for congenital infection and damage (185). The French government decided some time ago to reject CMV-seropositive donors for artificial insemination. It then pulled back from this decision, deciding instead to reject only donors with recent seroconversion.

Two independent laboratories tested for CMV in 178 cryopreserved sperm samples from 97 healthy donors, 34% of whom were CMV seronegative, 52.6% of whom were seropositive with no recent contamination (absence of immunoglobulin M), and 13.4% of whom had unknown serological status. They detected CMV in 2.8% of the samples after culture and in 5.6% by PCR, thereby demonstrating that CMV can be detected in the semen in the absence of recent contamination (200).

CMV is also one of the most common opportunistic infections in AIDS patients, and it has been suggested that persistent CMV infection of the semen increases the risk of AIDS, possibly by activating CD4 + cells such that HIV-1 is produced (93). CMV has been isolated from the semen of homosexual men (202, 301), into which it was excreted intermittently (73), and CMV levels are associated with HIV seropositivity (186, 270). However, Rinaldo et al. found no association between CMV shedding and a higher risk of developing AIDS (269). The shedding of HIV was more closely associated with the concomitant shedding of CMV than with the CD4 + cell count (174). In a study investigating the relationship between CMV infection and the progression of HIV-1 disease, a group of 234 asymptomatic HIV-1 antibody-positive homosexual men were...
tested for CMV. CMV was isolated from the semen of 45% of the men. CD4+ cell levels were significantly lower in those in whom CMV had been isolated from semen. Similarly, an inverse relationship was observed between the concentration of whom CMV had been isolated from semen. Similarly, an

verse relationship was observed between the concentration of CMV in semen and the CD4+ cell levels (190). Leach et al. (191) later concluded from junctional hybridization experiments that the presence of multiple CMV strains in HIV-1-positive homosexual men was associated with the progression to AIDS, possibly via activation of HIV-1-infected CD4+ cells. Hematopoietic cells are the only cells in the semen to have been identified as being infected (265).

Epstein-Barr virus. Epidemiological studies have shown that Epstein-Barr virus (EBV) infection is most common in the age group at which sexual activity begins, strongly indicating that it is sexually transmitted (334). The presence of EBV in semen has not yet been investigated, but several studies have reported that human seminal plasma activates replication of this virus. Thus, EBV early-antigen expression is stronger in infected cells cultured in the presence of semen (149, 151, 330, 357). In vitro inhibition by seminal plasma of both the T- and B-lymphocyte responses to infection has also been described (193, 334). These results suggest that seminal plasma may facilitate EBV replication in the cervix of the uterus and may therefore have some relevance to the etiology of cervical cancer.

Papillomavirus. Papillomaviruses, like EBV, cause cancer. There has been extensive testing for these viruses in the male genital tract, especially in the prostate, since they are thought to be a possible cause of prostate cancer; this is discussed below (see “Viruses and the prostate and other accessory glands”). Human papillomavirus (HPV) DNA was detected in the semen of three patients, and the data obtained were consistent with the contention that HPV can be transmitted sexually via semen, as suggested by epidemiological data on the sexual transmission of HPV (247). Since that first report, papillomaviruses have been detected in semen in several studies (130, 131, 150, 181, 184). These results conflict with early work by Nieminen, which claimed that papillomavirus DNA was not transmitted by semen since the semen of the sexual partners of 17 women positive for HPV DNA was uninfected (238). However, these results actually show only that transmission from the woman to the man is rather inefficient. Indeed, other studies have indicated that HPV type 16 and 18 DNA is present in sperm cells and may be transmitted to the partner (64, 181, 183, 184). Thus, HPV type 16 DNA and RNA were detected in the semen of 25 and 8% of 24 randomly selected patients, respectively, whereas the prevalence of detection for HPV type 18 DNA and RNA was higher (46 and 21%, respectively). The incidence of asthenozoospermia is significantly higher in patients with HPV in their semen (183).

Hepatitis viruses. Hepatitis viruses may cause acute diseases (fulminant hepatitis) or chronic diseases such as liver cancer and cirrhosis. The ability of human semen to transmit hepatitis B virus (HBV) was first demonstrated by the inoculation of gibbons (291). HBV antigens were subsequently detected in human semen (155), and it is now well established that this biological fluid is a vector for the spread of hepatitis B (84, 107, 115, 153, 158). However, few studies have tried to identify the contaminated cells within semen. Hachchuel et al. (135) showed that HBV DNA was integrated into the DNA of sper-
from a cohort of 54 HIV-1-infected homosexual men (143). The pathological significance of HGV is unknown.

**Herpesvirus and adenovirus.** HSV is a sexually transmitted agent that may be associated with cervical cancer and may cause high morbidity and mortality in perinatal infection (7, 233). Centifanto et al. (60) were the first to suggest that it was present in the male genital tract: inclusion bodies that resembled those of herpesvirus were found in tissue cultures of semen from two subjects, but no infectious virus could be cultured directly from the samples (94). Moore et al. (226) described an individual in a therapeutic donor insemination program who asymptptomatically acquired a primary HSV-2 infection and transmitted it to one of two HSV-seronegative partners, in whom a primary HSV-2 infection developed, providing evidence for the sexual transmission of HSV-2. HSV DNA was recently detected in the semen of men with genital HSV-2 infection, mainly during recurrences of herpes (345). Concerning the type of cell infected within semen, an early electron microscopy study found no ultrastructural relationship between the virion and the spermatozoon (302). However, HSV DNA has recently been detected in human spermatozoa by in situ hybridization (172). The association of HSV DNA with spermatozoa has been linked to fertility problems, because HSV infection was found to be almost three times as common in the semen of patients with a low sperm count attending an infertility clinic as in individuals with a normal sperm count (172). A significant association was also found between infertility and positive tests for HSV in another study performed on 153 men (109). A possible relationship between infertility and the presence of the adeno virus in semen has also been a matter of concern. Csata and Kulcsar (80) detected HSV or adenovirus in the semen of 40% of infertile patients tested and found that these viruses were present in a latent form in 60% of their spermatozoa.

Further studies are required to confirm these data and to elucidate the link between the presence of these viruses in semen and fertility problems.

**Animal Semen**

Several viruses have been detected in the semen of a number of animal species (Table 3). DNA from Rous sarcoma virus binds to *Xenopus laevis* spermatozoa and is transferred to ova during fertilization, inducing developmental malformations in 25 to 30% of embryos (134). In gibbons, sperm abnormalities are frequently encountered and have often been shown to be related to the presence of herpesvirus (36, 327).

Feline immunodeficiency virus is shed into the semen of both experimentally and naturally infected cats (154). Simian immunodeficiency virus (SIV) is also present in the semen of monkeys and is transmitted by this vector (217).

In mice, high concentrations of retroviral particles have been detected in the epididymal fluid (162–164). These viruses are mostly endogenous retroviruses, but some exogenous infectious retroviruses (mouse ecotropic virus and Friend ecotropic virus) have also been described (254), and the association of retroviral particles with spermatozoa may lead to congenital infection. Murine leukemia virus has been found free in the seminal fluid, fixed to spermatozoa, and associated with macrophages (164, 194). The main site of virus synthesis within the male genital tract is the epithelial cells lining the epididymis duct. Murine CMV DNA has also been detected in spermatozoa, and the affected mice remained fertile (31).

The porcine reproductive respiratory syndrome (PRRS) was
first recognized in the United States in 1987 and in Europe in 1990 and has now spread worldwide. The PRRS virus (PRRSV) has been detected in semen (341) and is transmitted sexually (6, 237). Attempts have been made to determine the origin of PRRSV in semen by inoculating vasectomized and nonvasectomized boars intranasally with the virus. PRRSV RNA was detected in semen from all the boars and was most consistently found within macrophages. Therefore, macrophages in semen are probably infected via the infection of local tissue macrophages or via infected circulating monocytes or macrophages (69). In contrast, porcine parvovirus DNA, detected in the epididymal semen of oronasally inoculated boars, was found to bind to spermatozoa (128) but seemed to have no negative effect. Lastly, porcine rubulavirus has been reported to reduce sperm quality in sexually mature boars by decreasing spermatozoon motility and concentration (263).

Since viral contamination of semen is common in bulls and since frozen semen is widely distributed and is of major economic importance, national and international organizations have laid down guidelines aimed at establishing disease-free bull studs producing semen free from potential pathogens. Many different viruses have been detected in bull semen. In particular, bovine diarrhea virus has been detected at high titers in semen (28) and is transmitted by semen (214, 215, 285). This virus does not seem to cause sperm defects (170). Bluetongue virus (BTV) has also been isolated from bull semen (51, 146, 250, 252), and a positive relationship has been found between the infectivity of semen samples from bulls infected with latent BTV and semen abnormalities (118), with virus-like particles occasionally observed in the heads of affected spermatozoa (118). BTV is associated with reproductive disorders including early embryo death, abortion, malformedness of fetal calves and lambs, and transient infertility in bulls and rams coinciding with the shedding of virus into semen (245). Bulls were experimentally inoculated with BTV to investigate the frequency, duration, and pathogenesis of virus shedding into the semen. The shedding of BTV into semen in infected bulls closely followed peak virus titers in blood, and in no case was the virus isolated from semen without also being isolated from blood. Three of nine heifers inseminated with semen from a bull shedding BTV became viremic, seroconverted, and became pregnant. However, there was no evidence of fetal infection (48, 49). Bovine herpesvirus 1 (BHV-1), one of the most common viral pathogens in bovine semen, may also be associated with a decrease in semen quality, and bull semen is therefore systematically tested for this virus before being used for insemination (106). BHV-1 is present in the seminal plasma rather than the cell fraction (337). The risk of transmitting BHV-1 to cows by insemination has been investigated, and such transmission has been shown to lead to fertility disturbances (337–339). Foot-and-mouth disease virus has also been detected in bull semen and is transmitted by artificial insemination (35, 58, 78, 86, 285, 292, 300, 307). In contrast, bovine leukemia virus is present in semen but does not seem to be transmitted by it (35, 225, 271, 313, 332), possibly due to the presence of an inhibitory factor in fresh semen that prevents bovine leukemia virus infection from becoming established (271). Finally, bovine immunodeficiency virus, a lentivirus, is associated with a lymphoproliferative disease and is prevalent in dairy and beef cattle in the southeastern United States. Its mode of transmission is unknown, but semen is suspected to be a potential vector of infection. Indeed bovine immunodeficiency virus DNA has been detected in the leukocyte fraction of cryopreserved semen specimens (234).

### VIRUSES AND THE TESTIS

Semen is known to be an important vector in the dissemination of viral diseases, and several viruses are present in cell-free semen and in seminal cells including spermatozoa, macrophages and lymphocytes (see “Viruses and semen” above). Paradoxically, testing for viruses in the testis has not been extensive despite the possibility that they are involved in some of the disorders of this organ, such as orchitis, decrease in semen quality, azoospermia, and testicular carcinoma. The blood-testis barrier makes it likely that the testis acts as a reservoir for viruses (which may be protected against antiviral treatments), and this is another crucial reason for considering the testis to be an organ of special interest in the context of viral infection and STDs. Viruses present in the testis are listed in Fig. 1.

The mammalian testis has two main compartments: (i) the interstitium, which contains the Leydig cells responsible for testosterone production, macrophages, fibroblasts, blood, and lymphatic vessels; and (ii) the seminiferous tubules, which are bordered by the peritubular myoid cells and are composed of the various generations of germ cells (from spermatogonia [the stem germ cells] to the meiotic spermatocytes and the haploid spermatids that differentiate into spermatozoa) that are associated with the somatic Sertoli cells (152). Viruses have been found in both these compartments in humans and other mammals.

### The Human Testis

The best-known viruses causing testicular disorders in humans are mumps virus and HIV. A few studies have considered the role of oncogenic viruses, such as EBV or papillomavirus, in the etiology of testicular carcinoma even though testicular germ cell tumors are the most common malignant tumors in young adults and the incidence of testicular cancer has been steadily increasing in all countries for which epidemiological data are available (2, 39, 331). The viruses found in the human testis are listed in Table 4; see also Table 6.

**Mumps virus.** Mumps is caused by an RNA virus of the paramyxovirus group. In prepubertal boys, the symptoms of mumps are usually limited to infectious parotitis, but, in men, orchitis is the most common complication (29, 34). Orchitis develops in 5 to 37% of all adult patients infected with mumps (120). Within the first few days of infection, the virus directly attacks the testes, destroying the testicular parenchyma (44, 242) and decreasing androgen production (4). This accounts for the testicular atrophy observed in 40 to 70% of patients with orchitis (29, 34). Unilateral involvement is the most common, while bilateral involvement occurs in 15 to 30% of the patients with orchitis (201). Bilateral orchitis leads to hypofertility with oligospermia and testicular atrophy in 13% of those patients (59). Morphological studies of mumps-associated orchitis were carried out in the 1940s by Gall (122) and Charny and Meraney (65). They showed the focused nature of the inflammation and described the sequential stages of the dis-
TABLE 4. Viruses found in the human testis and their consequences

<table>
<thead>
<tr>
<th>Virus (reference)</th>
<th>Cells infected</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mumps virus</td>
<td>Leydig cells (4), germ cells</td>
<td>Orchitis (29, 34, 120), testicular atrophy (29, 34), sterility (3, 34, 159), decrease in androgen secretion (4), testicular cancer ? (34, 243)</td>
</tr>
<tr>
<td>HIV</td>
<td>Lymphocytes and macrophages (257), germ cells (228, 240)</td>
<td>Orchitis, interstitial fibrosis, lymphocyte infiltration, change in Leydig cell number, decrease in germ cell number (63, 220, 236, 266, 348), change in spermatogenesis (63, 82, 83, 92, 257, 273, 354)</td>
</tr>
<tr>
<td>EBV (67)</td>
<td>NA*</td>
<td>Orchitis (268), testicular cancer ? (10, 262, 305)</td>
</tr>
<tr>
<td>Parvovirus B19 (129)</td>
<td>NA</td>
<td>Testicular cancer ? (129)</td>
</tr>
<tr>
<td>HSV-2 (95)</td>
<td>NA</td>
<td>Viral reservoir ? (95)</td>
</tr>
<tr>
<td>HSV-1 (80)</td>
<td>NA</td>
<td>Infertility ? (80)</td>
</tr>
<tr>
<td>Adenovirus (80)</td>
<td>NA</td>
<td>Infertility ? (80)</td>
</tr>
<tr>
<td>Coxsackie virus</td>
<td>NA</td>
<td>Orchitis (216)</td>
</tr>
<tr>
<td>Influenza virus</td>
<td>NA</td>
<td>Orchitis (216)</td>
</tr>
<tr>
<td>Endogenous retroviruses</td>
<td>Germ cells (103, 187)</td>
<td>Testicular cancer ? (320)</td>
</tr>
</tbody>
</table>

* NA, not available.

During the initial stage, an interstitial edema was commonly detected. Blood vessels were congested and surrounded by lymphocytes. Increased permeability of the blood vessels was found to lead to local interstitial hemorrhage and to exudation of leukocytes and fibrin. The seminiferous epithelium degenerated, but the Sertoli cells seemed little affected. Repair of the damage caused by infection involved the deposition of collagen within the interstitium, followed by tubular atrophy and peritubular concentric fibrosis, the usual residue of mumps orchitis. Sterility may be transient or definitive and is due to the loss of the germinal epithelium (290). There are several possible explanations for the mumps-induced degeneration of germ cells. Since mumps virus does not seem to induce germ cell transformation or proliferation in vitro, it may have an indirect effect (243): (i) high fever associated with the disease leads to a change in testicular temperature, contributing to germ cell degeneration (the most common hypothesis); (ii) germ cell degeneration may be caused by seminiferous tubule congestion following the interstitial edema (201); or (iii) a modification of testosterone production by Leydig cells may have a deleterious effect on seminiferous tubule function. Data concerning the consequences of orchitis on testicular endocrine function are rare. Adamopoulos et al. (3) described a severe alteration of Leydig cell function during the acute phase of the disease. These authors observed a drop in the testosterone level together with an increase in luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels in 27 patients suffering from mumps orchitis, suggesting a testicular failure. In another study (4) examining three patients, testicular atrophy, libido decrease, impotence, and gynecomasty were associated with the reduction of testosterone and the increase in LH and FSH levels. Thus, in some cases, the Leydig cell function seems to be damaged by the mumps infection. Whether this alteration is due to a direct or indirect effect of the virus on the cells is unknown. Such a dysfunction would also account for the higher incidence of testicular cancer in men who have had mumps than in control men (34). Two publications suggested that mumps virus replicates within the testis: Bigazzi et al. (42, 43) showed viral replication in the interstitial tissue in organotypic culture of monkey testis following virus inoculation through the testicular vein, and Bjorvatn et al. (44) rescued mumps virus by needle aspiration of testis biopsy specimens from mumps-infected patients. Two studies (113, 177) showed that systemic treatment with interferon (IFN) prevented the testicular atrophy caused by mumps orchitis in all treated patients (4 of 4 and 13 of 13, respectively). In the second study, where patients were randomly assigned into two groups, atrophy of the testes was observed in three of eight men in the control group. However, asthenospermia was still detected in four patients 7 years after interferon treatment (177).

**Human immunodeficiency virus type 1.** Several endocrine and testicular dysfunctions have been reported in men infected with HIV-1, depending, in part, on the stage of the disease. High levels of testosterone have been found in men during the early stages of HIV infection (68, 212), whereas low testosterone levels have been found in men with AIDS (100, 261, 287, 343). Hypogonadism is common in HIV disease, and the decrease in testosterone levels certainly contributes to the weight loss observed in AIDS patients. Indeed, in men with low testosterone levels, testosterone replacement via a transdermal system is followed by a gain in lean body mass (41). The testosterone deficiency probably results from lymphocyte infiltration and fibrosis of the interstitial tissue and from a decrease in Leydig cell number (82, 83, 257, 273).

AIDS patients often suffer from orchitis, hypogonadism, oligospermia, or azospermia (20, 100, 253, 257, 325) and, in some cases, from testicular germ cell tumor or lymphoma (54, 116, 244, 351). Among 3,015 HIV-positive men, the incidence of testis tumors has been reported to be 0.2%, which is 57 times that of US. average of 3.5 cases/100,000 men (351). Several autopsy reports for deceased AIDS patients have shown a high rate of testicular abnormalities (63, 220, 236, 266, 348). Several groups have studied testis histology in men with AIDS and spermatogenesis dysfunction is invariably reported. This was first described by Chabon et al. (63), who showed major changes in the testes of 20 patients, resulting in a “Sertoli cell only” pattern, and interstitial inflammation in 53% of the patients. Rogers and Klatt (273) also described germ cell depletion, a decrease in tubule diameter, and an increase in the thickness of the tubule boundary wall. Yoshikawa et al. (354) set up a classification system for the disorders observed in the testes of 31 patients who had died from AIDS. Five categories were established: Sertoli cell only (42%), germ cell degeneration (27%), peritubular fibrosis associated with tubular hyalinization (15%), maturation arrest (12%), and normal appear-
ance (3%). These observations were confirmed in 57 autopsy cases in another study (92). Since then, other authors have described the arrest of spermatogenesis at various points, numerous foci of degenerating germ cells, and epididymis block. Interestingly, a study of 140 testicular autopsy specimens from AIDS patients with and without antiviral treatment showed that treatment and prolongation of survival in AIDS patients is associated with a shift in the histologic findings for testes toward a more pronounced loss of germ cells (304).

These morphological observations have raised questions about the mode of action of HIV on the testis. The virus was first thought to have an indirect effect due to the chronic debilitating illness and cachexia of the patients. It has also been suggested that opportunistic infections are involved. However, another study indicated that only 32% of patients with opportunistic infections had CMV, Mycobacterium avium-intracellulare, or Toxoplasma gondii in the testes. This suggested that these infections were probably not the key factors causing the observed hypogonadism (91). HIV may also act indirectly via changes in the hypothalamic-pituitary axis. Men with AIDS often have low testosterone levels (see above), and a dysfunction in hypothalamic gonadotropin releasing hormone secretion may account for this phenomenon (100). However, other studies found no significant abnormality in the hypothalamic-pituitary axis (253, 286). The low serum testosterone levels in men with AIDS are in some cases associated with high serum LH and FSH levels, implying that there is primary testicular failure (79). A possible reason for the testosterone secretory defect in these men is suggested by reports that cytokines released by the activated phagocytic cells of the immune system inhibit the steroidogenic response to human chorionic gonadotropin in vitro and presumably also the response to LH in vivo (57).

Recent studies have shown that physiologic testosterone replacement in HIV-infected men with weight loss who have low testosterone levels can increase muscle mass and effort-dependent strength (for a review, see reference 40). Testosterone therapy also helps to alleviate the symptoms of hypogonadism (260). However, further studies are needed to determine whether androgen therapy can significantly and durably improve physical function and health-related outcomes in HIV-infected men.

HIV infection of the testis has now been described in several studies, indicating that direct local action may be responsible for the observed damage within the gonads. The HIV p17 protein was first detected within the testis by immunohistochemistry using monoclonal antibodies (83). Pudney and Anderson (257) detected the CD4 receptor on the cell surface of lymphocytes and macrophages infiltrating the testis, which suggests that these cells have the potential to be infected by HIV. Indeed, in 9 of the 23 cases in which immunocytochemistry was used to test for HIV-1 protein, HIV-infected cells of the lymphocytic/monocytic type were found in the seminiferous tubules and interstitium of the testis. Such cells were also found in the semen (257). Using in situ PCR, several other studies have detected HIV-1 DNA within testicular germ cells. (i) The infection of spermatogonia, spermatocytes, and a small number of spermatids in 11 of 12 men with AIDS was described by Nuovo et al. (240). Another study with seropositive asymptomatic subjects reported the presence of HIV-1 DNA in the nuclei of spermatogonia and other germ cells at all stages of differentiation, suggesting clonal infection (288). In these subjects, the presence of provirus did not cause cell damage and was associated with normal spermatogenesis, whereas in AIDS patients, spermatogenesis was arrested and there were few infected spermatogonia and spermatocytes. (iii) HIV-1 DNA was recently found in 25 to 33% of the residual germ cells in the testes of AIDS patients, with testicular changes including all variants from decreased spermatogenesis to the Sertoli-cell-only pattern (303). In contrast, HIV-1 DNA was not detected in the testes of any of three preadolescent boys who acquired HIV in utero (303).

The way in which HIV enters germ cells is unknown. The CD4 receptor has still not been unequivocally detected on the germ cell surface, nor have the chemokine coreceptors triggering HIV entry in other cell types (e.g., monocytes/macrophages, lymphocytes, and microglial cells). The virus may also enter germ cells via an alternative galactoglycerolipid receptor, as suggested (52). Tissue-specific HIV-1 quasispecies have been identified in the testis, indicating that there may be a tissue tropism for this organ (341). This is a very important issue for HIV treatment because triple therapy seems to be unable to eradicate the virus completely. Thus, the testis could act as a viral reservoir isolated by the blood-testis barrier which cannot be reached by drugs (71) (see the section on HIV-1 in “Human semen” above).

Oncogenic viruses. Orchitis has been described as a symptom of infectious mononucleosis caused by EBV, indicating that this virus may have a direct effect on the testis (268). EBV DNA was detected in the testes of three individuals with no EBV-related disease, showing that the testis may be a target organ (67). Rajpert De Meyts et al. (262) found that EBV was not directly involved in the testicular germ cell tumors of 20 patients but thought that germ cell proliferation might be stimulated by testicular EBV-transformed lymphocytes. However, more recently, Shimakage et al. (305) detected EBV RNA and EBV-related proteins in all of the 27 seminomas they tested but in none of 25 nonmalignant testes, providing further support for the hypothesis that EBV is associated with testicular tumors. In addition, transgenic mice expressing papillomavirus genes develop germ cell tumors resembling human seminoma (171). Recently, 39 patients with testicular germ cell tumors were screened for EBV, CMV, and parvovirus B19 DNA by PCR. Neither EBV nor CMV DNA was detected in the testis. In contrast, parvovirus B19 DNA sequences were found in the testicular tissues of 85% of patients with testicular cancers but in none of the normal testicular tissue samples (129). However, the cellular targets of parvovirus B19 within normal and cancerous testes are unknown and the possible role (direct or indirect) of this virus in the development of testicular germ cell tumors is unclear.

Other viruses. HSV-2 was detected in the testes of 4 of 10 corpses at autopsy, suggesting that this organ acts as a reservoir for transmission of the virus (95). Csata and Kulcsar (80) studied the relationship between male infertility and the presence in the semen of testis of HSV-1 or adenovirus. In 40% of patients with infertility either HSV-1 or adenovirus was present within the testis. Testicular cells were also infected in vitro by either HSV-1 or adenovirus, and these two viruses were found to penetrate and replicate (80).

Several viruses are suspected of inducing orchitis, based on
epidemiological or clinical association. There is, however, little evidence that any known human viral pathogen, apart from mumps virus, has selective or exclusive organotropism for the testis. The association between viral infection and orchitis was reviewed in 1962 (268) and in 1982 (216). It appears that coxsackievirus, dengue virus, and bat salivary gland virus infections are associated with a significant incidence of clinical orchitis. Other viruses, such as influenza virus, EBV, lymphocytic choriomeningitis virus, phlebotomus fever virus, adenovirus, echovirus, smallpox virus, vaccinia virus, rubella virus, and chickenpox virus, also cause orchitis, albeit more rarely (216, 268). Atrophy of the testis is common in viral orchitis, but since atrophy is unilateral in most cases, sterility is extremely rare. Rubella virus is thought to alter the development of some aspects of the reproductive tract, as shown by a study reporting that 12% of 316 boys with congenital rubella suffered from cryptorchidism (256). The virus may also affect the adult testis: testalgia, an indicator of orchitis, was found in 5 of 68 adults during an epidemic of rubella (255). However, it is unclear whether this clinical symptom does indeed reflect testicular inflammation or is just a nonspecific manifestation of viremia.

**Endogenous retroviruses.** Endogenous retroviruses (ERV) are chromosomal elements that have a genomic organization analogous to that of exogenous retroviruses. Their relevance to this review is that they may originate from ancient infections of germ cells by exogenous retroviruses (199), thus demonstrating that viral genomes can be incorporated into the germline genome. Retrotransposons are closely related to ERV, but differ from them in that they do not have the env gene and, therefore, produce particles that are intracellular and not infectious (178).

The first human ERV (HERV) was cloned in 1981 (204). Since then, more than 20 HERV families have been identified, cloned, and partially characterized (199, 336). No HERV has ever been shown to be infectious, but they may be associated with germ cell tumors (320). HERV genes are often highly expressed in the testis due to the hypomethylation of testicular genes. Hence, HERV-K, the most biologically active member of the HERV family in terms of the coding of viral protein and particles, preferentially expressed in cell lines derived from teratocarcinomas (199, 336), is also closely associated with active seminomas (199, 283). One study also reported the detection of antibodies against HERV-K0 Gag protein in 45% of seminoma patients (283), whereas no positive tests were observed in seminoma patients tested months or years after treatment. The HERV-K Gag protein was detected only in tumor cells, with no reaction being observed in the surrounding testicular tissue (283). However, the authors found no conclusive association between HERV and other testicular neoplasms, including teratomas (283). In contrast, HERV gag and env were detected by in situ hybridization in 100% of a series of germ cell tumor specimens (140). Samples of testicular carcinoma, a precursor of germ cell tumor, also consistently tested positive for HERV-K mRNA in situ (140), whereas the surrounding testicular tissues mostly tested negative. However, HERV-K mRNA has been detected in normal testicular parenchyma in other studies using the more sensitive reverse transcription-PCR technique (272). Herbst et al. (140) observed that a consistent absence of transcripts was observed in a series of immature and mature teratomas. They suggested that this inhibition of expression might result from the higher level of differentiation of the cells, a characteristic that distinguishes teratomas from other germ cell tumors. Indeed, the HERV gag sequence was found to be more highly methylated in primary germ cell testicular tumors (127). It is therefore possible that HERV-K may be involved in seminomas, due to the high level of gene expression resulting from demethylation. Another endogenous retrovirus, ERV-3, also has high levels of gene expression, at least at the mRNA level, in germ cells during the early phases of spermatogenesis but not in the somatic Sertoli cells or Leydig cells (187). However, ERV-3 env gene expression was almost undetectable in the two cases of seminoma studied so far (187).

The transcription of cellular retrotransposons is induced by a variety of physiological stimuli. A study reported that VL30 retrotransposons are expressed in steroidogenic cells within all four endocrine tissues engaged in the synthesis of steroid hormones in response to pituitary-derived trophic hormones (284). In the testis, the transcription of VL30 retrotransposons is activated in the testosterone-producing Leydig cells. Specific activation of retrotransposons has also been observed in spermatogenic stem cells. This may help to increase genomic 2plasticity within the germ line (103). Indeed, the testis is the only organ in which retrotransposon long terminal repeats LTRs are hypomethylated (320).

**The Animal Testis**

The viruses found in the animal testis and their consequences are listed in Table 5. The effects of viruses on the reproductive endocrine systems in men and animals are listed in Table 6.

**Viruses and the seminiferous epithelium.** In rhesus monkeys experimentally infected with SIV, many seminiferous tubules were found to be necrotic and infiltrated with neutrophils due to opportunistic CMV infection. No inclusion-bearing cells (characteristic of CMV infection) were observed in the interstitial tissue, but considerably more were present in the seminiferous tubules than anywhere else in the body (33). CMV antigens were detected by immunohistochemistry in the testes of SIV-infected monkeys in another recent study (179). Malignant lymphoma associated with SIV-induced immunodeficiency in macaques is a high-grade malignant tumor that most frequently affects the viscera, skin, central nervous system, and testes. It has been associated with an EBV-like simian herpesvirus (116). Testicular atrophy is a relative common finding in monkeys infected by SIV (218).

Several studies have investigated CMV infection in mice because of its similarity to disorders in humans. Dukto and Oldstone (105) detected murine CMV in a latent form in the testes of mice. Acutely infected male adult mice homozygous for the nude gene produced infectious virus in their testes, whereas their heterozygous littermates contained significantly less virus than did nude/nude mice. Viral DNA was restricted to the testicular germ cells and spermatozoa. This demonstrates that murine CMV may be harbored in the testes during both acute and latent infections and may replicate in male germ cells. In another study (235), CMV was recovered from both epididymal sperm and seminal vesicles. CMV DNA was detected by in situ hybridization in the spermatozoa and sper-
Influenza virus (mouse) ..............................................................................
CMV (mouse) .......................................................................................................
PRRSV (pig) ........................................................................................................
Myxoma virus (rabbit) ............................................................................................
SIV (monkey) ........................................................................................................
EHV-1 (pony) ...........................................................................................................
Porcine rubulavirus (pig) .........................................................................................
Maedi/visna virus (ram) .........................................................................................

**TABLE 5. Viruses found in the animal testis and their consequences**

<table>
<thead>
<tr>
<th>Virus and species infected</th>
<th>Cells infected</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECMV (mouse, hamster)</td>
<td>Sertoli cells (335), germ cells (335)</td>
<td>Orchitis (335), necrosis and degeneration of germ cells including spermatogonia (141), increase in Leydig cell number (141)</td>
</tr>
<tr>
<td>Influenza virus (mouse)</td>
<td>Spermatocytes (299)</td>
<td>Chromosomal abnormalities (299)</td>
</tr>
<tr>
<td>CMV (mouse)</td>
<td>Germ cells (31, 105), Leydig cells (30)</td>
<td>No effect on fertility (31)</td>
</tr>
<tr>
<td>PRRSV (pig)</td>
<td>Germ cells (315), macrophages (315)</td>
<td>Germ cell apoptosis (315)</td>
</tr>
<tr>
<td>Myxoma virus (rabbit)</td>
<td>Interstitial cells (119)</td>
<td>Orchitis (119), impaired steroidogenesis (119), impaired spermatogenesis (119)</td>
</tr>
<tr>
<td>SIV (monkey)</td>
<td>Lymphocytes and macrophages in interstitium (32)</td>
<td>Degeneration of seminiferous tubules, hypospermatogenesis (32)</td>
</tr>
<tr>
<td>EHV-1 (pony)</td>
<td>Endothelial cells (323)</td>
<td>Necrotizing vasculitis, thrombosis (323)</td>
</tr>
<tr>
<td>Porcine rubulavirus (pig)</td>
<td>NA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Interstitial mononuclear cell infiltration (263), seminiferous tubule degeneration (263), infertility (263)</td>
</tr>
<tr>
<td>Maedi/visna virus (ram)</td>
<td>NA</td>
<td>Interstitial mononuclear cell infiltration and fibrosis (248), atrophy of seminiferous tubules (248), disturbances in spermatogenesis (248)</td>
</tr>
</tbody>
</table>

<sup>a</sup> NA, not available.

The induction of feline acquired immune deficiency syndrome by feline leukemia virus (FeLV) in cats alters hormone production in the hypotalamic-pituitary-gonadal system. Thus, 12 weeks after the infection of male cats with FeLV, much lower levels of testicular testosterone production were observed following the injection of human chorionic gonadotropin in infected cats than in uninfected cats (346). Other glands, such as the hypothalamus and pituitary were also affected, with lower levels of pig testicular germ cells and macrophages, affecting spermatogenesis by inducing germ cell apoptosis (315).

Avian leukemia virus (ALV) is frequently congenitally transmitted by hens to their progeny. Interestingly, although the testes of congenitally infected roosters contain large amounts of the virus, ALV does not seem to be transmitted by semen (277). A more thorough study tried to resolve this apparent contradiction by attempting to identify the infected testicular cells involved and to detect free virus particles in the male genital duct system (99). The authors used virus assays and electron microscopy and showed that the viral buds were seen only in Sertoli cells and never on germ cells. This may account for the lack of male congenital transmission, although the techniques used do not exclude the possibility of latent viral infection of the germ cells. Very few free virus particles were shed into the duct system (99).

**Viruses and the interstitial compartment.** The induction of feline acquired immune deficiency syndrome by feline leukemia virus (FeLV) in cats alters hormone production in the hypotalamic-pituitary-gonadal system. Thus, 12 weeks after the infection of male cats with FeLV, much lower levels of testicular testosterone production were observed following the injection of human chorionic gonadotropin in infected cats than in uninfected cats (346). Other glands, such as the hypothalamus and pituitary were also affected, with lower levels of pig testicular germ cells and macrophages, affecting spermatogenesis by inducing germ cell apoptosis (315).

Influenza virus also has cytogenetic effects on male mouse germ cells, inducing the induction of chromosomal abnormalities in spermatocytes (299). Similarly, PRRSV, which is shed into the genital duct system (99). The authors used virus assays and electron microscopy and showed that the viral buds were seen only in Sertoli cells and never on germ cells. This may account for the lack of male congenital transmission, although the techniques used do not exclude the possibility of latent viral infection of the germ cells. Very few free virus particles were shed into the duct system (99).

**TABLE 6. Effects of viruses on the reproductive endocrine system in men and animals**

<table>
<thead>
<tr>
<th>Virus (species affected)</th>
<th>Effect (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mumps (human)</td>
<td>Decrease in androgen production (4)</td>
</tr>
<tr>
<td>HIV (human)</td>
<td>Increase in testosterone level at the early stage of infection (68, 212); decrease in testosterone level and increase in LH and FSH production (79) in men with AIDS</td>
</tr>
<tr>
<td>FeLV (cat)</td>
<td>Decrease in testosterone level (346); decrease in LH, FSH, and LHRH levels (346)</td>
</tr>
<tr>
<td>EHDV-2/BTV ? (Mule deer)</td>
<td>Decrease in testosterone production (328); increase in LH and FSH levels (328)</td>
</tr>
<tr>
<td>Myxoma virus (rabbit)</td>
<td>Impaired steroidogenesis (119)</td>
</tr>
<tr>
<td>EMCV (hamster)</td>
<td>Apparent increase in Leydig cell number (141)</td>
</tr>
<tr>
<td>CMV (mouse)</td>
<td>Leydig cell infection (30)</td>
</tr>
<tr>
<td>Murine leukemia virus (mouse)</td>
<td>Leydig cell infection (249)</td>
</tr>
<tr>
<td>Aujesky's virus (boar)</td>
<td>Interstitial cell infection (219)</td>
</tr>
<tr>
<td>ALV (rooster)</td>
<td>Interstitial cell infection (99)</td>
</tr>
<tr>
<td>Retrotransposons (human)</td>
<td>Activation in testosterone-producing Leydig cells (284)</td>
</tr>
</tbody>
</table>
release of LH, FSH, and luteinizing hormone-releasing hormone LHRH (346).

The monitoring of mule deer on a former plutonium production site revealed that 27% of the 116 adult males had abnormally developed testicles associated with low levels of testosterone in serum and compensatory high levels of LH and FSH (328). The severity of the atrophy and the absence of other affected tissues suggested that radiation might not be the cause. Indeed, 90% of 10 affected animals were seropositive for epizootic hemorrhagic disease virus and BTV, versus only 63% of 19 unaffected animals.

One of the most common lesions observed in SIV-infected monkeys is hypospermatogenesis with degeneration of the seminiferous tubules (32). Staining for SIV antigen identified small numbers of positive lymphocytes and macrophages in the interstitium (32). In contrast, a study of macaques inoculated with SIV concluded that spermatogenesis was impaired but that this was due to cachexia rather than to a direct effect of the virus on the testes (232). However, this conclusion may not be fully justified because the study was performed on prepubertal monkeys in which spermatogenesis was not fully established and the authors did not test for SIV in the testes.

Furthermore, CMV is a common opportunistic infection in rhesus monkeys experimentally infected with SIV. CMV causes changes in several organs including the testis (33). In 3 of 11 SIV-infected monkeys with CMV infection, the testicles were found to be severely affected: massive infiltration of the interstitial area was observed with patchy areas in which all elements were necrotic.

Intratesticular or intraperitoneal injection of CMV in mice leads to Leydig cell infection (30), as does injection of the murine leukemia retrovirus (249).

In boars experimentally infected with porcine rubulavirus, interstitial mononuclear cell infiltration was observed in atrophic testes and was identified as the most probable cause of seminiferous tubule degeneration (263). Similar testicular lesions were previously observed in rams infected with maedi/visna virus, in which the atrophy of seminiferous tubules was associated with disturbances in spermatogenesis (248). In contrast, intratesticular inoculation of boars with a high titer of a virulent strain of Belgian Aujesky’s disease virus had much less dramatic effects (219). Only a few small foci of viruses were detected by immunofluorescence in the interstitium, and no viral replication, necrosis, or inflammatory lesions were detected in the seminiferous tubules (219).

Rabbits infected with an attenuated strain of myxoma virus develop interstitial orchitis and have impaired steroidogenesis and spermatogenesis, with the virus being restricted to the interstitial cells (119). Equine herpesvirus 1 was found to replicate in the testes of ponies and to be shed into semen (323). Endothelial cells are the main testicular target cells of this virus, and there is associated necrotizing vasculitis and thrombosis, but no productive viral infection of the seminiferous epithelium (323). Venereal shedding may thus be facilitated by focal tissue ischemia and leakage of free virus of infected cells across the disrupted blood-testis barrier. Finally, ALV has been found to multiply in the connective tissue stroma and interstitial cells of the testis of mature roosters (99).

VIRUSES AND THE PROSTATE AND OTHER ACCESSORY GLANDS

The effects of viruses on the human and animal prostate are listed in Table 7.

Human Prostate

In terms of incidence, prostate cancer is one of the most important cancers in men throughout the world. Putative causative agents have therefore been extensively studied. The etiology of prostate cancer is still unknown, but there are two main hypotheses. The first and most popular is the hormonal hypothesis, i.e., that the disease is caused directly or indirectly by endogenous or exogenous androgenic hormonal factors. The other hypothesis is that prostate cancer is caused by an oncogenic virus that may be transmitted venereally. There is indeed some evidence supporting an association between prostate cancer and STD, in particular the link between a high level of sexual activity and prostate cancer reported in some studies. Before reviewing the various viruses detected in the prostate, we should bear in mind that there are two different clinical entities: benign prostate hyperplasia and carcinoma of the prostate. It is unknown whether benign prostate hyperplasia coexists with prostate cancer and has a common etiology or

<table>
<thead>
<tr>
<th>TABLE 7. Viruses and the human and animal prostate</th>
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<tr>
<td>Viruses found in the human prostate</td>
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<tr>
<td>HPV DNA (18, 81, 101, 148, 210, 211, 282, 333, 350)</td>
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<td>HSV Viral particles (60, 227)</td>
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<td>CMV Antigens (123, 264)</td>
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<td>HIV Antigens (83)</td>
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<td>DNA (240)</td>
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<tr>
<td>KSHV/HHV-8 DNA, only in HIV-infected patients (77)</td>
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whether it is a totally separate condition, etiologically un-
related to prostate cancer. According to Sokol-Roseman et al.
(306), latent carcinoma of the prostate and prostate cancer are
etiologically related and the latent carcinoma may correspond
to a stage in the natural history of prostate cancer.

Papillomavirus. A link between HPV, a family of oncogenic
viruses, and prostate cancer has been sought. McNicol and
Dodd (210) detected type 16 and 18 HPV DNA by Southern
blotting in prostate tissue from 4 of 12 Canadian patients with
benign prostate hyperplasia and in 3 of 4 patients with prostate
carcinoma. The viral DNA was in an episomal or integrated
form, and the authors concluded that this may have important
implications for the etiology of prostate disease. In the follow-
ing year, they published their results with a larger series of 88
prostate biopsy specimens and found a much higher prevalence
of type 16 HPV than of type 18 (211). However, no significant
difference was observed between patients with benign disease
and those with evidence of malignancy, with nearly half of
the patients testing positive in each case. Furthermore, the preva-
ience of type 16 HPV DNA in diseased prostates did not differ
significantly from that in normal prostates. Several studies on
the prevalence of HPV in the prostate were published in 1992
and are reviewed in detail elsewhere (81, 279). When the data
were combined, 32% of the cancers tested positive for HPV,
versus 49% of the benign lesions and 9% of the normal tissue
samples. The highest rates of detection were found in earlier
studies (probably due to problems of PCR contamination),
whereas more recent results have been predominantly negative
(81). In particular, one study of 84 American patients carried
out using PCR and in situ hybridization (148) reported a low
prevalence of type 16 HPV in both normal and cancerous
prostates and, in two other studies, no HPV DNA was detected
in 30 benign and malignant prostate tissue samples, even by
PCR (108, 297). A secondary adult human prostate epithelial
cell culture, on transfection with a plasmid containing the
entire type 18 HPV genome, acquired an indefinite, life span in
culture but did not undergo malignant conversion (267). Thus,
although there are strong arguments in favor of the prostate
being an important reservoir for the transmission of HPV (18,
101, 282, 333, 350), the involvement of HPV in the pathogen-
esis of prostate cancer is still a subject of great controversy.
For example, two laboratories with extensive experience in HPV
research recently tested specimens from two populations with
different risks of prostate carcinoma, using three different PCR
assays and two serologic assays for HPV. They concluded that
HPV was not associated with prostate carcinoma (239, 314).
However, another group found that a subgroup of prostate
tumors (10 of 47) had a significantly larger number of copies of
type 16 HPV sequence than did control tissue samples (1 of 37)
(298). Thus, we cannot rule out the possibility that HPV is
involved in the etiology of a minority of prostate cancers, as
recently suggested by a serological and epidemiological study
(98).

Herpes simplex virus. The importance of HSV in prostate
disease was first suggested when the virus was isolated from 2
men with prostatitis and from none of 10 men with no prostatic
symptoms (227). A later study in which HSV was isolated from
the prostate tissue of 15% of the men in a randomly selected
population of 190 men suggested that the prostate acts as a
reservoir of the virus (60). HSV antigens have also been found
in prostate cancer cells (62), and antibodies against an HSV
antigen were detected in the sera of all eight patients with
prostate cancer tested (280). However, a larger serological
epidemiological study investigating whether there was an as-
ociation between antibodies against HSV-2 and cancer of the
prostate showed no significant difference in the level of HSV-2
antibodies between patients with cancer of the prostate and
those with benign prostate hypertrophy (139). Specific immu-
nofluorescence was used to test for HSV-2 in prostate carci-
noma in 305 patients: 5% of the patients had HSV-2 antigen in
the prostate, and a higher prevalence was observed in patients
with carcinoma of the prostate than in controls with benign
prostate hypertrophy (27). This higher prevalence of HSV-2
was not confirmed in another study of 27 prostate carcinoma
and 33 benign prostate hyperplasia patients (136). It therefore
seems that HSV-2 infection of the prostate is common (25% in
the most recent study), but no causal relationship with prostate
carcinoma has yet been demonstrated. However, in vitro trans-
formation of hamster embryo cells by tumor-associated HSV-2
from a human prostate carcinoma has been reported (61).

Cytomegalovirus. CMV was first reported to be present in
the prostate in a 3-year-old male donor from whom a cell line
was obtained that expressed CMV-specific antigen at the cell
surface (264). Geder et al. (124) reported that human prostate
cancer cell lines expressed CMV-specific membrane antigen
and suggested that CMV might be associated with prostate
cancer. Primary cells were then transformed in vitro by a strain
of CMV isolated from a normal prostate (123). These authors
also showed that prostate cancer cells in culture possessed
intraepithelial antigens specific for CMV but produced no infec-
tious virus particles. However, normal human prostate tissue
yielded a CMV isolate that transformed cells, and lymphocytes
from prostate cancer patients were found to have specific re-
activity against CMV-transformed cells (123). Antibodies
against CMV have also been found at higher titers in patients
with prostate cancer than in controls with benign prostate
hyperplasia (123). Although CMV DNA has been detected in
both normal and cancerous prostates, CMV RNA and antigens
have only been detected in benign prostate hyperplasia and
prostate carcinoma. This suggests that CMV may be associated
with prostatic abnormality and that latent CMV may be har-
bored by normal prostates (46). However, in another study,
CMV antigens were not detected in any of the 21 tissue sam-

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Human immunodeficiency virus type 1. HIV-1-related pro-
teins have been found in several adjacent glandular epithelial
cells in prostate sections from patients who died from AIDS
(82), indicating that this organ may act as a reservoir of the
virus (1). However, morphologic analyses of reproductive tis-
sues obtained at autopsy from the bodies of 43 male AIDS
patients revealed no major abnormalities of the prostate (257).
In another study, viral nucleic acids were not detected by PCR
amplification in the epithelium of the prostate but a few mac-
rophages infected with the virus were observed (240).

Human herpesvirus 8. Initially, two studies from the same
group claimed to have detected HHV-8 in normal, hyperplas-
tic, and neoplastic prostate glands from HIV-negative individ-
uals (222, 224), but other studies did not find HHV-8 to be
associated with this site in HIV-negative individuals (76, 77, 192, 278, 321). However, HHV-8 was detected by PCR in the prostates of all five HIV-infected KS-positive patients tested (76), whereas most other organs tested negative. This indicates that the prostate gland may be a preferential site of viral latency and persistence. HHV-8 was found not to establish latent infection in prostate cancer cell lines (229), indicating that it is probably not involved in prostate pathogenesis. A study recently identified the location of the infected cells more precisely within the prostate: HHV-8 DNA was detected in the nuclei of more than 90% of glandular epithelial cells, and in situ hybridization showed that the virus was mostly latent, with only 1 to 5% of cells harboring viral transcripts produced by HHV-8 replication (96).

**Human Seminal Vesicles and Epididymis**

Although malignant lymphoma of the testis involves the adjacent epididymis in approximately 60% of patients, isolated epididymal lymphoma is rare. One case report, however, described bilateral epididymal enlargement in a 34-year-old man, epididymal lymphoma is rare. One case report, however, described bilateral epididymal enlargement in a 34-year-old man, with minimal involvement of the epididymis, prostate, or seminal vesicles (32). Another study found that within the male genital tract of infected monkeys, the SIV-infected T cells and macrophages were present mostly in the epididymis (218). Atrophy of the glandular elements and an interstitial fibrosis of the prostate were also observed (218).

In bulls, bovine diarrhea virus is transmitted by semen (see “Animal semen” above). Virolological studies of the reproductive tracts of bulls during acute transient infection suggest that the most productive sites for virus replication are the seminal vesicles and the prostate gland (168).

Swelling of the head of the epididymis was observed in boars inoculated with porcine rubulavirus, which causes “blue eye” disease, resulting in infertility in sows and boars and corneal opacity in pigs of all ages (263). The lesion was associated with mononuclear cell infiltration and interstitial fibroplasia. Porcine rubulavirus antigen was detected by immunofluorescence in the head of the epididymis in pigs killed during acute infection (263). Mice transgenic for the type 18 HPV long control region were found to have enlarged seminal vesicles due to distension by fluid, suggesting that the transcript is specifically produced in the genital tract of mice (74).

The inoculation of guinea pigs with HSV-2 causes vesicular lesions in the genital area. During acute infection, the virus has been detected in the epididymis, seminal vesicles, prostate, and testis, but persistent detection of the virus is rare in these organs (except the prostate) (310).

Endogenous MMTV is present not only in the mammary glands and the prostate but also in the seminal vesicles, the vas deferens, and the epididymis, as shown by electron microscopy (274), radioimmunoassay (246), immunocytochemistry (344), and in situ hybridization (138).

**TESTICULAR ANTIVIRAL DEFENSE SYSTEM**

Viral infection of the testis, as described above, can have damaging effects on spermatogenesis and may lead to testicular cancers. It may also be sexually transmitted. Therefore, elucidation of the natural mechanisms of defense of the testis against viral attack is of major importance (Fig. 2).

IFNs and IFN-induced proteins were the first elements of the testicular defense system to be investigated. IFNs are small proteins well known for their crucial involvement in cellular antiviral defenses. They bind to specific cellular receptors, either on the producing cells themselves or on neighboring cells. They then induce the synthesis of several proteins, three of which, 2’,5’-oligoadenylate synthetase (2’,5’-AS), double-stranded RNA-activated protein kinase (PKR), and Mx protein, have attracted a great deal of attention due to their ability to generate an antiviral state (for reviews, see references 281 and 308).

Attempts were first made to detect these molecules within the rat seminiferous tubules. The protection of the germ cells in these seminiferous tubules is of prime importance because their infection may result in the total destruction of the germ line or the dissemination of the virus or viral DNA to all germ cell classes, including the spermatogonia themselves. Spermatogonia are one of the most important potential targets because, unlike the meiotic and postmeiotic germ cells, they are not isolated from the molecules and agents produced by the interstitial compartment. The basal tubular position of these germ
line precursors, a priori, exposes them to the risk of contamination in cases of viral infection. Despite the high potential risk of infection of spermatogonia, these cells do not constitutively express biologically active IFNs, and they respond only weakly to stimulation by Sendai virus in vitro (89). Similarly, early spermatids and pachytene spermatocytes, although expressing low levels of IFNs under basal conditions, respond very weakly if at all to exposure to Sendai virus (88). It was therefore suggested that the germ cell antiviral protection system may involve mainly the somatic peritubular and Sertoli cells within the seminiferous tubules and the somatic Leydig cells and macrophages within the interstitium, all of which have subsequently been shown to express high levels of IFN-α/β in response to viral infection (88). Of all the testicular cell types tested, Leydig cells and Sertoli cells have by far the strongest antiviral potential.

The synthesis of the main antiviral IFN-induced proteins, 2′,5′-AS, PKR, and Mx, was then studied. 2′,5′-AS was not detected in meiotic and postmeiotic germ cells, but it was found in Sertoli cells under basal conditions, and its synthesis was increased by IFN and Sendai virus. A pattern similar to that in Sertoli cells was observed in peritubular cells, except that there was no basal expression (87).

No PKR protein or mRNA was detected in pachytene spermatocytes and early spermatids, even after exposure to IFNs or to Sendai virus. In contrast, Sertoli and peritubular cells constitutively expressed the PKR gene, and this expression was stimulated by IFN-α/β and IFN-γ (87).
Sertoli cells constitutively synthesize small amounts of Mx2 and Mx3 but not Mx1 (87). Mx1 was detected only after IFN or Sendai virus stimulation, and the synthesis of Mx2 and Mx3 was increased by these stimuli. This was the first time that Mx proteins were reported to be constitutively synthesized in a particular cell type, and the pattern of synthesis seemed to differ for the three proteins. Moreover, IFN-γ usually has no effect on Mx protein regulation, but it induced Mx1 gene expression and stimulated Mx2 and Mx3 gene expression in Sertoli cells. In contrast to what was observed in Sertoli cells, no Mx protein or mRNA was detected in peritubular cells cultured under basal conditions. However, Mx1, Mx2, and Mx3 were also detected in peritubular cells after IFN or Sendai virus exposure. As with 2′5′-AS and PKR, no Mx mRNA or protein was detected in pachytene spermatocytes and early spermatids (87). Our latest results establish that Leydig cells and testicular macrophages produce high levels of 2′5′-AS, PKR, and Mx while late spermatids and spermatogonia express only Mx (N. Melaine, unpublished data).

Thus, the first line of testicular defense against viruses arriving in the bloodstream is the Leydig cells and testicular macrophages. The second line of defense involves the myoid cells lining the seminiferous tubules and Sertoli cells. These two barriers may be fundamental for the protection of both spermatogenesis and androgen production, which is essential not only for testicular function but also for other crucial functions such as the development and maintenance of the secondary male sex characteristics, control of the musculoskeletal system, and key aspects of the nervous and cardiovascular systems.

CONCLUSION

A number of viruses are able to infect both the reproductive tract tissues and the semen in humans and animals (see the summary in Fig. 1), and the consequences of these viral infections may be extremely serious in terms of organ integrity, the development of diseases, and changes in the reproductive and endocrine systems. The immunosuppressive properties of the seminal plasma (designed to prevent a reaction against sperm antigens in the female reproductive tract) may play a critical role in STDs, not only because semen is a vector of viral propagation but also because the immune response of the female genital tract to infectious agents present in this biological fluid is certainly affected by the high level of immunosuppressive factors present in seminal plasma (160). In all animal species, but particularly in humans, viral infection of the germ cells may result not only in changes in testicular function (a serious risk for the fertility and general health of the individual) but also in the possible transmission of virus-induced mutations to subsequent generations. There are several obvious examples illustrating the lack of attention paid to studies of viral infection of the genital tract in men: (i) although the deleterious effects of mumps virus on fertility have been known for at least five decades, the mechanism by which the seminiferous epithelium is destroyed in testes infected with mumps virus is still unknown, and (ii) similarly, although the presence of HIV within the testis and spermatozoa themselves is still a matter for debate, key questions have been raised about the safety of insemination with spermatozoa from HIV-positive men and the possible existence of a testicular or genital tract reservoir for HIV. Another consequence of the superficial attention paid to viral infection of the testis in all species is the relative paucity of data concerning its effects on the endocrine system.

It is therefore urgent for both health and economic reasons to develop the scientific and medical interface between endocrinology and virology. In our opinion, the key priorities include (i) identification of the routes of entry of viruses into the male genital tract, (ii) identification and characterization of viral receptors in the various cell populations, (iii) studies of the infectivity of the virus in the male genital tract and its precise consequences on the endocrine system, (iv) determination of the mode of replication of the virus in the corresponding tissues, (v) determination of the nature of possible genital reservoirs, (vi) elucidation of the antiviral defense system of the reproductive tract and investigation of why this system is permissive for a number of viruses, and (vii) assessment of the efficacy of antiretroviral therapies in the genital tract and of the possible side effects of these treatments on testicular cells.

All this requires the establishment of close cooperation between clinicians/andrologists, virologists and biologists. Progress in these domains is essential for the development of new treatment strategies to eradicate viruses and to correct the virus-induced dysfunction of the endocrine system.

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