3
Vaginal Microbicides for the Prevention of HIV Transmission

KARL MALCOLM*, DAVID WOOLFSON, CLARE TONER AND DEBORAH LOWRY

School of Pharmacy, The Queen's University of Belfast, Belfast, BT9 7BL, Northern Ireland, UK

Introduction

The human immunodeficiency virus (HIV) kills more people worldwide than any other infectious disease. Approximately 42 million people, mostly in Africa and Asia, are currently infected with HIV (*Figure 3.1*), and 5 million new infections occur every year (AIDS Epidemic Update, 2002). It is estimated that 22 million people have died since the first clinical evidence of AIDS (acquired immunodeficiency syndrome) emerged in 1981 ('Mobilization for Microbicides', The Rockfeller Foundation). HIV is generally transmitted in one of three ways: through unprotected sexual intercourse, blood-to-blood contact, and mother-to-child transmission. Once the virus has entered the body, it invades the cells of the immune system and initiates the production of new virus particles with concomitant destruction of the immune cells. As the number of immune cells in the body slowly declines, weight loss, debilitation, and eventually death occur due to opportunistic infections or cancers. Although AIDS is presently incurable, highly active antiretroviral therapy (HAART), where a cocktail of potent antiretroviral drugs are administered daily to HIV-positive patients to control the viral load, has resulted in dramatic reductions in HIV-related morbidity and mortality in the developed world (King *et al.*, 2003; Portsmouth *et al.*, 2003).

*To whom correspondence may be addressed (k.malcolm@qub.ac.uk)

Abbreviatons: AIDS, acquired immunodeficiency syndrome; CONRAD, Contraceptive Research and Development; DNA, deoxyribonucleic acid; FDA, Food and Drug Administration (US); GRAS, generally recognized as safe; HAART, highly active antiretroviral therapy; HEC, hydroxyethylcellulose; HIV, human immunodeficiency virus; HSV, herpes simplex virus; hu-SCID, human severe combined immunodeficiency; IND, investigational new drug; MAP, momordica anti-human immunodeficiency virus protein; N9, nonoxynol-9; NIAID, National Institute of Allergies and Infectious Diseases; NNRTI, non-nucleoside reverse transcriptase inhibitor; PMPA, (R)-(9-(2-phosphonomethoxypropyl)adenine; RNA, ribonucleic acid; RT, reverse transcriptase; SDS, sodium dodecyl sulfate; SHIV, simian/human immunodeficiency virus; SIV, simian immunodeficiency virus; STD, sexually transmitted disease; TOPCAD, Topical Prevention of Conception and Disease.

Biotechnology and Genetic Engineering Reviews – Vol. 21, November 2004
0264–8725/04/21/81–121 $20.00 + $0.00 © Intercept Ltd, 9 Farriers Close, Colehill, Wimborne, Dorset, BH21 2UA, UK

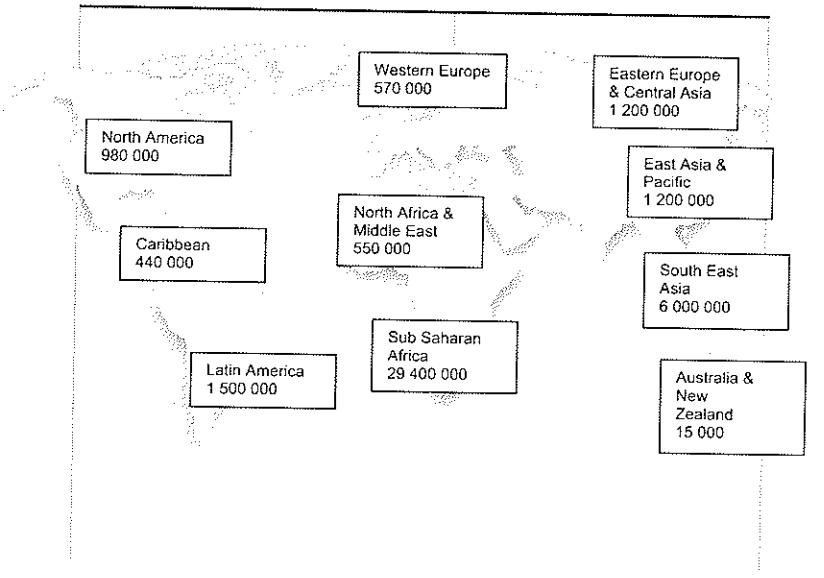

Figure 3.1. Adults and children estimated to be living with HIV/AIDS at the end of 2002 (image used and modified with kind permission from 'Aids Epidemic Update: December 2002, UNAIDS 0.246E').

Over the years, numerous strategies have been proposed to reduce or prevent sexually-acquired HIV infection, including abstinence, monogamy, condom use, reduction in number of sexual partners, and treatment of sexually related infections. Unfortunately, these strategies have had only limited success in controlling the pandemic, and are not always feasible, particularly for women within the developing world. The most obvious solution to stopping the spread of HIV/AIDS is an effective HIV vaccine. However, over two decades into the HIV pandemic, researchers are still struggling with the daunting scientific challenges involved in developing a safe and effective HIV vaccine ('HIV Vaccines', The NIAID Division of AIDS). There are currently three types of HIV vaccines under investigation; subunit vaccines, recombinant vaccines, and DNA vaccines. The ideal HIV vaccine must be inexpensive, easy to store and administer, and must provide a strong, long-lasting immunity against the many different strains of the HIV virus. Although encouraging clinical data is beginning to surface, it is highly unlikely that a HIV vaccine will be available for human use within the next decade.

Given the devastating effects of the HIV/AIDS epidemic, especially within the developing world, other preventative strategies are urgently needed. It has been estimated recently that heterosexual transmission of HIV now accounts for over 90% of adult infections worldwide, and that male-to-female transmission of HIV is eight times more likely than female-to-male transmission (Lamptey, 2002). A clear scientific rationale therefore exists for the development of female-controlled preventative strategies.

The most promising approach is the development of effective vaginal microbicides. These chemical substances have the potential to either prevent or reduce HIV transmission when applied to the vagina prior to intercourse. Although no effective

Table 3.1. Discovery of HIV

Year	
1950s	HIV emerged in sub-Saharan Africa (?)
1981	Five cases of *Pneumocystis carinii* reported in young homosexual men from Los Angeles
Dec 1981	Patients noted to have decreased $CD4^+$ counts
Early 1982	Disease named Acquired Immunodeficiency Disease
1983–1984	Virus discovered independently by Montagnier, Gallo and Levy
1986	Causative agent named Human Immunodeficiency Virus (HIV)
1986	HIV-2 and SIVs discovered

microbicidal products are presently available, there is currently a massive push to have an effective first-generation microbicide on the market by 2007.

This review will include a brief discussion of the salient features of HIV, the human vagina, and vaginal transmission of HIV, before describing the current state of affairs relating to the scientific and clinical development of the current lead vaginal HIV microbicide candidates.

The human immunodeficiency virus (HIV)

HIV belongs to the genus of retrovirus known as lentivirus, which have genes composed of ribonucleic acid (RNA) molecules. Like all viruses, HIV replicates inside cells. However, unlike many viruses, it does so by using an enzyme called reverse transcriptase to convert the viral RNA into DNA, which can then be incorporated into the host cell's genome. The virus was first detected, isolated, and shown to be the causative agent in the development of acquired immunodeficiency syndrome (AIDS) by Gallo (Gallo *et al.*, 1984; Popovic *et al.*, 1984; Sarngadharan *et al.*, 1984; Schupbach *et al.*, 1984), Montagnier (Barré-Sinoussi *et al.*, 1983), and Levy (Levy *et al.*, 1984) (*Table 3.1*).

HIV is a spherical virus of approximately 100 nm in diameter, containing an electron dense core surrounded by a phospholid bilayer envelope (*Figure 3.2*). The major HIV proteins associated with the envelope are gp120 and gp41. These function as the viral attachment proteins, binding to the CD4 receptors on certain host cells, and facilitating viral entry. gp120 is a heavily glycosylated glycoprotein, which is bound to the outside of the membrane and is non-covalently attached to the transmembrane protein, gp41. The HIV proviral genome contains *gag*, *pol* and *env* genes, which code for the core proteins, reverse transcriptase and envelope proteins, respectively. On average, 72 copies of the protein Env, containing three to four gp120 and three to four gp41 molecules, exist on the virus surface. Within the envelope of a mature HIV particle is a bullet-shaped core made of 2000 copies of the viral protein, p24. This capsid holds two single strands of HIV RNA that encode for the virus' nine genes – *gag* (group specific antigen), *pol* (polymerase), *env* (envelope), *tat* (transactivator), *rev* (regulator of viral expression), *vif* (viral infectivity), *vpr* (viral protein R), *vpu* (viral protein U), and *nef* (negative-regulation factor). HIV RNA contains long terminal repeats, which control the synthesis of new virions when activated by the host cell or HIV. The core of HIV also includes protein p7, the

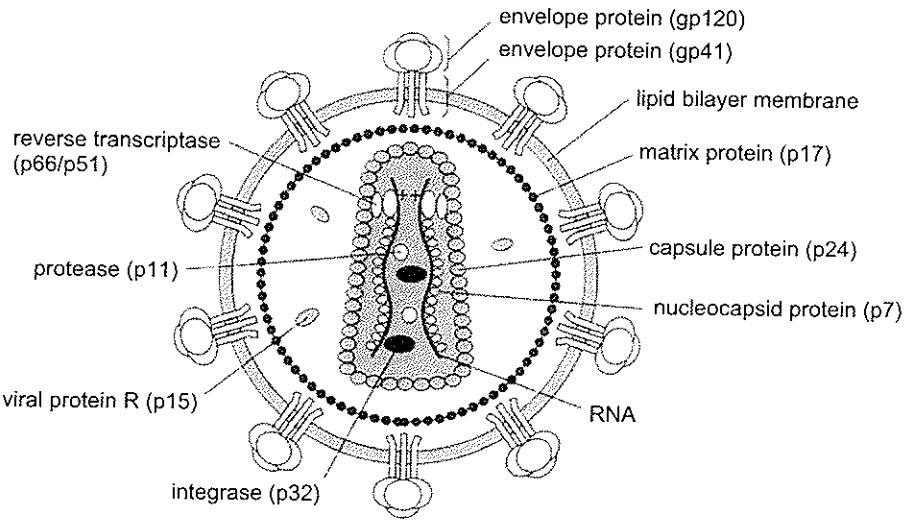

Figure 3.2. Structure of human immunodeficiency virus.

HIV nucleocapsid protein, and copies of three enzymes that carry out later steps in the virus's life cycle: reverse transcriptase, integrase, and protease.

There are several, well-defined steps in the replicative cycle of HIV – attachment, fusion, penetration, uncoating, reverse transcription, nuclear importation, integration, assembly of new virions, viral budding, and maturation. Initial attachment of the virus to the host cell involves non-specific interactions between the viral envelope and molecules (such as heparan sulfate proteoglycans, lectins, and various adhesion molecules) on the surface of human cells, followed by a specific interaction between the gp120 protein on the viral envelope, and the CD4 receptor on the surface of the host cell. The gp120/CD4 interaction thereby induces changes in the molecular conformation of chemokine co-receptor proteins on the cell surface (the most important of which are CCR3, CXCR4 and CCR5) (Alkhatib *et al.*, 1996; Choe *et al.*, 1996; Doranz *et al.*, 1996; Drajic *et al.*, 1996; Feng *et al.*, 1996; Zhang *et al.*, 1998), which facilitate further binding of the virus to the cell via these co-receptors. The HIV envelope then fuses with the cell in a process mediated by the gp41 transmembrane protein in the HIV envelope. Once fusion has taken place, and the viral capsid (the inside of the virus which contains the RNA and important enzymes) is released into the host cell, the matrix and capsid proteins of the virus disassemble, resulting in the release of viral RNA into the cell cytoplasm. HIV genes are carried in the two strands of RNA, while the genetic material of human cells is DNA. In order for the virus to infect the cell, a process called 'reverse transcription' must take place, whereby the viral RNA is converted into complementary DNA (cDNA) by the reverse transcriptase enzyme. This new DNA, also known as 'proviral DNA', is then imported into the nucleus, where it undergoes specific cleavages at the 5' and 3' termini before being integrated into the host DNA with the assistance of the viral enzyme integrase. The DNA then synthesizes new viral RNA, which is transported out of the nucleus and translated, in the case of mRNA, or incorporated into new virions, in the case of genomic RNA. Finally, new virus

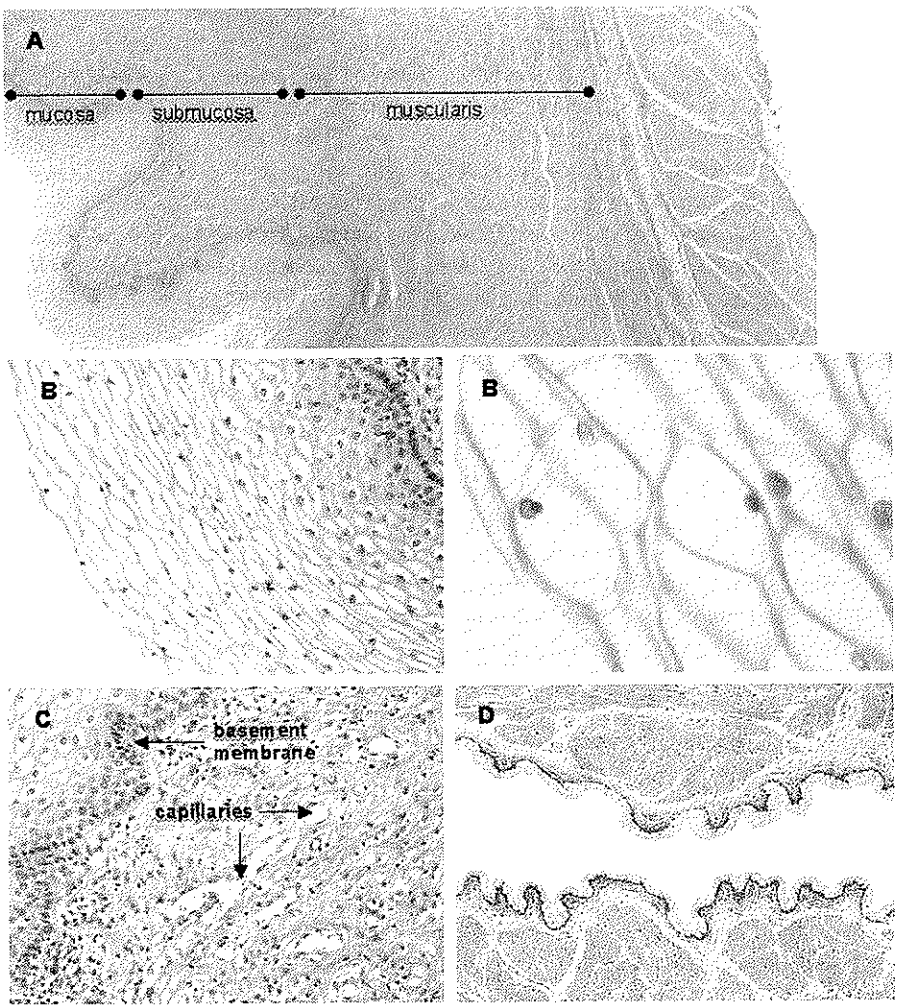

Figure 3.3. (a) Histology of the vagina showing the mucosa, submucosa and muscularis; (b) non-keratinised stratified squamous epithelium of the vagina; (c) submucosa of the vagina containing abundance of connective tissue and capillaries; (d) the cervix lined by simple, highly columnar epithelium containing mucous cells (images used and modified with kind permission of Rose M. Chute, Biology Department, North Harris College, Houston, Texas, US (http://science.nhmccd.edu/biol/reproductive/vagina.htm)).

particles are assembled. The protein molecules are cut to size by the viral enzyme protease; these smaller proteins serve a variety of functions – some become structural elements of new HIVs, while others become enzymes, such as reverse transcriptase. Once the new viral particles are assembled, they bud off from the host cell, and create new viruses which, once matured, are capable of infecting new cells.

The human vagina

Sexual transmission of HIV to the human female occurs when the virus enters the mucosal tissues of the cervix and/or vagina and infects host lymphocytes, macrophages, and dendritic cells expressing the CD4 protein. A knowledge of the physiology, histology, and ecology of the vagina will therefore be useful in developing an understanding of the mechanistic details of HIV transmission.

The vagina consists of three distinctive tissue layers: the mucosa, the muscularis, and the tunica adventitia (*Figure 3.3*). A more detailed description of the histological and immunological characteristics of these layers are provided in *Table 3.2*. The thickness of the vaginal epithelium and the immune cell populations in vaginal tissue have been reported to remain relatively constant during the menstrual cycle (Patton *et al.*, 2000).

Vaginal fluid consists primarily of transudate, which passes through the vaginal wall from the blood vessels. It is mixed with vulval secretions from sebaceous and sweat glands, with minor contributions from Bartholin's and Skene's glands (Burgos and de Vargas-Linares, 1978; Deshpande *et al.*, 1992). The fluid then becomes contaminated with cervical mucus and sloughed cells from the vaginal epithelia. Endometrial and oviductal fluids may also contribute to its chemical composition. Vaginal fluids may also contain several enzymes, enzyme inhibitors, protein, carbohydrates, amino acids, alcohols, hydroxy-ketones, and aromatic compounds. The chief component of vaginal fluid is cervical mucus, produced by glandular units within the cervical canal. The amount, composition, and physical characteristics of cervical mucus change with the menstrual cycle, making its production oestrogen-dependent. The mucus is minimal immediately after menstruation, and becomes more transparent, viscous, and elastic prior to ovulation. At the time of ovulation, the amount of cervical secretions further increases, resulting in an increase in the overall volume of vaginal fluid. Consequently, there is an increase in fibrosity, pH, and mucin content, and a decrease in the viscosity, cellularity, and albumin concentration. Previous studies suggest that approximately 6 g of vaginal fluid are produced daily with approximately 0.5–0.75 g present at any one time in the vagina (Lissimore and Currie, 1939; Lapan and Friedman, 1950; Perl *et al.*, 1959; Odeblad, 1964; Dusitsin *et al.*, 1967; Preti *et al.*, 1979; Wagner, 1979; Wagner and Levin, 1980; Godley, 1985; Owen and Katz, 1999).

These physiological parameters will almost certainly have implications for the development of anti-HIV vaginal microbicides. It is also apparent that any drug intended for application as a vaginal microbicide will require at least some solubility in aqueous vaginal fluid, to a degree dependent on its potency, while, in certain cases, also requiring sufficient lipid solubility to permit uptake across the viral and/or vaginal epithelial membranes. Also, changes in the volume and physical make-up of the fluid, particularly in response to sexual excitement, may further influence drug release/solubility/permeation characteristics (Masters and Johnson, 1966).

The vaginal fluid in healthy mature women is maintained at a pH of between 3.5 and 5 by the commensal microorganism *Lactobacillus acidophilus,* which produces lactic acid from glycogen contained in the sloughed mature cells of the vaginal mucosa. The acidic nature of the vaginal fluid is of great practical importance as it offers natural resistance to the colonization of various microorganisms (Graves *et al.*,

Table 3.2. Histological description of the human vagina

Layers	Sublayers	Histological description	Immune cells present in normal vaginal mucosa*
Mucosa	*Epithelium* 15–200 nm	Approximately 25 layers (menstrual cycle dependent) of non-keratinised, stratified, squamous cells (Patton *et al.*, 2000; Burgos and de Vargas-Linares, 1978); five cell types identified:	T8 lymphocytes
		(i) basal – small, typically columnar or squamous in shape with small nuclei, very little cytoplasm, and microvilli present on cell surface)	T4 lymphocytes
		(ii) parabasal – polygonal in shape, similar in size and structure to basal cells	Langerhans cells
		(iii) transitional	
		(iv) intermediate – largest cells, exhibit microvilli, abundant in cytoplasm and glycogen	Macrophages
		(v) superficial – outermost layer during follicular phase of cycle	Neutrophils
	Lamina propria	Loose, highly vascularised connective tissue (extremely rich in blood vessels and lymphatics)	Plasma cells
	Submucosa	Connective tissues made of collagen and elastic fibres, and blood vessels	B lymphocytes
Muscularis		Smooth muscle fibres arranged in an outer longitudinal layer and an inner circular layer	
Adventitia		Thin, fibrous layer composed of dense sheath of collagen and elastic fibres; contains blood vessels, lymph vessels and nerves	

* Patton *et al.*, 2000

1980; Sturn and Zanen, 1984; Croughan and Behbehani, 1988; Nagy *et al.*, 1991; Pettit *et al.*, 1999; Sparling, 1999). The pH of vaginal fluid rises during menstruation, but it may also increase after periods of frequent acts of coitus as both vaginal transudate, formed during coitus, and ejaculate are alkaline. Elevated pH has been associated with increased transmissibility of HIV infection (Martin *et al.*, 1985; Ongradi *et al.*, 1990; O'Connor *et al.*, 1995). Physiologically, the anterior fornix of the vagina has the lowest pH, which gradually rises toward the vestitube. Intravaginal pH may also be affected by the presence of cervical mucus, which has a pH in the range 6.5 to 9, and by the amount of lubricating vaginal secretions. These changes could influence the solubility, uptake, and release profile of pH-sensitive substances within the vagina.

Biochemical activity in the vagina may also be an important factor that may affect the stability of certain microbicidal substances. The basal layer of the vaginal epithelium has a high activity of enzymes found in the citric acid cycle, in fatty acid metabolism, and in 17-ketosteroidogenesis, e.g. succinic dehydrogenase, diaphorase, acid phosphotase, β-glucuronidase, and phosphoamidase. The outer cell layers

of the vagina contain β-glucuronidase, acid phosphatase, and smaller quantities of α-naphthylesterase, diaphorase, phosphoamidase, and succinic dehydrogenase (Yu and Chien, 1982).

Vaginal transmission of HIV

Several excellent reviews on the sexual/vaginal transmission of HIV have been published (Osmond, 1998; Miller and Shattock, 2003), and therefore only a brief overview will be provided here.

Although HIV may be transmitted by several non-sexual modes, including mother-to-child transmission, transfusion of contaminated blood, and drug injection, the current AIDS pandemic is largely driven by heterosexual/vaginal transmission, which accounts for some 90% of new HIV-1 infections globally (Royce *et al.*, 1997). This is despite the fact that vaginal fluid is the least infectious of body fluids (attributed to the relatively low concentration of $CD4^+$ cells in vaginal fluid compared to other fluids).

It is well documented that vaginal intercourse can transmit HIV either from male-to-female or female-to-male (Padian *et al.*, 1991, 1997; European Study Group, 1992; Mastro *et al.*, 1994; Nicolosi *et al.*, 1994). However, in the absence of other risk factors, such as sexually transmitted diseases, men are two to three times more likely to transmit HIV to women than vice versa (Peterman *et al.*, 1988; European Study group, 1992), and eight times more likely when sexually transmitted diseases are taken into consideration (Padian *et al.*, 1997). Several facts help in explaining this observation, including: 1) a larger exposed area of susceptibility to infection in women (vagina, cervix, and uterus) compared with men (head of the penis, exposed urethra); 2) women are exposed to a larger quantity of infectious fluid (ejaculate, typically 1.5 to 5 ml) than men (vaginal fluids, typically >1 ml (reviewed in Owen and Katz, 1999)); 3) the concentration of HIV virions in vaginal fluid tends to be less than in semen; and 4) women retain the secretions within the body after sex while men are only exposed during the sexual act. Semen has been shown to contain both free infectious HIV-1 and HIV-1-infected cells (Mermin *et al.*, 1991; Pudney and Anderson, 1991; Vernazza *et al.*, 1994, 1996; Zhang *et al.*, 1998), and thus transmission might occur by cell-free and/or cell-associated HIV modes. Studies of SIV transmission in Rhesus macaques suggest that cell-associated virus does not contribute significantly to sexual transmission (Miller, 1992). Instead, free virus binds to $CD4^+$ Langerhans cells and macrophages in the genital mucosa before being transported to the lymph nodes. Given that CD4-expressing Langerhans cells, dendritic cells, and macrophages are also present in the human vaginal epithelia, it is possible that these are the main target cells for HIV infection via heterosexual vaginal intercourse in the human female. However, a number of studies have demonstrated that HIV infection of epithelial cells does not occur, but is instead limited to cells within the sub-epithelial mucosa, strongly suggesting that establishment of HIV infection requires epithelial penetration (Pomerantz *et al.*, 1988; Miller *et al.*, 1992; Nuovo *et al.*, 1993; Palacio *et al.*, 1994; Spira *et al.*, 1996). The current consensus is that at least two main routes are available for HIV to cross the vaginal epithelium – transepithelial migration of infected Langerhans cells, and penetration of the virus through damaged epithelial tissue (Miller and Shattock, 2003)

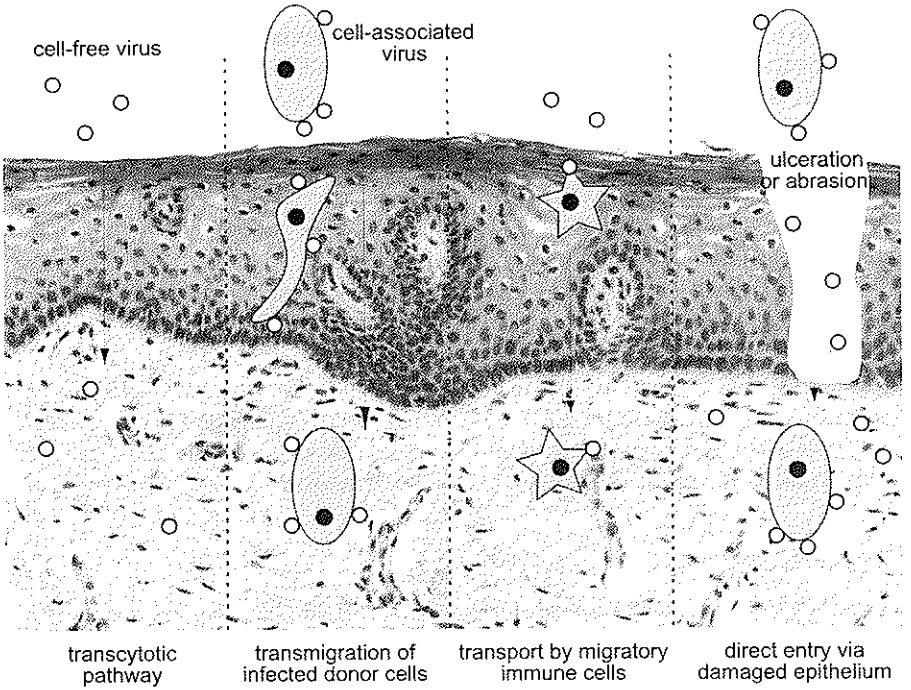

Figure 3.4. Diagram illustrating the proposed four major mechanisms by which HIV can be transported across the vaginal epithelium and initiate infection in the submucosal tissue.

(*Figure 3.4*). Damage to the vaginal epithelium is detected in most women following consensual intercourse (Novell *et al.*, 1984), and would potentially provide direct viral access to susceptible T cells, dendritic cells, and macrophages in the vaginal lamina propria.

There is also evidence to suggest that the cervix might be more susceptible to HIV than vaginal tissue, a consequence of its fragility, the fact that it is commonly compromised by a number of sexually transmitted diseases, and, like the vagina, the presence of specific HIV receptors (Howell *et al.*, 1997; Zhang *et al.*, 1999; Moench *et al.*, 2001). The importance of the cervix as a site for HIV infection has been demonstrated in experiments in which rhesus macaques were infected vaginally with HIV. Cervical cells were observed to be infected by day 3, while the vaginal mucosa was not infected until day 12 (Zhang *et al.*, 1999). In contrast, it has also been reported that cervical ectopy, an immature area of the cervix lined by fragile columnar rather than more protective squamous epithelium, is not independently associated with HIV infection (Moscicki *et al.*, 2001).

In the absence of hard *in vivo* data, researchers are still uncertain as to the type of cells that *initially* become infected in the reproductive tract of women after exposure to HIV – in chronically HIV-1-infected women, T cells, macrophages, and Langerhans cells in cervical tissue are all infected. Indeed, as already alluded to, one of the major problems in studying HIV transmission is the lack of a suitable *in vitro* model. For example, in studies employing the simian immunodeficiency virus (SIV)–rhesus monkey model, Langerhans cells (Spira *et al.*, 1996), $CD4^+$ T cells (Zhang *et al.*,

1999), and dendritic cells (Spira *et al.*, 1996; Hu *et al.*, 2000) have all been implicated as the first cell type to become infected upon inoculation with SIV. However, more recent studies using a cervical tissue-derived organ culture model to study HIV transmission across the mucosal surface (designed to more closely mimic the human *in vivo* situation) have shown that memory $CD4^+$ T cells were first to become infected (0.2, 2.7, and 2.3% after 6, 24 and 96 h, respectively) followed by macrophages (0, 0.8 and 1.2%, respectively) and dendritic cells (0, 0.1, 0.7%, respectively) (Gupta *et al.*, 2002). Such a model may prove useful in the preclinical screening of potential vaginal microbicides.

An increased risk of HIV susceptibility and infectiousness in females has also been positively associated with a wide range of cervicovaginal infections (Royce *et al.*, 1997), including *Neisseria gonorrhoeae* (Martin *et al.*, 1998), *Chlamydia trachomatis* (Laga *et al.*, 1993), *Candida* infection (Martin *et al.*, 1998), and bacterial vaginosis (Martin *et al.*, 1998). Progesterone therapy and oral contraception have also been correlated with increased risk of vaginally-acquired HIV (Ungchusak *et al.*, 1996; Mostad *et al.*, 1997; Martin *et al.*, 1998).

Vaginal microbicides

Vaginal microbicides are chemical substances, or formulations thereof, that prevent, or at least reduce, the transmission of sexually transmitted diseases, including HIV, when applied to the human vagina prior to intercourse. Several characteristics typify the ideal vaginal HIV microbicide – broad spectrum of activity; activity against both cell-free and cell-associated HIV; minimal systemic absorption; non-toxic to vaginal tissue; retention of activity in the presence of semen; activity over a broad pH range (typically pH 3–8); available in contraceptive and non-contraceptive forms; compatibility with condoms; retention in the vagina over prolonged periods of time; low cost; long shelf life; ease of use/acceptability/patient compliance.

The first potential microbicide formulations to be evaluated contained the non-ionic surfactant, nonoxynol-9. It has long been employed as the active ingredient in various marketed spermicides and lubricating gels, and has GRAS (generally recognized as safe) status. Although various initial studies suggested that nonoxynol-9 was effective *in vitro* against a wide range of sexually transmitted microorganisms through disruption of the viral/bacterial membrane (Singh *et al.*, 1972; Hicks *et al.*, 1985; Judson *et al.*, 1989), its surfactant characteristics were also shown to be responsible for damaging the vaginal epithelium, resulting in an *increased* risk of HIV transmission (Kreiss *et al.*, 1992; Roddy *et al.*, 1998; Stephenson, 2000; Fichorova *et al.*, 2001; Rustomjee and Abdool Karim, 2001; Wilkinson *et al.*, 2002).

Although a vaginal microbicide may potentially act at any stage of the HIV replicative cycle, it is preferable to disrupt the cycle as early as possible. For this reason, the most promising microbicides currently being evaluated are attachment/binding/fusion/entry inhibitors, inhibitors of reverse transcription, or compounds/formulations that enhance the natural vaginal defence mechanisms (*Figure 3.5*). There are currently 34 products in preclinical development, 15 in Phase I safety trials, 4 in Phase II expanded safety and preliminary effectiveness trials, and 3 in Phase III trials (*Table 3.3, Figure 3.6*).

To date, little thought has gone into optimizing vaginal microbicide formulations

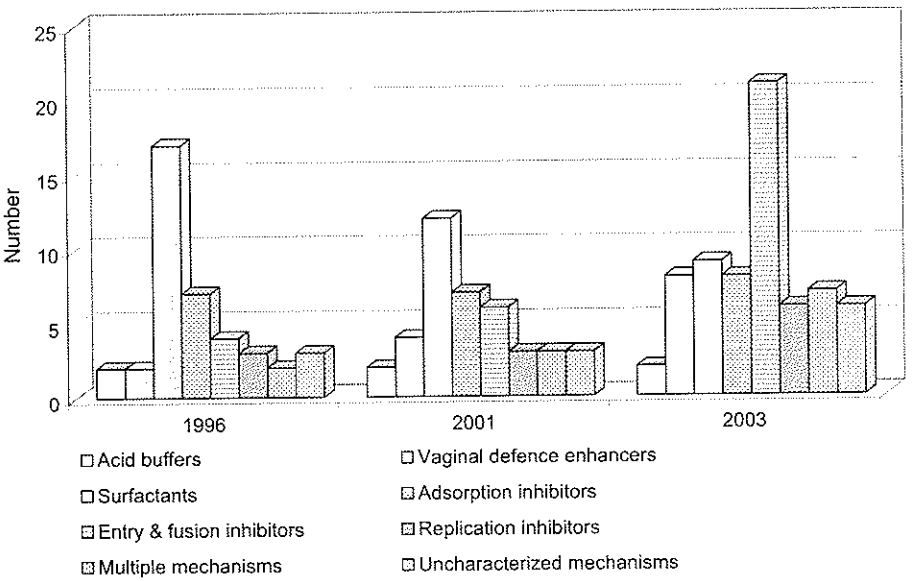

Figure 3.5. Mechanisms of action of vaginal microbicides.

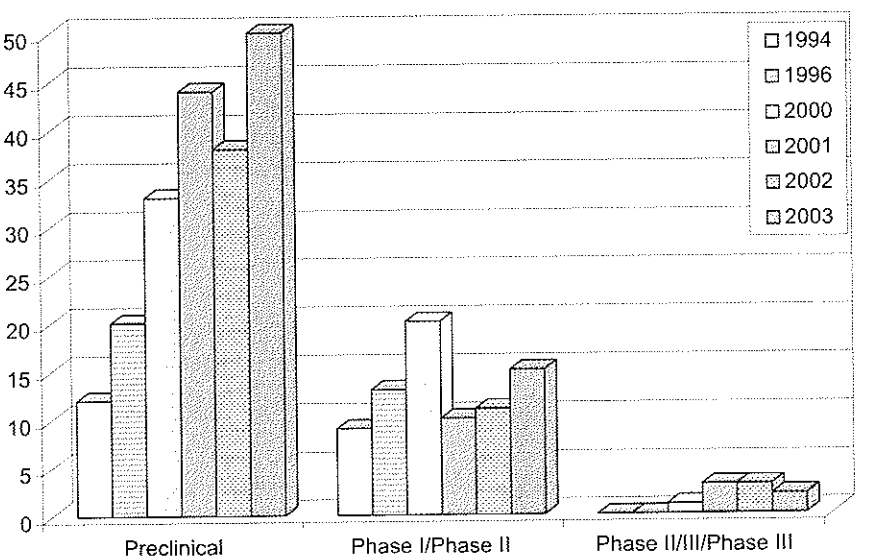

Figure 3.6. Development profile for vaginal microbicides.

Table 3.3. Description of microbicides currently under development

Mechanism of microbicide action	Product type/compound	Other name/trade name (developer)	Clinical status	Cont.*
Compounds that kill or inactivate HIV				
Acidic buffers	*See later section of table*			
Antibiotic peptides	*See later section of table*			
Lipids	Synthetic lipids adapted from human breast milk			
Monoclonal antibodies	*See later section of table*			
Peroxidases/peroxides	Haloperoxidases			
	Halides			
Plant extracts	Praneem (polyherbal oil)	(Inst. Res. & Reprod., India)		
	Gossypol			
Surfactants/detergents/lipids	Benzalkonium chloride	(Biofem)	Phase I	C
	C31G (Savvy)	(Biosyn Inc.)	Phase I	C
	Chlorhexidine			
	Nonoxynol-9		Phase III	C
	Octoxynol-9			
	Sodium dodecyl sulfate			
	Sodium lauryl sulfate			
Inhibitors of HIV binding/entry				
Peptides/proteins	B69 (modified beta-lactoglobulin)	(Lab. Bioche. Virol. LFK Res. Inst. NY Blood Centre)	Preclinical	NC
	Cyanovirin-N	(Biosyn Inc.)	Preclinical	NC
	T-20 (gp41 fusion inhibitor)	Roche and Trimeris	Preclinical	NC
Sulfated polysaccharides	Carrageenan (seaweed derivative)	Carraguard™ PC515 (Pop. Council)	Phase III	NC
	Cellulose sulfate	Ushercell™ (Polydex Pharmaceuticals Ltd., GMP)	Phase III	C
	Dextrin-2-sulfate	Emmelle™ (ML Laboratories)	Phase I	NC
Sulfonated polymers	Naphthalene sulfonate polymer	PRO-2000 (Interneuron Pharm, Inc.)	Phase II/III	C
	Polystyrene sulfonate	Program for the Topical Prevention of Conception and Disease (TOPCAD), Global Microbicide Project (GMP)	Phase I	C
Others	CCR-5 inhibitors			
	Cellulose acetate phthalate (CAP			
	n-docosanol	Lidakol (Lidak Pharmaceuticals)		
	Monoclonal antibodies (MAb)	e.g. broadly neutralizing human MAb b12		NC

Inhibitors of HIV replication				
Non-nucleoside reverse transcriptase inhibitors	Calanolide A/B	Sarawak MediChem		
	DABO	(Indenix Pharmaceuticals)		
	Nevirapine	Viramune		
	TMC-120	Dapivirine (Tibotec-virco)	Preclinical	NC
	UC-781	(Biosyn Inc.)	Preclinical	NC
Nuceloside reverse transcriptase inhibitors	3TC	Lamivudine		NC
	AZT	Zidovudine		NC
	ddI	Didanosine		NC
Nucelotide reverse transcriptase inhibitors	Tenofovir (PMPA)	Viread™ (Gilead)	Phase I	NC
Protease inhibitors	Doxovir (CTC-96)	(Redox Pharmaceutical Corp.)	Preclinical	NC
Enhancers of natural vaginal defence mechansims				
Acidity regulators	Bioadhesive acidic aqueous gel/ Carbomer 974P formulation	Buffergel™ (ReProtect LLC)	Phase II/III	C
	Bioadhesive acidic aqueous gel	AcidForm™ (TOPCAD, GMP)	Phase I	C
Maintenance of vaginal ecology	*Lactobacillus crispatus*		Phase II	NC
	CD4-expressing *Lactobacillus*	Viroshield™		
Antibiotic peptides	Protegrins			
	Defensins	e.g. retrocyclin (RC-100)		
	Magainins	e.g. squalamine		
	Gramicidin			
Monoclonal antibodies	Plantibodies™			
Other	Hydrogen peroxide/peroxidases			
	Estrogens	e.g. estradiol		NC
Multiple mechanisms				
Antiviral proteins	MAP30 (Momordica anti-HIV protein)	(NY University School of Medicine)		NC
	GAP31 (Gelonium anti-HIV protein)	(NY University School of Medicine)		NC
Others	Cellulose acetated phthalate			
	Monocaprin with PAVAS			
	Naphthalene urea derivatives	(Mt. Sinai Medical Centre)		
Others				
Herbal substances	Alatavir			
	Kolaviron			
	Pokeweed antiviral protein			
	Praneem			C
	Tinosporin			
Uncharacterized	Anionic dendrimers			
	Porphyrins and protoporphyrins			
	Sodium dimandelic acid ether			
	Zinc gluconate gel			
	Cobalt containing redox complex	Doxovir-M™		
	n-cyclodextrin			
	Gossypol			

* C – contraceptive; NC – non-contraceptive.

for effective delivery within and distribution throughout the vagina. The vast majority of candidate microbicides are formulated in traditional semi-solid topical gels or creams for the purposes of clinical evaluation. However, the advantages of such gel systems, such as their inexpensiveness and ease of manufacture, need to be considered alongside their inherent disadvantages, which include relatively poor retention in the vagina (even for so-called 'bioadhesive' formulations), messiness in their application, requirement for applicator use, poor user acceptability, and interference with coitus (Woolfson *et al.*, 2000). In order to provide protection, semi-solid formulations are most likely to require application prior to every act of intercourse, which is also likely to lead to poor user compliance. A number of alternative formulations for the delivery of vaginal microbicides are currently being evaluated, such as controlled-release intravaginal rings (Woolfson *et al.*, 1999, 2003; Malcolm *et al.*, 2001, 2003a,b; Malcolm, 2003), akin to those currently being marketed for contraception (Nuvaring®) and hormone replacement therapy (Estring®, Femring®).

The remainder of this review describes in more detail the scientific literature relating to 19 microbicidal substances, presented in alphabetical order, that are currently being evaluated for the prevention of heterosexual transmission of HIV. In order to provide the reader with a broad perspective of the current state of play within the field, compounds have been selected representing all stages of the research and development pipeline.

Acidform

Acidform™ is an aqueous-based, bioadhesive, acid-buffering gel formulation designed to keep the vagina acidic in the presence of semen. The gel, which has been developed by the Programme for Topical Prevention of Contraception and Disease (TOPCAD), consists of water, gelling agents, buffer salts, humectants, and preservatives in a proprietary mixture buffered to pH 3.55. All the ingredients are 'generally recognized as safe' (GRAS) except for one, which is currently used in a number of marketed vaginal products. The low pH of Acidform, in addition to having the potential to inactivate HIV and other pathogens, is also spermicidal, immobilizing 100% of the spermatozoa even when diluted 10-fold before addition to semen (Zaneveld *et al.*, 1996). Like many gel-based vaginal microbicide formulations, Acidform may also create a physical barrier that inhibits the passage of pathogens into the vaginal and cervical epithelia. A Phase I clinical study designed to evaluate the vaginal tolerance of Acidform has demonstrated that it does not irritate the vaginal epithelium (Amaral *et al.*, 1999). However, when Acidform was co-formulated with 2.5 and 5.0% w/w nonoxynol-9, vaginal erythema and/or abrasions were observed in 50% of women after two days use, and in 100% after seven days use (Amaral *et al.*, 1999). The *in vitro* acid-buffering, bioadhesive, viscosity and stability characteristics of Acidform have been evaluated, and compared to those of other marketed vaginal gels (Garg *et al.*, 2001). The study not only indicated that Acidform has excellent semen-buffering ability, but that its high viscosity and bioadhesive characteristics should ensure that it will coat and be retained within the vagina.

B69

The New York Blood Centre has shown that chemical modification of bovine beta-lactoglobulin, a major protein of milk and whey, resulted in the generation of a potent inhibitor of infection by HIV, including clinical isolates. The compound, designated B69 or 3HP-β-LG, is a biological candidate compound for chemical barriers against vaginal, oral, and rectal HIV transmission. Laboratory tests have shown that B69 blocked at nanomolar concentrations the binding to CD4 of human (HIV) and simian (SIV) immunodeficiency virus surface glycoproteins and monoclonal antibodies specific for the HIV binding site on CD4, and inhibited infection by HIV-1, including primary virus isolates, by HIV-2 and by SIV (Neurath *et al.*, 1996).

It has been reported previously that B69, suspended in phosphate-buffered saline and administered prior to and after intravaginal inoculation with SIV, was effective in preventing SIV transmission in 50% of the female rhesus monkeys tested (Wyand *et al.*, 1999). Studies have shown that B69 is not irritating to vaginal tissues and has no deleterious effects on bacteria corresponding to the natural vaginal flora. *In vitro* inhibition of the herpes simplex virus has also been indicated by B69 (Manson *et al.*, 2000).

BufferGel

BufferGel™ (ReProtect) is a spermicidal and microbicidal gel formulation (pH 3.9) based on Carbomer 974P, a synthetic, high molecular weight, pharmaceutical grade poly(acrylic acid) (*Figure 3.7*) cross-linked with a polyalkenyl polyether.

Carbomers are a class of poly(acrylic acid)s used in a wide range of cosmetic and pharmaceutical applications, at concentrations up to 50% w/w. The carboxylic acid functional groups in the Carbomer are capable of maintaining the protective acidic environment of the vagina in the presence of alkaline semen (Olmsted *et al.*, 2000; Maguire *et al.*, 2001; Zeitlin *et al.*, 2001) without adversely affecting the *Lactobacillus* species normally responsible for pH maintainence (Clarke *et al.*, 2002). Encouragingly, vaginal BufferGel use also significantly reduces the number of anaerobic pathogens present, whose associated disease states may have implications for HIV transmission; for example, the number of women presenting *Gardnerella vaginalis*, associated with bacterial vaginosis, was reduced by up to 87% after once-daily vaginal application of BufferGel for 14 days (van de Wijgert *et al.*, 2001; Clarke *et al.*, 2002). The pH required for HIV inactivation (and indeed many other sexually transmitted disease microorganisms) has been reported in different studies to be between 4 and 5.8 (Ongradi *et al.*, 1990; O'Connor *et al.*, 1995). Five millilitres

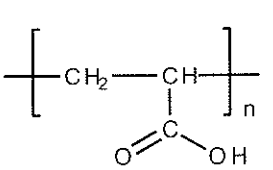

Figure 3.7. Structure of polyacrylic acid.

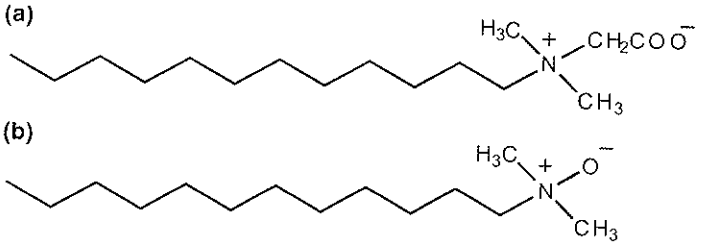

Figure 3.8. Structure of C31G.

of BufferGel is capable of buffering twice its volume of semen to a pH of 5.0, equivalent to approximately three times the average ejaculate volume (Olmsted *et al.*, 2000). In addition to its ability to directly inactivate sperm, HIV, and other microorganisms, the bioadhesive BufferGel formulation may also create a viscous physical barrier that inhibits the passage of pathogens into the vaginal and cervical epithelium. The safety of BufferGel in regard to vulvar, vaginal, and cervical epithelial toxicity has already been demonstrated in HIV/STD negative women in a multi-centre, international Phase I clinical trial (Mayer *et al.*, 2001; van de Wijgert *et al.*, 2001). Enrolment is currently in progress for a multinational, multiproduct Phase II/III effectiveness trial (see reference section – HPTN 0350).

The excellent mucosal safety profile of Carbomers and their widespread inclusion in various pharmaceutical formulations make BufferGel an obvious gel base for the formulation of other HIV microbicides. For example, UC-781, a potent experimental non-nucleoside, reverse transcriptase inhibitor currently being evaluated as a vaginal microbicide, has been formulated in BufferGel, and its efficacy in preventing HIV-1 transmission through cervical tissue evaluated (Zussman *et al.*, 2003).

C31G

C31G is an equimolar mixture of two amphoteric, surface-active molecules: N-*n*-dodecyl-N,N-dimethylamine-N-oxide (C12-N-oxide) and N-*n*-dodecyl-N,N-dimethyl-amine-N-glycine (C12-N-betaine) (*Figure 3.8*). It has broad spectrum antibacterial, antiviral, and antifungal activity (Corner *et al.*, 1988; Calis *et al.*, 1992; Thompson *et al.*, 1996; Wyrick, *et al.*, 1997; Krebs *et al.*, 1999; Birnie *et al.*, 2000). However, the surfactant mixture is not active against non-enveloped viruses, such as the sexually transmitted human papillomaviruses, which have been implicated in the genesis of cervical cancer (Malamud *et al.*, 1998; Howett *et al.*, 1999). The antimicrobial, and presumably antiviral, activity of alkyl betaines and alkyldimethylamine oxides have been reported to increase with increasing alkyl chain lengths up to approx. 15 carbon atoms, such that minimum inhibitory concentrations were directly correlated with critical micelle concentrations (Birnie *et al.*, 2000).

In vitro anti-HIV activity has been demonstrated for C31G in several studies. It has been reported to have greater capacity for inactivating both cell-free and cell-associated HIV-1 than nonoxynol-9 (N9) or sodium dodecyl sulfate (SDS) (Krebs *et al.*, 1999). For example, in studies to assess the effect of surfactant concentrations on the ability of various HIV strains to infect HeLa-CD4-LTR-β–gal (HCLB) cells, C31G, N9, and SDS inactivated at the 0.00625% concentration level were shown to

Table 3.4. Self-reported symptoms, results of colposcopic evaluation and product acceptability in women using 5 ml daily applications for 7 days of various gel formulations (Ballagh *et al.*, 2002)

	Microbicide formulation		
	1.2% C31G HEC gel	2% N9 gel (Gynol II)	HEC-only gel
Symptoms of vaginal/vulvar irritation	80%	45%	50%
Heat/burning	80%	25%	5%
New lesions: non-applicator-related	45%	50%	20%
Serious lesions: non-applicator-related	20%	10%	0%
Would use if effective contraceptive	25%	35%	55%

have inactivated 84, 67 and 24% of HIV-1 IIIB (T tropic) virions, respectively, 78, 44, and 23% of HIV-1 BaL (M tropic) virions, respectively, and 100, 72, and 23% of HIV 89.6 (dual tropic) virions, respectively, compared to an untreated control. At this concentration, cell viability was decreased to approximately 85% for C31G and N9, compared to 95% for SDS. Complete viral inactivation was achieved in all strains at C31G concentrations of 0.025% and above, but this was associated with a concomitant decrease in cell viability. The results demonstrate that all three agents could function either solely or in combination at concentrations that provide effective viral inactivation and low levels of cytotoxicity. In a subsequent study by the same investigators, primary human vaginal keratinocytes (cells found within the vaginal epithelium) were five times more sensitive to N9 than either C31G or SDS during continuous 48-hr exposure (Krebs *et al.*, 2000). In a more realistic multiple exposure mode, cells were considerably more sensitive to C31G than N9 or SDS at lower concentrations within the range tested. Furthermore, in time-sensitivity studies, exposure to C31G for 18 hrs decreased cell viability to a greater extent than exposure to N9 or SDS for at least 24 hrs. These concentration and time-sensitivity considerations are clearly important factors impacting the *in vivo* efficacy of vaginal micobicides. Other *in vitro* experiments have been performed in which cell lines of human immune and epithelial origin are used as surrogate markers for primary cells to evaluate the effects of microbicides on cell metabolism, membrane composition, and integrity, and the effects of cell-type, proliferation, and differentiation on microbicide sensitivity (Krebs *et al.*, 2002).

In the first Phase I human trial of C31G (60-women, randomized, double-blind, 7-day tolerance study), a C31G hydroxyethylcellulose (HEC) gel formulation, previously shown to exhibit antimicrobial properties *in vitro* (Birnie *et al.*, 2001), presented significant differences in the incidence of vaginal symptoms and colposcopic findings, compared to a positive control nonoxynol-9 gel (Gynol II) and a negative control HEC gel (*Table 3.4*) (Ballagh *et al.*, 2002). The most common complaint was a burning sensation in the vagina, reported by 80% of C31G users. It is also clear that the active agents, C31G and N9, caused increased incidence of epithelial disruption in these formulations, compared to the HEC-only gel, whether reported in the form of lesions, oedema, or erythema. Given the physicochemical

characteristics of surfactant molecules, and in particular their ability to solubilize lipids, such damage to vaginal epithelia is not wholly unexpected. However, the study also highlights two additional issues pertinent to the development of vaginal microbicide formulations. First, much more research needs to be conducted into the design and use of vaginal applicators; the results of this and other studies have reported a significant incidence of applicator-related epithelial damage. Second, the results reiterate the generally poor acceptability of gel-based vaginal formulations, associated with leakage, messiness, and partner acceptability.

In a follow-up Phase I study, designed to assess the tolerability and user acceptability of five reformulated C31G gel/cream formulations, a marked decrease in the incidence of self-reported symptoms, compared with that of the 1.2% C31G HEC formulation, was reported (Bax *et al.*, 2002). For example, the best tolerated and most accepted formulation amongst the 105 women enrolled, a 1.0% C31G poloxamer gel formulation (poloxamers are co-polymers of propylene and ethylene glycols), caused a heating/burning sensation in only 13% of users, compared with 80% for the HEC formulation. It is unlikely that the improved acceptability of the co-polymer product is related solely to the decreased C31G concentration or the decreased vaginal dose used in this study (3.5 ml cf. 5.0 ml). However, it will be imperative to conduct both colposcopy studies and imaging studies with the co-polymer gel to evaluate the integrity of the vaginal epithelium during use, and to ensure that the reduced dose volume is sufficient to provide protective coverage within the vagina.

Carrageenan

Carrageenan is a linear polysaccharide, derived from red seaweed, and composed of repeating sulfated and non-sulfated D-galactose and 3,6-anhydro-D-galactose units joined by alternating $\alpha(1\rightarrow3)$, $\beta(1\rightarrow4)$ glycosidic linkages. Its polymeric structure and various sources and extraction methods ensure that many structural variations exist; for the sake of simplicity, three main types are defined – kappa, iota, and lambda carrageenans – which are idealized molecules assigned definitive structures (*Figure 3.9*). Most seaweeds produce a range of structural variations, rather than these idealized structures.

Carrageenan is the most active sulfated polysaccharide against HIV (PearcePratt and Phillips, 1996). In its native form, it has a (weight average) molecular weight of ~2 million, although it is usually prepared in low molecular weight form to enhance absorption. It is stable, even at elevated temperatures, is not degraded by bacteria, and has been used for hundreds of years as a thickener in foods, and as an emulsifier in topical creams and lotions. Carrageenan compounds are on the US Food and Drug Administration's list of GRAS (generally recognized as safe) products for consumption and topical application. This classification gives carrageenan-based formulations a number of advantages, including the need for fewer standard preclinical tests for regulatory approval, and a greater likelihood that they could be made available widely and easily.

Carraguard™ (formerly known as PC-515), a 3% w/w aqueous gel formulation of carrageenan, is the Population Council's lead candidate microbicide. It has been shown to block infection by HIV, HSV-2, HPV and *N. gonorrhoeae* both *in vitro* and

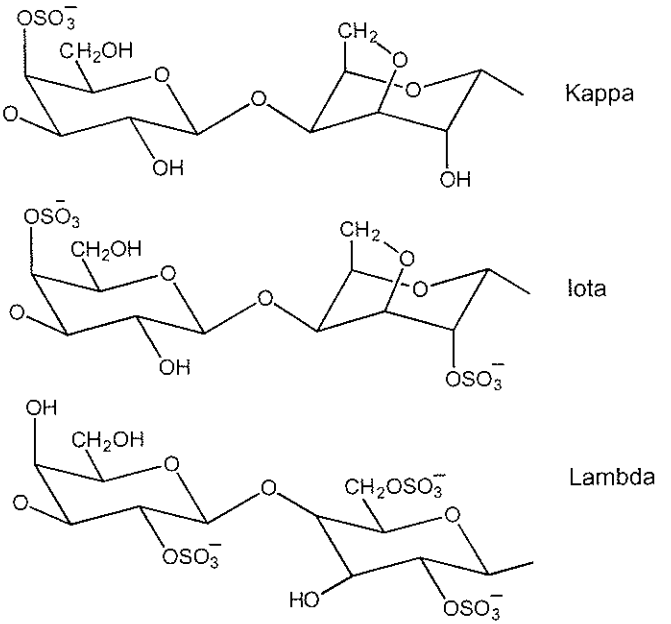

Figure 3.9. Idealized chemical structures of kappa, iota, and lamba carrageenans.

in vivo (Zacharopoulos and Phillips, 1997; Bourne *et al.*, 1999a; Maguire *et al.*, 2001). A carrageenan solution, however, provided no protection in a mouse model against *Chlamydia trachomatis*, a sexually transmitted infection implicated in increased risk of HIV infection (Achilles *et al.*, 2002). Phase I human safety studies of related carrageenan compounds (PC-213 and PC-503), and data from pilot studies of Carraguard (PC-515), have shown that carrageenan is not harmful when used vaginally (Elias *et al.*, 1997; Coggins *et al.*, 2000). Interim safety data from the Population Council's expanded safety, acceptability, and preliminary effectiveness trials also show Carraguard to be safe and acceptable when used for up to 12 months. A Phase III effectiveness trial of Carraguard is scheduled to begin in 2004, and will last approximately four years. If it is shown to be effective, a New Drug Application will be filed with the US Food and Drug Administration and regulatory authorities in the other countries where it will be available.

Polymer-drug conjugates of *kappa*-carrageenan have also been synthesized using a succinate spacer to covalently link 3'-azido-3-deoxythymidine (AZT, a potent, FDA-approved anti-HIV agent) to the polymer backbone (Vliege *et al.*, 2002) (*Figure 3.10*). Synergism between the carrageenan and AZT was demonstrated when MT-4 cells were preincubated with the conjugate prior to HIV-1 infection, although a threshold loading of AZT was required to achieve this effect. Although such conjugated molecules have not yet been considered for vaginal application, they could offer many advantages, including: i) increased activity through synergism; ii) decreased drug toxicity; iii) controlled release potential; and iv) delay in emergence of drug-resistant strains.

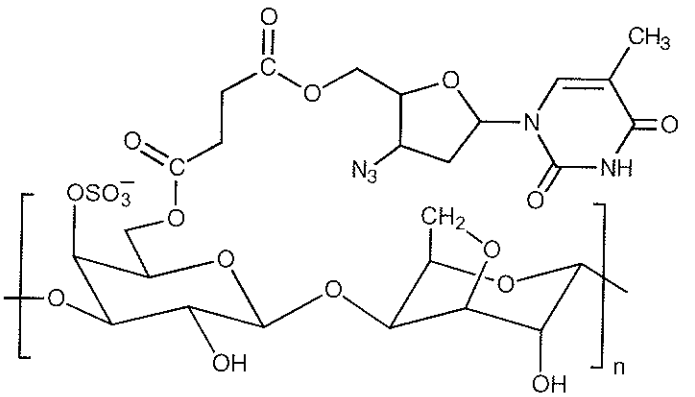

Figure 3.10. Polymer-drug conjugate of *kappa*-carrageenan and the antiretroviral drug, AZT.

Cellulose acetate phthalate

Cellulose acetate phthalate (CAP) is a high molecular weight compound (average molecular weight, M_w ~60 000) (*Figure 3.11*) commonly used as an enteric film coating material or a matrix binder for tablets and capsules (Lee, 1994). It is a promising microbicide candidate for prevention of infection by sexually transmitted disease pathogens, including HIV-1 (Neurath *et al.*, 1999). CAP inactivates HIV-1 and blocks the co-receptor binding site on the virus envelope glycoprotein gp120, while leaving the site for the primary cellular receptor CD4 accessible (Deng *et al.*, 1996; Neurath *et al.*, 2001). CD4 was shown to inhibit HIV-1 infection by two mechanisms: reversible blockage of virus binding to receptors, and irreversible inactivation of virus activity.

When formulated in a vehicle, a micronized form of CAP has been shown to inactivate HIV-1, herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), cytomegalovirus, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Haemophilus ducreyi*,

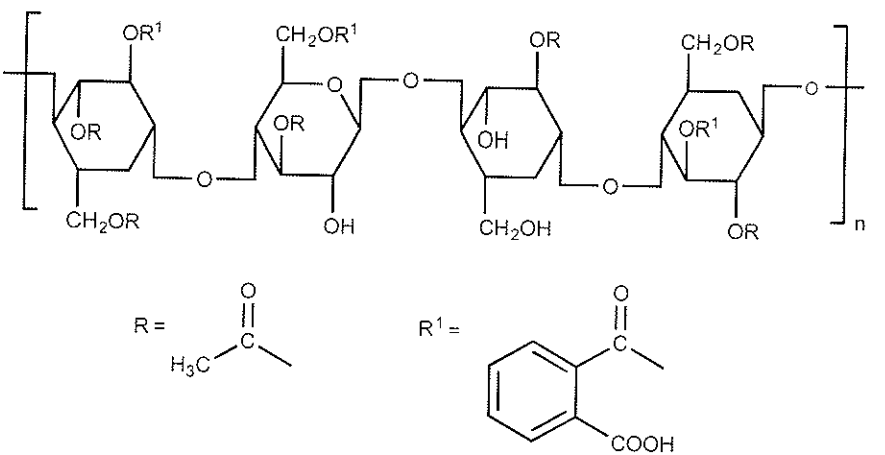

Figure 3.11. Structure of cellulose acetate phthalate.

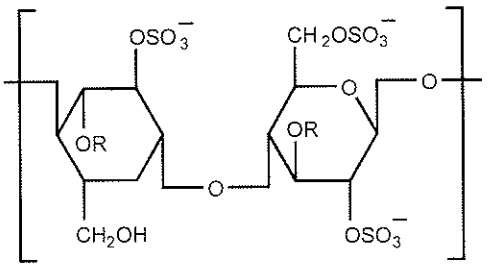

Figure 3.12. Structure of cellulose sulfate.

and *Chlamydia trachomatis in vitro*. Another advantage of CAP is that it does not appear to affect lactobacilli, part of the natural vaginal flora that contributes to resistance to STDs (Neurath *et al.*, 1999).

Cellulose sulfate

Cellulose sulfate, which has a backbone consisting of (1→4) linked glucose units with adjacent trans-hydroxyl groups (Gohdes *et al.*, 1997) (*Figure 3.12*), is being developed as a vaginal microbicide by the Programme for the Topical Prevention of Conception and Disease (TOPCAD, Chicago) in collaboration with the Contraceptive Research and Development (CONRAD) programme (Washington DC), and is currently in clinical trials. The mechanism by which cellulose sulfate effects microbical activity against STD's could be either by binding to the infectious agent or by binding to the target cell, thus preventing successful infection of susceptible host cells or tissues (Christensen *et al.*, 2001).

In addition to exhibiting *in vitro* antimicrobial activity against sexually transmitted pathogens (Mauck *et al.*, 2001), cellulose sulfate has been found to be also a contraceptive in rabbits, both when a 0.1% formulation is mixed with sperm prior to vaginal administration, or when a 5% formulation is applied vaginally prior to sperm introduction (Anderson *et al.*, 2000b).

Ushercell is a high molecular weight cellulose sulfate envisioned for vaginal use primarily in the prevention of heterosexual transmission of HIV infection and other sexually transmitted diseases (Anderson *et al.*, 2002). HIV-1 inhibition by Ushercell correlates with earlier data obtained with low molecular weight forms of this polymer (Mizumoto *et al.*, 1988; Yamamoto *et al.*, 1990), although Ushercell activity has been reported to be greater (Anderson *et al.*, 2002). The data provide convincing evidence that, *in vitro*, Ushercell is highly effective against HIV. Ushercell has a broad spectrum of antimicrobial activity *in vitro*. Inhibited microbes include HIV (IC_{50} values range from 3 to 78 μg/ml), HSV-1 (IC_{50} = 59 ng/ml) and HSV-2 (IC_{50} = 24 ng/ml), *Neisseria gonorrhoeae* (IC_{50} = 2 μg/ml), and *Chlamydia trachomatis* (IC_{50} = 78 μg/ml). Importantly, Ushercell does not inhibit growth of the beneficial vaginal bacteria, *Lactobacillus gasseri*, at 5 mg/ml. These results suggest that the antimicrobial effects of Ushercell are selective, and not likely mediated by non-specific cytotoxic mechanisms (Anderson *et al.*, 2002).

Currently, a vaginal contraceptive containing 100 mg cellulose sulfate and 230 mg nonoxynol-9 is marketed in Germany under the name A-Gen53. Clinical studies

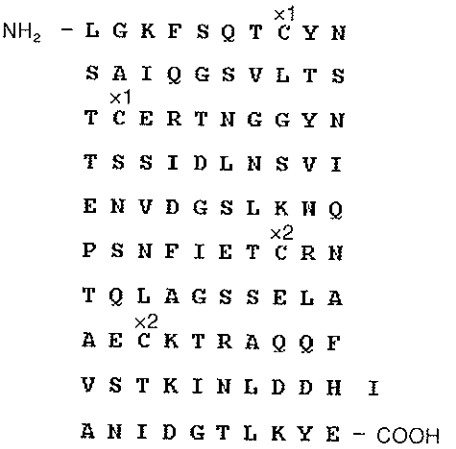

Figure 3.13. Structure of cyanovirin-N.

of this product report a high degree of safety, with a slight burning sensation as the only reported side effect (Martinez Sausor and Royo, 1984).

Cyanovirin-N

Cyanovirin-N is an 11 kDa protein, consisting of a single 101 amino acid chain containing two intrachain disulfide links (important for HIV activity) between its four cysteine residues (*Figure 3.13*) (Gustafson *et al.*, 1997). Isolated from the cyanobacterium (blue-green alga), *Nostoc ellipsosporum* (Boyd *et al.*, 1997), cyanovirin is a highly potent virucidal agent that has generated interest as a lead candidate for the prevention and chemotherapy of HIV infection by preventing viral entry into host cells (Henderson, 1997). Its antiviral activity is mediated through high-affinity interactions with N-linked high-mannose oligosaccharides found on gp120 viral surface envelope glycoproteins (Esser *et al.*, 1999; O'Keefe *et al.*, 2000; Bolmstedt *et al.*, 2001). It is interesting to note that the Ebola virus, which is also susceptible to inhibition by cyanovirin-N, contains similar oligosaccharide constituents on its surface envelope, and thus further implicates these carbohydrate moieties as viral molecular targets (Barrientos *et al.*, 2003).

Dextran sulfate

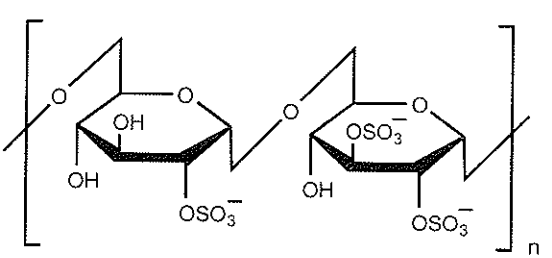

Figure 3.14. Structure of dextran sulfate.

Dextran is an α(1→6)-linked, glucose polymer containing approximately 5% branching through α(1→3)-linkages (Rankin and Jeanes, 1954). The size of the dextran polymer can vary, and hence the sulfated derivatives of dextran that are produced have variable molecular weights. Dextran sulfate is a heparin-like polysaccharide containing approximately 17% sulfur, with up to three sulfur groups per glucose molecule (Araki *et al.*, 2001) (*Figure 3.14*).

Polyanionic sulfated polysaccharides, such as dextran sulfate, contain negatively charged glycoprotein surfaces which affect both viral binding and uncoating. Dextran sulfate potently inhibits the absorption of HIV to $CD4^+$ cells. Synthetic peptides, negatively charged glycoproteins, and polysaccharides, as well as antisera and monoclonal antibodies raised against gp120, bind to the neutralizing domains of gp120, and prevent HIV-1 entry into the target cells (Batinic and Robey, 1992). It has been demonstrated that negatively charged sulfated polysaccharides, such as dextran sulfate, bind to the positively charged side chain groups of amino acids of gp120 to inhibit HIV-1 replication *in vitro* (Baba *et al.*, 1988, 1990). Dextran sulfate has also been shown to block the attachment of HIV particles to T lymphocytes (Baba *et al.*, 1988; Mitsuya *et al.*, 1988; Schols *et al.*, 1989).

The activity of dextran sulfate against various strains of HIV has been shown to be dependent upon the molecular weight of the dextran sulfate (Busso and Resnick, 1990; Witvrouw *et al.*, 1991). Although no marked differences in inhibition of HIV were observed, for dextran sulfates within the weight average molecular range of 3000–500 000, significant differences were observed when the molecular weight was decreased to 1000.

Docosanol

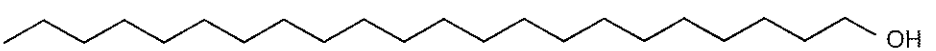

Figure 3.15. Structure of docosanol.

n-Docosanol, $[CH_3(CH_2)_{21}OH]$, is a 22-carbon saturated primary alcohol (*Figure 3.15*) with antiviral activity against a broad spectrum of lipid-enveloped viruses *in vitro*, including HSV-1, HSV-2, CMV, VZV, HHV-6, and HIV-1 (Katz *et al.*, 1991, 1994; Pope *et al.*, 1998; Scolaro *et al.*, 2001). The compound has been shown to lack any of the toxic, mutagenic, and teratogenic properties normally associated with most other antiviral therapeutic drugs currently available, and is therefore under investigation as a possible anti-HIV microbicide (Katz *et al.*, 1991). Studies have demonstrated that *n*-docosanol is not directly virucidal in nature, but that cells pretreated with the drug are rendered resistant to infection by many lipid-enveloped viruses (Katz *et al.*, 1991, 1994; Marcelletti *et al.*, 1996). Similarly, it has been shown that non-enveloped viruses, such as the poliovirus, are not susceptible to the inhibitory action of *n*-docosanol (Katz *et al.*, 1991). These observations suggest that the inhibitory effect of *n*-docosanol is related to the viral entry process of lipid-enveloped viruses into their target cells.

Inhibition studies have demonstrated that *n*-docosanol has the ability to inhibit HIV-1 in cultures of peripheral blood mononuclear cells (PBMCs) in a dose-dependent

manner (Marcelletti *et al.*, 1996). The drug demonstrated a 50% effective concentration (EC50) of ~10 mM, and preincubation of the PBMCs with *n*-docosanol was shown to significantly increase the anti-HIV activity of the drug. In contrast, *n*-docosanol added to the PBMC cultures at the same time as the HIV-1 infection did not demonstrate any significant HIV inhibition. Additional studies of an *n*-docosanol topical cream have clearly demonstrated that prevention of SIV mac251 vaginal transmission in rhesus macaques is possible (Miller *et al.*, 1995). These observations indicate that *n*-docosanol may be useful as a prophylactic treatment to prevent the transmission of HIV in human candidates and, therefore, the drug warrants further investigation as a potential topical therapy, particularly in light of its low toxicity and broad inhibitory effect against most lipid-enveloped viruses.

Human monoclonal antibody b12

There has been much speculation as to the potential efficacy of antibodies in preventing mucosal transmission of HIV. Several recent studies have demonstrated that both intravenous (Parren *et al.*, 2001) and vaginal administration (Veazey *et al.*, 2003) of broadly neutralizing monoclonal antibody b12 (MAb b12) specific for HIV-1 gp120 provides dose-dependent protection to progestogen-treated macaques vaginally challenged with the chimeric simian/human immunodeficiency virus (SHIV-162P4). (SHIV, like HIV, employs the chemokine CCR5 receptor to enter cells, and is therefore a useful model for HIV-1 transmission.) In the later study, initial *in vitro* experiments showed that MAb b12 could inhibit infection of macaque peripheral-blood mononuclear cells, immature human dendritic cells, and human cervical explants. *In vivo*, 3 of 12 rhesus macaques became infected after having received b12 vaginally, either in a gel or saline formulation, compared with 12 out of 13 that received a control human monoclonal antibody. The protective effect was also shown to be dose-dependent.

MAP30

MAP30 (**M**omordica **A**nti-human immunodeficiency virus **P**rotein) is a 30 kDa single-stranded plant protein from the bitter melon, *Momordica charantis*, possessing anti-HIV (Lee-Huang *et al.*, 1990, 1995a; Bourinbaiar and Lee-Huang, 1995; Huang *et al.*, 1999), anti-HSV (Bourinbaiar and Lee-Huang, 1996), and anti-tumour potential (Lee-Huang *et al.*, 1995b; Huang *et al.*, 1999). It seems to inhibit multiple stages of the viral life cycle, on acute infection as well as replication in chronically infected cells. Recombinant MAP30 (re-MAP30) has been shown to inhibit HIV-1 to the same extent as its native counterpart, natural MAP30 (nMAP30) (Lee-Huang *et al.*, 1995b), and exhibits little toxicity to the uninfected viral target cells and other normal human cells. re-MAP30, like nMAP30, is also active in topological inactivation of viral DNA, inhibition of viral DNA integration, and cell-free ribosome inactivation. The cloning and expression of the gene encoding biologically active re-MAP30 provides an abundant source of homogeneous material for clinical investigations, as well as structure-function studies. Early studies have also demonstrated that MAP30 enhances the anti-HIV activity of the weak HIV antagonists, dexamethasone and indomethacin, suggesting that MAP30, in combination with low

pharmacological doses of these drugs, may improve the efficacy of anti-HIV therapy. The non-spermicidal properties of MAP30 have also been reported – the protein has no effect on the motility or vitality of human sperm cells over a dose range of 0.1 to 100 mg/ml, a range that encompasses the HIV-1/HSV inhibitory doses (Schrieber *et al.*, 1999). The structural basis for MAP30's biological activities has been investigated by solution state ^{1}H, ^{13}C and ^{15}N nuclear magnetic resonance spectroscopies, and demonstrates similarity in secondary structure and P-sheet topology with those of the ricin A chain (Wang *et al.*, 1999).

PMPA (tenofovir)

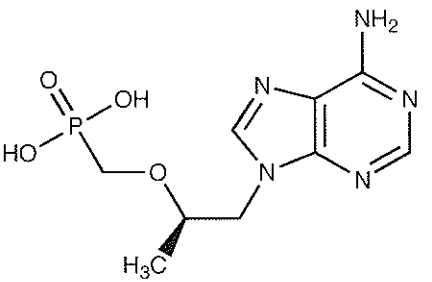

Figure 3.16. Structure of PMPA (tenofovir).

PMPA ((R)-9-(2-**ph**osphono**me**thoxy**p**ropyl)**a**denine) (*Figure 3.16*) is an acyclic adenosine nucleotide analogue reverse transcriptase inhibitor being developed by Gilead Sciences (Foster City, California, USA). It is the parent compound of tenofovir disoproxil fumarate (Viread), the orally bioavailable prodrug FDA approved in 2001 for treatment of HIV infection. Tenofovir is phosphorylated by adenylate kinase to the pharmacologically active metabolite, tenofovir diphosphate, which inhibits viral replication by competing with deoxyadenosine 5'-triphosphate for incorporation into newly synthesized DNA. Upon incorporation, tenofovir diphosphate results in chain termination.

Applied intravaginally, tenofovir may be absorbed by cells in the vaginal epithelium, therefore potentially blocking local viral replication once infection has occurred. In a study of 4 rhesus macaques challenged intravaginally with simian immunodeficiency virus (SIV) and administered intravaginal tenofovir gel (10% w/w) at 24 hours before and 0, 24, 48 hours after challenge, 100% of the treated animals remained free of evidence of systemic SIV infection. In an additional study, 1% w/w tenofovir gel administered 24 hours before, 15 minutes before, and 24 hours after a single intravaginal inoculation of SIV resulted in protection of 4 out of 5 rhesus macaques. Sixty per cent protection was achieved in a third group of macaques which received only a single application of 1% w/w PMPA gel 15 minutes before challenge (Bischofberger *et al.*, 1996; Miller *et al.*, 1996). Post-exposure prophylaxis for 28 days with PMPA, initiated after 12 to 72 hours, has also been demonstrated in macaques after intravaginal exposure to HIV-2 (Otten *et al.*, 2000). Systemic infection was not evident in the 12 and 36 hour groups, while breakthrough infection was detected in the 72 hour group 16 weeks post-exposure. The results clearly demonstrate that early intervention after high risk sexual exposure may prevent infection. A Phase I Safety and Acceptability Study is currently in progress with 0.3% and 1.0% w/w tenofovir gel formulations.

Polystyrene sulfonate

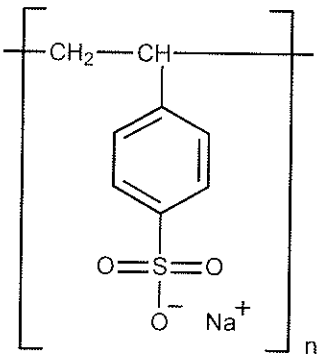

Figure 3.17. Structure of the sodium salt of polystyrene sulfonate.

Several studies have demonstrated the *in vitro* activity of polystyrene sulfonate, or its sodium salt (*Figure 3.17*), against a range of vaginal pathogens including *Chlamydia trachomatis* (Herold *et al.*, 1999; Anderson *et al.*, 2000a), *Gardnerella vaginalis* (associated with bacterial vaginosis) (Herold *et al.*, 1999; Simoes *et al.*, 2002), *Neisseria gonorrhoeae* (Anderson *et al.*, 2000a), bovine papillomavirus (Christensen *et al.*, 2001), and herpes simplex virus (Herold *et al.*, 1999). Importantly, the growth of *Lactobacillus* species normally present in the vagina was not inhibited; lactobacilli break down glycogen in the vaginal epithelial cells to lactic acid, and thus maintain the acidic environment in which HIV and other sexually transmitted disease microorganisms cannot survive. However, in a mouse model, polystyrene sulfonate provided no protection against *Chlamydia trachomatis* innoculation (Achilles *et al.*, 2002).

Praneem

Praneem is an intravaginal cream or pessary formulation containing extracts of neem leaves (*Azadirachta indica*), *Sapindus mukerossi* oil, and mentha citrate oil, and having both spermicidal and antimicrobial activity. Unlike nonoxynol-9 formulations, however, it does not damage the vaginal mucosa. Numerous studies have reported the spermicidal characteristics of Praneem (Garg *et al.*, 1993, 1994; Talwar *et al.*, 1995; Raghuvanshi *et al.*, 2001) – each individual component is inherently spermicidal, but their combination results in a synergistic effect, enhancing spermicidal activity eight- to twelve-fold relative to the single components. Praneem has also been demonstrated to act as a broad spectrum microbicide, killing a wide range of genital pathogens (Talwar *et al.*, 1995; Talwar, 2001). It has been reported to inhibit the growth in culture of clinical isolates of *Candida albicans*, *Candida krusei*, *Candida tropicalis*, various strains of *Escherichia coli* and *Neisseria gonorrhoeae*, and HIV-1. In addition, vaginal application of the cream and pessary formulations in progestin-sensitized mice prevented lesions and vaginal transmission of HSV-2 and *Chlamydia trachomatis* (Talwar *et al.*, 2000).

PRO 2000

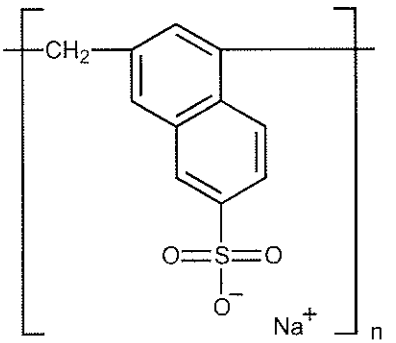

Figure 3.18. Structure of naphthalene sulfonate polymer PRO 2000.

PRO 2000 is a stable, colourless, odourless, water-soluble naphthalene sulfonate polymer synthesized by the condensation reaction between 2-naphthalene sulfonic acid and formaldehyde. The polymerization mechanism produces polymer molecules having a broad range of molecular weights; PRO 2000 is defined as the fraction having molecular weight 4000–5000 Daltons (n = approx. 20, *Figure 3.18*) (Rusconi *et al.*, 1996; Bourne *et al.*, 1999b). The formaldehyde condensation reaction produces a range of methylene substitution patterns on the naphthalene moiety. PRO 2000 has been shown to effectively block binding of recombinant HIV envelope glycoprotein gp120 to recombinant CD4 at nanomolar concentrations (IC_{50} = 400 ng/ml) (Rusconi *et al.*, 1996). Similarly, PRO 2000 has been shown to inhibit infection in a wide range of HIV isolates in a variety of cell types (Rusconi *et al.*, 1996). PRO 2000 has also been evaluated in a cervical explant model, where a concentration of 100 µg/ml (400 times less than that currently proposed for intravaginal use – see following two paragraphs) efficiently blocked HIV infection under conditions that mimic both compromised epithelial integrity and inflammatory conditions (Greenhead *et al.*, 2000).

The ability of PRO 2000 to protect against HSV-2 infection and disease onset in a mouse model has also been demonstrated (Bourne *et al.*, 1999b). PRO 2000 Carbopol 1382 gel formulations (0.5% and 4% w/w) were shown to protect against infection in a concentration-dependent manner (81% and 100% protection, respectively) when administered 20 seconds prior to viral inoculation; a similarly administered placebo gel offered protection in only 18% of mice. Although such studies demonstrate the efficacy of the microbicide, administration of a microbicidal gel 20 seconds before viral challenge is unlikely and impractical in a human context; microbicidal formulations are more likely to be used minutes or hours before intercourse. Thus, the authors have also reported on the effect of time of vaginal adminstration of a 4% w/w PRO 2000 gel on efficacy. The results show that efficacy decreased from 100% to 92% to 58% with gel administered 5, 15 and 30 minutes, respectively, prior to inoculation.

Two clinical studies have evaluated the safety and tolerability of PRO 2000 gel formulations in sexually active HIV-negative, sexually inactive HIV-negative, and

sexually inactive HIV-positive women (van Damme *et al.*, 2000; Mayer *et al.*, 2003). In the earlier Phase I study, where the 4%, 0.5% w/w or placebo gel was administered once daily for 2 weeks, cervicovaginal abrasion was observed colposcopically in 3 out of 73 subjects (two in the 4% and one in the placebo), and genital ulceration was not observed at all, thereby demonstrating the topical acceptability of the formulations. Furthermore, plasma levels of PRO 2000 showed no evidence of systemic absorption. In the latter study, 2% and 4% w/w PRO 2000 vaginal gel formulations applied once or twice daily were evaluated for epithelial disruption. Almost three-quarters of the women had at least one adverse effect, although most of these were classified as mild. Not surprisingly, more adverse effects were reported for users of the 4% twice daily gel. Phase II/III efficacy trials are currently being planned.

SPL7013

SPL7013 is a dendrimer molecule being developed by an Australian company, Starpharma, that has been shown to possess high potency against a range of sexually transmitted disease pathogens, including HIV, HSV, hepatitis B and chlamydia (Bourne *et al.*, 2000). (Dendrimers are novel, spherical-shaped macromolecules having a fractal-type architecture radiating out from a central core.) In recent *in vitro* studies, SPL7013 has prevented cell infection when challenged with various HIV-1 and HIV-2 strains isolated from HIV-positive patients (Witvrouw *et al.*, 2000). In order to assess the ability of the dendrimer to prevent HIV/SIV and chlamydia infection, two clinical studies using a macaque model and a gel formulation of the dendrimer are currently ongoing in the US, while a Phase I human trial is planned for the near future, subject to an Investigational New Drug (IND) application to the US Food and Drug Administration (FDA).

Sulfated polysaccharides

It is well understood that sexual transmission of HIV is mediated to vaginal epithelia via mononuclear cells found in semen and cervical–vaginal secretions. The first critical step in this process is cell–cell adhesion. Therefore, compounds that prevent this adhesion process may be effective when administered vaginally in reducing the probability of infection. The potential of sulfated polysaccharides in this regard was first described in the late 1980s (Ito *et al.*, 1987; Nakashima *et al.*, 1987; Baba *et al.*, 1988), and since then several such compounds (*Table 3.3*) have been investigated for activity against a range of enveloped viruses (PearcePratt and Phillips, 1993, 1996; Lynch *et al.*, 1994; Witvrouw and de Clercq, 1997; Yamada *et al.*, 1997, 2000; Bourne *et al.*, 1999b; Coggins *et al.*, 2000; Schaeffer and Krylov, 2000; Haslin *et al.*, 2001; Maguire *et al.*, 2001; Achilles *et al.*, 2002; Neurath *et al.*, 2002). The negatively charged functionalities associated with the sulfate groups in these polysaccharides are responsible for inhibiting the binding of the virions to the cell by shielding the positively charged sites of amino acids in the V3 loop of the viral envelope glycoprotein (gp120) (Schols *et al.*, 1990; Witvrouw and de Clercq, 1997); cell-to-cell adhesion/fusion is prevented in a similar fashion (Baba *et al.*, 1990). Information relating to specific sulfated polysaccharides can be found elsewhere in this review.

TMC-120

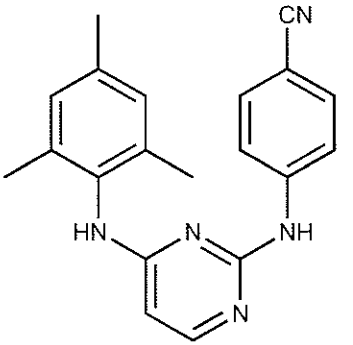

Figure 3.19. Structure of TMC-120.

TMC-120 (a dianilinopyramidine derivative, *Figure 3.19*) is a second-generation, non-nucleoside reverse transcriptase inhibitor (NNRTI), currently being developed by Tibotec-Virco Belgium, with high potency against wild-type HIV-1 (EC_{50} = 0.3 ng/ml, EC_{90} = 0.9 ng/ml, CC_{50} (MT4 cells) = 0.7 mg/ml, selectivity index = 2400). Its antiretroviral activity in HIV-positive patients has been tested in a randomized double blind Phase I/II study (Pauwels, 2001), in which 43 participants received 50 or 100 mg twice daily or placebo as monotherapy for 7 days, followed by triple antiretroviral therapy with a number of new antiretroviral agents. The mean viral load decrease at day 8 was −1.44 log in the 50 mg group and −1.51 log in the 100 mg group. The only adverse events reported were somnolence or insomnia, which occurred in three individuals. Activity against strains with NNRTI resistance mutations K103N, Y181C, and G190A/S is below 10-fold resistance, compared to wild type. When passaged against a laboratory strain, resistance to the TMC compounds emerged either more slowly, or not at all, compared to efavirenz and nevirapine, the two NNRTIs in common use at present. Non-GLP vaginal irritation experiments in rabbits have shown no irritation, and systemic absorption was only detected with very high concentrations (1M concentration). The potential effectiveness of TMC120 as a vaginal microbicide has recently been demonstrated in the humanized severe combined immunodeficient (hu-SCID) mouse model (di Fabio *et al.*, 2003), where intravaginal application of a TMC-120 gel formulation administered 20 minutes prior to non-invasive vaginal challenge with cell-associated HIV prevented systemic infection. Importantly, the work provides the first *in vivo* evidence that a vaginally-administered non-nucleoside reverse transcriptase inhibitor is capable of acting as a HIV microbicide.

UC-781

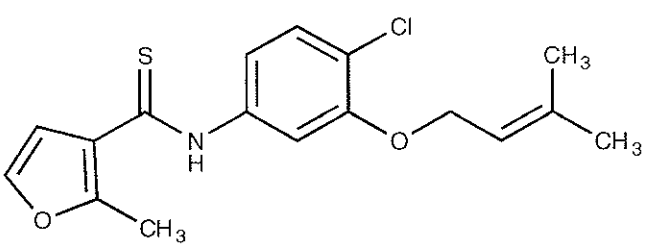

Figure 3.20. Structure of the non-nucleoside reverse transcriptase inhibitor UC-781.

UC-781 (N-[4-chloro-3-(3-methyl-2-butenyloxy)phenyl]-2-methyl-3-furancarbothioamide) (*Figure 3.20*) belongs to a class of thiocarboxanilide derivatives and is a potent and selective non-nucleoside reverse transcriptase inhibitor (NNRTI) of HIV-1 in cell culture (Balzarini and de Clercq, 1996). It is a tight-binding inhibitor of the HIV-1 reverse transcriptase enzyme at low non-toxic concentrations (Buckheit *et al.*, 1997), and is currently the only NNRTI with this unique tight-binding characteristic, making it an ideal anti-HIV microbicidal candidate. The hydrophobic nature of the UC-781 molecule means it is able to readily transverse the HIV-1 membrane envelope and penetrate the capsid core, where it binds rapidly to the RT. Once bound, UC-781 does not readily dissociate from the enzyme, even after excess drug is removed and is effectively trapped within the virion (Borkow *et al.*, 1997; Zussman *et al.*, 2003). This tight-binding property means that UC-781 shows considerable potential as a virucidal agent for the prevention of the sexual transmission of HIV from an infected to a non-infected individual.

Exposure of HIV-1 chronically infected cells to UC-781 has been shown to attenuate the infectivity of nascent virus subsequently produced by the UC-781 treated cells, even after the removal of exogenous drug, therefore preventing any further cell-to-cell transmission by the virus. Similarly, incubation of uninfected lymphocytic cells with UC-781 renders these cells refractory to subsequent HIV-1 infection for several days, even after the removal of residual drug. Only short-term exposure to UC-781 is necessary to rapidly exert this potent, long-term microbicidal activity (Borkow *et al.*, 1997, 2002; Parniak, 2001).

Studies of a 5% UC-781 lipophilic Replens gel administered vaginally in rabbit models showed no signs of irritation, inflammation, or toxicity to vaginal mucosa or epithelial tissue (Balzarini *et al.*, 1998). This is of particular importance, as irritation and damage to the epithelial tissue and mucosal membranes have been shown to facilitate the sexual transmission of the virus. In addition, the drug was found to lack any significant inhibitory activity against normal vaginal flora (Candida and Lactobacillus strains) and other closely related RNA and DNA viruses, such as HIV-2 or simian immunodeficiency virus. These observations are not surprising, due to the high pathogen specificity and selectivity of UC-781 for HIV-1 reverse transcriptase (Balzarini *et al.*, 1998). In the same study, UC-781 has also been shown to be both temperature and pH stable over prolonged periods of time. UC-781 incorporated into a Replens gel formulation was stable up to 50°C over a one month time period. At pH 2.3, a pH value well below that of the normal human vagina (pH 3.5), UC-781 demonstrated no loss of antiviral activity after a 4-hr exposure period (Balzarini *et al.*, 1998).

Conclusions

There is much optimism in the microbicide field that an effective, first-generation vaginal microbicide will be available within the next five years to reduce/prevent the heterosexual transmission of HIV. The optimism appears to be well founded. Not only have great strides been made in understanding the mechanisms of sexual transmission of the virus, but a wealth of new, potent microbicidal candidates are also being rationally developed and evaluated in infectivity models that closely mimic the situation in humans. The importance of developing an effective microbicide product

soon has also been brought into sharp focus in the light of recent disappointing Phase III clinical results relating to Vaxgen's gp120 vaccine. However, many challenges remain. Most of the current research and evaluation is being carried out by small biotech companies, not-for-profit research entities, university research groups, and other public sector organizations. Large pharma are reluctant to get involved, at least in these early stages of development, owing to concerns regarding the potential market for microbicides (The Economics of Microbicide Development), regulatory uncertainty, and concerns over liability and safety. Also, the implementation of ethical clinical trials, where condom use is promoted alongside microbicide use, necessitates very large numbers of volunteers to provide statistically relevant data, which translates into significantly higher costs. The challenge of ensuring availability of a clinically effective microbicide product to the women who need it is also a very real one. While many questions remain to be answered, it is clear that vaginal microbicides offer the most optimistic short-term answer to the AIDS pandemic. A clear, focused, and multi-disciplinary strategy is imperative to ensure that they are made available to the women who need them as soon as possible.

References

ACHILLES, S.L., SHETE, P.B., WHALEY, K.J., MOENCH, T.R. AND CONE, R.A. (2002). Microbicide efficacy and toxicity tests in a mouse model for vaginal transmission of *Chlamydia trachomatis*. *Sexually Transmitted Diseases* **29**, 655–664.

AIDS EPIDEMIC UPDATE (2002). UNAIDS/WHO, December 2002. http://www.who.int/hiv/pub/epidemiology/epi2002/en/.

ALKHATIB, G., COMBADIERE, C., BRODER, C. *ET AL.* (1996). CC CKR5: a RANTES, MIP1-alpha, MIP1-beta receptor as a fusion co-factor for macrophage-tropic HIV-1. *Science* **272**, 1955–1958.

AMARAL, E., FAUNDES, A., ZANEVELD, L., WALLER, D. AND GARG, S. (1999). Study of the vaginal tolerance to Acidform, an acid-buffering, bioadhesive gel. *Contraception* **60**, 361–366.

ANDERSON, R.A., FEATHERGILL, K., DIAO, X.H. *ET AL.* (2000a). Evaluation of (polystyrene-4-sulfonate) as a preventative agent for conception and sexually transmitted diseases. *Journal of Andrology* **21**, 862–875.

ANDERSON, R.A., ZANEVELD, L.J. AND USHER, T.C. (2000b). Inventors, Polydex Pharmaceuticals Ltd., Rush-Presbyterian-St. Luke's Medical Centre, assignees. Cellulose sulfate for use as antimicrobial and contraceptive agent. *US Patent* 6,063,773.

ANDERSON, R.A., FEATHERGILL, K.A., DIAO, X. *ET AL.* (2002). Preclinical evaluation of sodium cellulose sulfate (Ushercell) as a contraceptive antimicrobial agent. *Journal of Andrology* **23**, 426–438.

ARAKI, Y., ANDOH, A., FUJIYAMA, Y. *ET AL.* (2001). Application of 2-aminopyridine fluorescence labelling in the analysis of *in vivo* and *in vitro* metabolism of dextran sulfate sodium by size-exclusion high performance liquid chromatography. *Journal of Chromatography B* **753**, 209–215.

BABA, M., SNOECK, P., PAUWELS, R. AND DE CLERCQ, E. (1988). Sulfated polysaccharides are potent and selective inhibitors of various enveloped viruses, including herpes simplex virus, cytomegalovirus, vesicular stomatitis virus and human immunodeficiency virus. *Antimicrobial Agents and Chemotherapy* **32**, 1742–1745.

BABA, M., SCHOLS, D., PAUWELS, R., NAKASHIMA, H. AND DE CLERCQ, E. (1990). Sulfated polysaccharides as potent inhibitors of HIV-induced syncytium formation: a new strategy towards AIDS chemotherapy. *Journal of Acquired Immune Deficiency Syndromes* **3**, 493–499.

BALLAGH, S.A., BAKER, J.M., HENRY, D.M. AND ARCHER, D.F. (2002). Safety of a single daily use for one week of C31G HEC gel in women. *Contraception* **66**, 369–375.

BALZARINI, J. AND DE CLERCQ, E. (1996). The thiocarboxanilides UC-10 and UC-781 have an additive inhibitory effect against human immunodeficiency virus type 1 reverse transcriptase and replication in cell culture combined with other antiretroviral drugs. *Antiviral Chemistry and Chemotherapy* **8**, 197–204.

BALZARINI, J., NAESENS, L., VERBEKEN, E. *ET AL.* (1998). Preclinical studies on thiocarboxanilide UC-781 as a virucidal agent. *Aids* **12**, 1129–1138.

BARRÉ-SINOUSSI, F., CHERMANN, J.C., REY, F. *ET AL.* (1983). Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* **220**, 868–871.

BARRIENTOS, L.G., O'KEEFE, B.R., BRAY, M., ANTHONY, S., GRONENBORN, A.M. AND BOYD, M.R. (2003). Cyanovirin-N binds to the viral surface glycoprotein, GP(1,2) and inhibits infectivity of Ebola virus. *Antiviral Research* **58**, 47–56.

BATINIC, D. AND ROBEY, F.A. (1992). The V3 region of the envelope glycoprotein of human immunodeficiency virus type 1 binds sulfated polysaccharides and CD4-derived synthetic peptides. *Journal of Biological Chemistry* **267**, 6664–6671.

BAX, R., DOUVILLE, K., MCCORMICK, D., ROSENBERG, M., HIGGINS, J. AND BOWDEN, M. (2002). Microbicides – evaluating multiple formulations of C31G. *Contraception* **66**, 365–368.

BIRNIE, C.R., MALAMUD, D. AND SCHNAARE, R.L. (2000). Antimicrobial evaluation of N-alkyl betaines and N-alkyl-N,N-dimethylamine oxides from semisolids. *Antimicrobial Agents and Chemotherapy* **44**, 2514–2517.

BIRNIE, C.R., MALAMUD, D., THOMULKA, K.W., SCHWARTZ, J.B. AND SCHNAARE, R.L. (2001). Antimicrobial and diffusional correlation of N-alkyl betaines and N-alkyl-N,N-dimethylamine oxides with variations in chain lengths. *Journal of Pharmaceutical Sciences* **90**, 1386–1394.

BISCHOFBERGER N., TSAI, C.C. AND FOLLIS, K.E. (1996). Antiviral efficacy of PMPA in macaques chronically infected with SIV. Ninth International Conference on Antiviral Research, Fukushima, May 19–24.

BOLMSTEDT, A.J., O'KEEFE, B.R., SHENOY, S.R., MCMAHON, J.B. AND BOYD, M.R. (2001). HIV-inhibitory natural products part 71 – Cyanovirin-N defines a new class of antiviral agent targeting N-linked, high mannose glycans in an oligosaccharide-specific manner. *Molecular Pharmacology* **59**, 949–954.

BORKOW, G., BARNARD, J., NGUYEN, TM., BELMONT, A., WAINBERG, M.A. AND PARNIAK, M.A. (1997). Chemical barriers to human immunodeficiency virus type 1 (HIV-1) infection: retrovirucidal activity of UC-781, a thiocarboxanilide non-nucleoside inhibitor of HIV-1 reverse transcriptase. *Journal of Virology* **71**, 3023–3030.

BORKOW, G., SALOMON, H., WAINBERG, M.A. AND PARNIAK, M.A. (2002). Attenuated infectivity of HIV type 1 from epithelial cells pre-treated with a tight-binding non-nucleoside reverse transcriptase inhibitor. *AIDS Research and Human Retroviruses* **18**, 711–714.

BOURINBAIAR, A.S. AND LEE-HUANG, S. (1995). Potentiation of anti-HIV activity of anti-inflammatory drugs, dexamethasone and indomethacin, by MAP30, the antiviral agent from bitter melon. *Biochemical and Biophysical Research Communications* **208**, 779–785.

BOURINBAIAR, A.S. AND LEE-HUANG, S. (1996). The activity of plant-derived antiretroviral proteins MAP30 and GAP31 against herpes simplex virus infection *in vivo*. *Biochemical and Biophysical Research Communications* **219**, 923–929.

BOURNE, K.Z., BOURNE, N., REISING, S.F. AND STANBERRY, L.R. (1999a). Plant products as topical microbicide candidates: assessment of *in vitro* activity against herpes simplex virus type 2. *Antiviral Research* **42**, 219–226.

BOURNE, N., BERNSTEIN, D.I., SONDERFAN, A.J., PROFY, A.T. AND STANBERRY, L.R. (1999b). The topical microbicide PRO 2000 protects against genital herpes infection in a mouse model. *Journal of Infectious Diseases* **180**, 203–205.

BOURNE, N., STANBERRY, L.R., KERN, E.R., HOLAN, G., MATHEWS, B. AND BERNSTEIN, D.I. (2000). Dendrimers, a new class of candidate topical microbicides with activity against herpes simplex virus infection. *Antimicrobial Agents and Chemotherapy* **44**, 2471–2474.

BOYD, M.R., GUSTAFSON, K.R., MCMAHON, J.B. *ET AL.* (1997). Discovery of cyanovirin-N, a novel human immunodefiency virus-inactivating protein that binds viral surface envelope

glycoprotein gp120; potential applications to microbicide development. *Antimicrobial Agents and Chemotherapy* **41**, 1521–1530.

BUCKHEIT, R.W., HOLLINGSHEAD, M., SINSON, S. *ET AL.* (1997). Efficacy, pharmacokinetics, and *in vivo* antiviral activity of UC-781, a highly potent, orally bioavailable non-nucleoside reverse transcriptase inhibitor of HIV type 1. *Aids Research and Human Retroviruses* **13**, 789–796.

BURGOS, M.H. AND DE VARGAS-LINARES, R. (1978). Ultrastructure of the vaginal mucosa. In: *The human vagina*. Eds. E.S.E. Hafez and T.N. Evans, pp 63–93. Amsterdam: Elsevier/ North-Holland Biomedical Press.

BUSSO, M. AND RESNICK, L. (1990). Anti-human immunodeficiency synergistic or antagonistic when dextran sulfate is given in combination with dideoxynucleosides. *Antimicrobial Agents and Chemotherapy* **34**, 1991–1995.

CALIS, S., YULUG, N., SUMNU, M., AYBAN, A. AND HINCAL, A.A. (1992). A non-antibiotic antimicrobial mixture (C31G): evaluation of the antimicrobial efficiency of C31G on vaginal cultures. *Bollettino Chimico Farmaceutico* **131**, 335–338.

CHOE, H., FARZAN, M., SUN, Y. *ET AL.* (1996). The β-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* **85**, 1135–1148.

CHRISTENSEN, N.D., REED, C.A., CULP, T.D. *ET AL.* (2001). Papillomavirus microbicidal activities of high-molecular-weight cellulose sulfate, dextran sulfate, and polystyrene sulfonate. *Antimicrobial Agents and Chemotherapy* **45**, 3427–3432.

CLARKE, J.G., PEIPERT, J.F., HILLIER, S.L. *ET AL.* (2002). Microflora changes with the use of a vaginal microbicide. *Sexually Transmitted Diseases* **29**, 288–293.

COGGINS, C., BLANCHARD, K., ALVAREZ, F. *ET AL.* (2000). Preliminary safety and acceptability of a carrageenan gel for possible use as a vaginal microbicide. *Sexually Transmitted Diseases* **76**, 480–483.

CORNER, A.M., DOLAN, M.M., YANKELL, S.L. AND MALAMUD, D. (1988). C31G, a new agent for oral use with potent antimicrobial and antiadherence properties. *Antimicrobial Agents and Chemotherapy* **32**, 350–353.

CROUGHAN, W.S. AND BEHBEHANI, A.M. (1988). Comparative study of inactivation of herpes simplex virus types 1 and 2 by commonly used antiseptic agents. *Journal of Clinical Microbiology* **26**, 213–215.

DENG, H., LIU, R., ELLMEIER, W., CHOE, S., UNUTMAZ, D. AND BURKHART, M. (1996). Identification of a major co-receptor for primary isolates of HIV-1. *Nature* **381**, 661–666.

DESHPANDE, A.A., RHODES, C.T. AND DANISH, M. (1992). Intravaginal drug delivery. *Drug Devices and Industrial Pharmacy* **18**, 1225–1279.

DI FABIO, S., VAN ROOEY, J., GIANNINI, G. *ET AL.* (2003). Inhibition of vaginal transmission of HIV-1 in hu-SCID mice by the non-nucleoside reverse transcriptase inhibitor TMC120 in a gel formulation. *AIDS* **25**, 1597–1604.

DORANZ, B.J., RUCKER, J., YI, Y. *ET AL.* (1996). A dual tropic primary HIV-1 isolate that uses fusion and the b-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion co-factors. *Cell* **85**, 1149–1158.

DRAJIC, T., LITWIN, V., ALLAWAY, G.P. *ET AL.* (1996). HIV-1 entry into $CD4^+$ cells is mediated by the chemokine receptor CC-CKR-5. *Nature* **381**, 667–673.

DUSITSIN, N., GREGOIRE, A.T., JOHNSON, W.D. AND RAKOFF, A.E. (1967). Histidine in human vaginal fluid. *Obstetrics and Gynecology* **29**, 125–129.

ELIAS, C.J., COGGINS, C., ALVAREZ, F. *ET AL.* (1997). Colposcopic evaluation of a vaginal gel formulation of iota-carrageenan. *Contraception* **56**, 387–389.

ESSER, M.T., MORI, T., SATTENTAU, Q.J. *ET AL.* (1999). Cyanovirin-N binds to gp120 to interfere with CD-4-dependent human immunodeficiency virus type 1 virion binding, fusion, and infectivity but does not affect the CD4 binding site on gp120 or soluble CD4-induced conformational changes in gp120. *Journal of Virology* **73**, 4360–4371.

EUROPEAN STUDY GROUP ON HETEROSEXUAL TRANSMISSION OF HIV (1992). Comparison of female-to-male and male-to-female transmission of HIV in 563 stable couples. *British Medical Journal* **304**, 809–813.

FENG, Y., BRODER, C.C., KENNEDY, P.E. AND BERGER, E.A. (1996). HIV-1 entry co-factor: functional cDNA cloning of seven trans-membrane domain, G-protein coupled receptor. *Science* **272**, 872–877.

FICHOROVA, R.N., TUCKER, L.D. AND ANDERSON, D.J. (2001). The molecular basis of nonoxynol-9-induced vaginal inflammation and its possible relevance to human immunodeficiency virus type 1 transmission. *Journal of Infectious Diseases* **184,** 418–428.

GALLO, R.C., SALAHUDDIN, S.Z., POPOVIC, M., SHEARER, G.M., KAPLAN, M. AND HAYNES, B.F. (1984). Frequent detection and isolation of cytopathic retroviruses (HLTV-III) from patients with AIDS and at risk from AIDS. *Science* **224**, 500–502.

GARG, S., TALUJA, V., UPADHYAY, S.N. AND TALWAR, G.P. (1993). Studies on the contraceptive efficacy of Praneem polyherbal cream. *Contraception* **48**, 591–596.

GARG, S., DONCEL, G., CHABRA, S., UPADHYAY, S.N. AND TALWAR, G.P. (1994). Synergistic spermicidal activity of neem seed extract, *reetha saponis* and quinine hydrochloride. *Contraception* **50**, 185–190.

GARG, S., ANDERSON, R.A., CHANY, C.J. *ET AL.* (2001). Properties of a new acid-buffering bioadhesive vaginal formulation (ACIDFORM). *Contraception* **64**, 67–75.

GODLEY, M.J. (1985). Quantification of vaginal discharge in healthy volunteers. *British Journal of Obstetrics and Gynaecology* **92**, 739–742.

GOHDES, M., MISCHNICK, P. AND WAGRNKNECHT, W. (1997). Methylation analysis of cellular sulfates. *Carbohydrate Polymers* **33**, 163–168.

GRAVES, S., GOTV, T. AND TREWARTHA, F. (1980). Effect of pH on the motility and virulence of *Treponema pallidum* (Nichols) and *Treponema paraluis cuniculi in vitro* under anaerobic conditions. *British Journal of Venereal Diseases* **56**, 269–270.

GREENHEAD, P., HAYES, P., WATTS, P.S., LAING, K.G., GRIFFIN, G.E. AND SHATTOCK, R.J. (2000). Parameters of human immunodeficiency virus infection of human cervical tissue and inhibition by vaginal microbicides. *Journal of Virology* **74**, 5577–5586.

GUPTA, P., COLLINS, K.B., RATNER, D. *ET AL.* (2002). Memory $CD4^+$ cells are the earliest detectable human immunodeficiency virus type 1 (HIV-1)-infected cells in the female genital mucosal tissue during HIV-1 transmission in an organ culture system. *Journal of Virology* **76**, 9868–9876.

GUSTAFSON, K.R., SOWDER, R.C., HENDERSON, L.E. *ET AL.* (1997). Isolation, primary sequence determination, and disulfide bond structure of cyanovirin-N, an anti-HIV (human immunodeficiency virus) protein from the cyanobacterium *Nostoc ellipsosporum*. *Biochemical and Biophysical Research Communications* **238**, 223–228.

HASLIN, C., LAHAYE, M., PELLEGRINI, M. AND CHERMANN, J.C. (2001). *In vitro* anti-HIV activity of sulfated cell-wall polysaccharides from gametic, carposporic and tetrasporic stages of the Mediterranean red alga *Asparagopsis armata*. *Planta Medica* **67**, 301–305.

HENDERSON, L.E. (1997). Discovery of cyanovirin-N, a novel human immunodeficiency virus-inactivating protein that binds viral surface envelope glycoprotein gp120: potential applications to microbicide development. *Antimicrobial Agents and Chemotherapy* **41**, 1521–1530.

HEROLD, B.C., BOURNE, N., MARCELLINO, D. *ET AL.* (1999). Polystyrene sulfonate is a safe and effective candidate topical antimicrobial for the prevention of sexually transmitted diseases. *Pediatric Research* **45**, 951–954.

HICKS, D., MARTIN, L. AND GETCHELL, J. (1985). Inactivation of HTLV-III/LAV-infected cultures of normal human lymphocytes by nonoxynol-9 *in vitro*. *Lancet* **2**, 1422–1423.

HIV VACCINES. The UNAIDS Division of AIDS, http://www.niaid.nih.gov/daids/vaccine.

HOWELL, A.L., EDKINS, R.D., RIER, S.E. *ET AL.* (1997). Human immunodeficiency virus type 1 infection of cells and tissues from the upper and lower human female reproductive tract. *Journal of Virology* **71**, 3498–3506.

HOWETT, M.K., NEELY, E.B., CHRISTENSEN, N.D. *ET AL.* (1999). A broad-spectrum microbicide with virucidal activity against sexually transmitted viruses. *Antimicrobial Agents and Chemotherapy* **43**, 314–321.

HPTN 0350: Phase II/IIb Safety and Effectiveness Study of the Vaginal Microbicides BufferGel and 0.5% PRO2000/5 Gel (P) for the Prevention of HIV Infection in Women. http://www.hptn.org/research_studies/study_details.asp?Protocol+%23=HPTN+035.

HU, J., GARDNER, M.D. AND MILLER, C.J. (2000). Simian immunodeficiency virus rapidly penetrates the cervicovaginal mucosa after intravaginal inoculation and infects intraepithelial dendritic cells. *Journal of Virology* **74**, 6087–6095.

HUANG, P.L., SUN, Y.T., CHEN, H.C., KUNG, H.F., HUANG, P.L. AND LEE-HUANG, S. (1999). Proteolytic fragments of anti-HIV and anti-tumour proteins MAP30 and GAP31 are biologically active. *Biochemical and Biophysical Research Communications* **262**, 615–623.

ITO, M., BABA, M., SATO, A., PAUWELS, R., DE CLERCQ, E. AND SHIGETA, S. (1987). Inhibitory effect of dextran sulfate and heparin on the replication of human immunodeficiency virus (HIV) *in vitro*. *Antiviral Research* **7**, 361–367.

JUDSON, F.N., EHRET, J.M., BODIN, G., LEVIN, M. AND RIETMEIJER, C. (1989). *In vitro* evaluation of condoms with and without nonoxynol-9 as physical and chemical barriers against *Chlamydia trachomatis*, herpes simplex virus type 2, and human immunodeficiency virus. *Sexually Transmitted Diseases* **16**, 51–56.

KATZ, D.H., MARCELLETTI, J.F., KHALIL, M.H., POPE, L.E. AND KATZ, L.R. (1991). Antiviral activity of 1-docosanol, an inhibitor of lipid-enveloped viruses including herpes simplex. *Proceedings of the National Academy of Sciences of the United States of America* **88**, 10825–10828.

KATZ, D.H., MARCELLETTI, J.F., POPE, L.E., KAHLIL, M.H., KATZ, L.R. AND MCFADDEN, R.R. (1994). Broad spectrum anti-viral activity against lipid-enveloped viruses. *Annals of the New York Academy of Sciences* **724**, 472–488.

KING, J.T., JUSTICE, A.C., ROBERTS, M.S. AND CHANG, C.C.H. (2003). Long-term HIV/AIDS survival estimation in the highly active antiretroviral therapy era. *Medical Decision Making* **23**, 9–20.

KREBS, F.C., MILLER, S.R., MALAMUD, D., HOWETT, M.K. AND WIGDAHL, B. (1999). Inactiviation of human immunodeficiency virus type 1 by nonoxynol-9, C31G, or an alkyl sulfate, sodium dodecyl sulfate. *Antiviral Research* **43**, 147–163.

KREBS, F.C., MILLER, S.R., CATALONE, B.J. *ET AL.* (2000). Sodium dodecyl sulfate and C31G as antimicrobial alternatives to nonoxynol-9: comparative sensitivity of primary human vaginal keratinocytes. *Antimicrobial Agents and Chemotherapy* **44**, 1954–1960.

KREBS, F.C., MILLER, S.R., CATALONE, B.J. *ET AL.* (2002). Comparative *in vitro* sensitivities of human immune cell lines, vaginal and cervical epithelial cell lines, and primary cells to candidate microbicides nonoxynol-9, C31G, and sodium dodecyl sulfate. *Antimicrobial Agents and Chemotherapy* **46**, 2292–2298.

KREISS, J., NGUGI, E., HOLMES, K.K., NDINYA-ACHOLA, J., WARYAKI, P. AND ROBERTS, P.L., (1992). Efficacy of nonoxynol-9 contraceptive sponge use in preventing heterosexual acquisition of HIV in Nairobi prostitutes. *Journal of American Medical Association* **268**, 477–482.

LAGA, M., MANOKA, A., KIVUVU, M., MALELE, B., TULIZA, M. AND NZILA, N. (1993). Non-ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women: results from a cohort study. *AIDS* **7**, 95–102.

LAMPTEY, P.S. (2002). Reducing heterosexual transmission of HIV in poor countries. *British Medical Journal* **324**, 207–211.

LAPAN, B. AND FRIEDMAN, M.M. (1950). Glycogen and reducing substances in vaginal mucus: gestational and cyclical variations. *American Journal of Obstetrics and Gynecology* **59**, 361–376.

LEE, J.C. (1994). Cellulose acetate phthalate. In: *Handbook of pharmaceutical excipients*. Eds. A. Wade and P.J. Weller, pp 91–93. Washington, DC, USA: American Pharmaceutical Association.

LEE-HUANG, S., HUANG, P.L., NARA, P.L. *ET AL.* (1990). MAP 30: a new inhibitor of HIV-1 infection and replication. *FEBS Letters* **272**, 8–12.

LEE-HUANG, S., HUANG, P.L., HUANG, P.L., BORRINBAIAR, A.S., CHEN, H.C. AND KUNG, H.F. (1995a). Inhibition of the integrase of human-immunodeficiency-virus (HIV) type 1 by anti-HIV plant proteins MAP30 and GAP31. *Proceedings of the National Academy of Sciences of the United States of America* **92**, 8818–8822.

LEE-HUANG, S., HUANG, P.L., CHEN, H.C. *ET AL.* (1995b). Anti-HIV and anti-tumour activities of recombinant MAP30 from bitter melon. *Gene* **161**, 151–156.

LEVY, J.A., HOFFMAN, A.D., KRAMER, S.M., LANDIS, J.A., SHIMABUKURO, J.M. AND OSHIRO, L.S. (1984). Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. *Science* **225**, 840–842.

LISSIMORE, N. AND CURRIE, D.W. (1939). Studies in vaginal fluid. Paper I. Pathological considerations. *Journal of Obstetrics and Gynaecology* **46**, 673–684.

LYNCH, G., LOW, L., LI, S. *ET AL.* (1994). Sulfated polyanions prevent HIV infection of lymphocytes by disruption of the CD4-gp120 interaction, but do not inhibit monocyte infection. *Journal of Leukocyte Biology* **56**, 266–272.

MAGUIRE, R.A., BERGMAN, N. AND PHILLIPS, D.M. (2001). Comparison of microbicides for efficacy in protecting mice against vaginal challenge with herpes simplex virus type 2, cytotoxicity, antibacterial properties, and sperm immobilization. *Sexually Transmitted Diseases* **28**, 259–265.

MALAMUD, D., HOWETT, M.K., WIGDAHL, B. *ET AL.* (1998). Anti-viral activity of microbicides in model systems. In: *Proceedings of the 12th World AIDS Conference, Epidemiology and Prevention*, pp 223–227. Switzerland: Monduzzi Editore International Proceedings Division.

MALCOLM, K. AND WOOLFSON, D. (2001). Blocking the heterosexual transmission of HIV: intravaginal rings for the controlled delivery of topical microbicides. *Drug Delivery Systems and Sciences* **1**, 117–121.

MALCOLM, K., WOOLFSON, D., RUSSELL, J., TALLON, P., MCAULEY, L. AND CRAIG, D. (2003a). Influence of silicone elastomer solubility and diffusivity on the *in vitro* release of drugs from intravaginal rings. *Journal of Controlled Release* **90**, 217–225.

MALCOLM, K., WOOLFSON, D., RUSSELL, J. AND ANDREWS, C. (2003b). *In vitro* release of nonoxynol-9 from silicone matrix intravaginal rings. *Journal of Controlled Release* **91**, 355–364.

MALCOLM, R.K. (2003). The intravaginal ring. In: *Modified-release drug delivery technology*. Eds. M.J. Rathbone, J. Hadgraft and M.S. Roberts, pp 759–774. Marcel Dekker.

MANSON, K.H., WYAND, M.S., MILLER, C. AND NEURATH, A.R. (2000). Effect of a cellulose acetate phthalate topical cream on vaginal transmission of simian immunodeficiency virus in rhesus monkeys. *Antimicrobial Agents and Chemotherapy* **44**, 3199–3202.

MARCELLETTI, J.F., LUSSO, P. AND KATZ, D.H. (1996). *n*-Docosanol inhibits *in vitro* replication of HIV and other retroviruses. *AIDS Research and Human Retroviruses* **12**, 71–74.

MARTIN, H.L., JR., NYANGE, P.M., RICHARDSON, B.A., LAVREYS, L., MANDALIYA, K. AND JACKSON, D.J. (1998). Hormonal contraception, sexually transmitted diseases, and risk of heterosexual transmission of human immunodeficiency virus type 1. *Journal of Infectious Diseases* **178**, 1053–1059.

MARTIN, L.S., MCDOUGAL, J.S. AND LOSKOSKI, S.L. (1985). Disinfection and inactivation of the human T lymphotropic virus type III /lymphadenopathy-associated virus. *Journal of Infectious Diseases* **152**, 400–403.

MARTINEZ SAUSOR, V. AND ROYO, A.P. (1984). Estdio de la eficacia y aceptabilidad de un espermicida espermostatico/vaginal en ovulos en portaduras de un dispositivo intrauterine. *El Medico* **91**, 82. [In Spanish.]

MASTERS, W.H. AND JOHNSON, V.E. (1966). *Human sexual response*. Boston, USA: Little, Brown and Company.

MASTRO, T., SATTEN, G. AND NAOPKESORN, T. (1994). Probability of female-to-male transmission of HIV-1 in Thailand. *Lancet* **343**, 204–207.

MAUCK, C., FREZIERES, R., ROBERGEAU, K. AND CALLAHAN, M. (2001). Cellulose sulfate: tolerance and acceptability of penile application. *Contraception* **64**, 377–381.

MAYER, K., PEIPERT, J.F., FLEMING, T. *ET AL.* (2001). Safety and tolerability of BufferGel, a novel vaginal microbicide, in women in the United States. *Clinical Infectious Diseases* **32**, 476–482.

MAYER, K.H., KARIM, S.A., KELLY, C. *ET AL.* (2003). Safety and tolerability of vaginal PRO 2000 gel in sexually active HIV-uninfected and abstinent HIV-infected women. *AIDS* **17**, 321–329.

MERMIN, J.H., HOLODINY, M., KATZENSTIEN, D.A. AND MERIGAN, T.C. (1991). Detection of human immunodeficiency virus DNA and RNA in semen by the polymerase chain reaction. *Journal of Infectious Diseases* **164**, 769–772.

MILLER, C.J. (1992). Use of the SIV/rhesus macaque model of the heterosexual transmission of HIV in vaccine research. *Vaccine Research* **1**, 295–301.

MILLER, C.J. AND SHATTOCK, R.J. (2003). Target cells in vaginal HIV transmission. *Microbes and Infection* **5**, 59–67.

MILLER, C., VOGEL, P., ALEXANDER, N., SUTJIPTO, S., HENDICKX, A. AND MARX, P. (1992). Localization of SIV in the genital tract of chronically infected rhesus macaques. *American Journal of Pathology* **141**, 655–660.

MILLER, C.J., GERSTEN, M.J., DAVIS, R.C. AND KATZ, D.H. (1995). *n*-Docosanol prevents vaginal transmission of SIVmac251 in rhesus macaques. *Antiviral Research* **26**, 277 (abstract).

MILLER, C., ROSENBERG, Z. AND BISCHOFBERGER, N. (1996). Use of topical PMPA to prevent vaginal transmission of SIV. Ninth International Conference on Antiviral Research, Fukushima, May 19–24.

MITSUYA, H., LOONEY, D.J., KUNO, S., UENO, R., WONG-STAAL, F. AND BRODER, S. (1988). Dextran sulfate suppression of viruses in the HIV family: inhibition of virion binding to CD4+ cells. *Science* **240**, 646–649.

MIZUMOTO, K., SUGAWARA, I., ITO, W., KODAMA, W., HAYAMI, M. AND MORI, S. (1988). Sulfated homopolysaccharides with immunodulating activities are more potent anti-HTLV-III agents than sulfated heteropolysaccharides. *Japanese Journal of Experimental Medicine* **58**, 145–151.

MOBILIZATION FOR MICROBICIDES: THE DECISIVE DECADE. The Microbicide Initiative, The Rockfeller Foundation. http://www.rockfound.org/Documents/488/rep1summary.pdf.

MOENCH, T.M., CHIPATO, T. AND PADIAN, N.S. (2001). Preventing disease by protecting the cervix: the unexplored promise of internal vaginal barrier devices. *AIDS* **15**, 1595–1602.

MOSCICKI, A.B., YONG, M.A., HOLLAND, C.A. AND VERMUND, S.H. (2001). Cervical ectopy in adolescent girls with and without human immunodeficiency virus infection. *Journal of Infectious Diseases* **183**, 865–870.

MOSTAD, S.B., OVERBAUGH, J., DEVANGE, D.M., WELCH, M.J., CHOHAN, B. AND MANDALIYA, K. (1997). Hormonal contraception, vitamin A deficiency, and other risk factors for shedding HIV-1 infected cells from the cervix and vagina. *Lancet* **350**, 922–927.

NAGY, E., PETTERSON, M. AND MARDH, P.-A. (1991). Antibiosis between bacteria isolated from the vagina of women with and without signs of bacterial vaginosis. *Acta Pathology Microbiology and Immunology Scandinavia* **99**, 739–744.

NAKASHIMA, H., KIDO, Y., KOBAYASHI, N., MOTOKI, Y., NEUSHUL, M. AND YAMAMOTO, N. (1987). Purification and characterization of an avian myeloblastosis and human immunodeficiency virus reverse transcriptase inhibitor, sulfated polysaccharides extracted from sea algae. *Antimicrobial Agents and Chemotherapy* **31**, 1524–1528.

NEURATH, A.R., JIANG, S., STRICK, N., LIN, K., LI, Y.Y. AND DEBNATH, A.K. (1996). Bovine beta-lactoglobulin modified by 3-hydroxyphthalic anhydride blocks the CD4 cell receptor for HIV. *Nature Medicine* **2**, 230–234.

NEURATH, A.R., STRICK, N., LI, Y.Y., LIN, K. AND JIANG, S. (1999). Design of a 'microbicide' for prevention of sexually transmitted diseases using 'inactive' pharmaceutical excipients. *Biologicals* **27**, 11–21.

NEURATH, A.R., STRICK, N., LI, Y.Y. AND DEBNATH, A.K. (2001). Cellulose acetate phthalate, a common pharmaceutical excipient, inactivates HIV-1 and blocks the co-receptor binding site on the virus envelope glycoprotein gp120. *BMC Infectious Diseases* **17**, 17.

NEURATH, A.R., STRICK, N. AND LI, Y.Y. (2002). Anti-HIV-1 activity of anionic polymers: a comparative study of candidate microbicides. *BMC Infectious Diseases* Article No. 27.

NICOLOSI, A., CORREA, L.M.L. AND MUSICCO, M. (1994). The efficiency of male-to-female and female-to-male sexual transmission of the human immunodeficiency virus: a study of 730 stable couples. Italian Study Group on HIV Heterosexual Transmission. *Epidemiology* **5**, 565–567.

NOVELL, M.K., BENRUDI, G.I. AND THOMPSON, R.J. (1984). Investigation of microtrauma after sexual intercourse. *Journal of Reproductive Medicine* **29**, 269–271.

NUOVO, G., FORDE, A., MACCONNELL, P. AND FAHRENWELD, R. (1993). *In situ* detection of PCR-amplified HIV-1 nucleic acids and tumour necrosis factor CDNA in cervical tissues. *American Journal of Pathology* **143**, 40–48.

O'CONNOR, T.J., KINCHINGTON, D., KANGRO, H.O. AND JEFFRIES, D.J. (1995). The activity of candidate virucidal agents, low pH and genital secretions against HIV-1 *in vitro*. *International Journal of Sexually Transmitted Diseases and AIDS* **6**, 267–272.

ODEBLAD, E. (1964). Intracavity circulation of aqueous material in the human vagina. *Acta Obstetrica et Gynecologica Scandanivica* **43**, 360–369.

O'KEEFE, B.R., SHENOY, S.R., XIE, D. *ET AL.* (2000). Analysis of the interaction between HIV-inactivating protein cyanovirin-N and soluble forms of the envelope glycoproteins gp120 and gp41. *Molecular Pharmacology* **58**, 982–992.

OLMSTED, S.S., DUBIN, N.H., CONE, R.A. AND MOENCH, T.R. (2000). The rate at which human sperm are immobilized and killed by mild acidity. *Fertility and Sterility* **73**, 687–693.

ONGRADI, J., CECCHERINI-NELLI, L., PISTELLO, M., SPECTER, S. AND BENDINELLI, M. (1990). Acid sensitivity of cell-free and cell-associated HIV-1: clinical implications. *AIDS Research and Human Retroviruses* **6**, 1433–1436.

OSMOND, D.H. (1998). Sexual transmission of HIV. HIV Insite Knowledge Base Chapter, http://hivinsite.ucsf.edu/InSite?page=kb-authors&doc=kb-07-02-01

OTTEN, R.A., SMITH, D.K., ADAMS, D.R. *ET AL.* (2000). Efficacy of postexposure prophylaxis after intravaginal exposure of pig-tailed macaques to a human-derived retrovirus (human immunodeficiency virus type 2). *Journal of Virology* **74**, 9771–9775.

OWEN, D.H. AND KATZ, D.F. (1999). A vaginal fluid simulant. *Contraception* **59**, 91–95.

PADIAN, N.S., SHIBOSKI, S.C. AND JEWELL, N.P. (1991). Female-to-male transmission of human immunodeficiency virus. *Journal of the American Medical Association* **266**, 1664–1667.

PADIAN, N., SHIBOSKI, S., GLASS, S. AND VITTINGHOFF, E. (1997). Heterosexual transmission of human immunodeficiency virus (HIV) in northern California: results of a ten-year study. *American Journal of Epidemiology* **146**, 350–357.

PALACIO, J., SOUBERBIELLE, R., SHATTOCK, R., ROBINSON, G., MANYONDA, I. AND GRIFFIN, G. (1994). *In vitro* HIV-1 infection of human cervical tissue. *Research in Virology* **145**, 155–161.

PARNIAK, M.A. (2001). Non-nucleoside reverse transcriptase inhibitors as anti-HIV-1 microbicides. *AIDS* **15**, S56.

PARREN, P.W., MARX, P.A., HESSEL, A.J. *ET AL.* (2001). Antibody protects macaques against vaginal challenge with a pathogenic R5 simian/human immunodeficiency virus at serum levels giving complete neutralization *in vitro*. *Journal of Virology* **75**, 8340–8347.

PATTON, D.L., THWIN, S.S., MEIER, A., HOOTON, T.M., STAPLETON, A.E. AND ESCHENBACH, D.A. (2000). Epithelial cell layer thickness and immune cell populations in the normal human vagina at different stages of the menstrual cycle. *American Journal of Obstetrics and Gynecology* **183**, 967–973.

PAUWELS, R. (2001). Tibotec-Virco, NV, Mechelen, Belgium. 8th Annual Retrovirus Conference. New Experimental HIV Drugs. http://www.natap.org/2001/spring2001reports/newexperimental.html

PEARCEPRATT, R. AND PHILLIPS, D.M. (1993). Studies of adhesion of lymphocytic cells – implications for sexual transmission of human-immunodeficiency-virus. *Biology of Reproduction* **48**, 431–445.

PEARCEPRATT, R. AND PHILLIPS, D.M. (1996). Sulfated polysaccharides inhibit lymphocyte-to-epithelial transmission of human immunodeficiency virus-1. *Biology of Reproduction* **54**, 173–182.

PERL, J.I., MILLES, G. AND SHIMOZATO, Y. (1959). Vaginal fluid subsequent to panhysterectomy. *American Journal of Obstetrics and Gynecology* **78**, 285–289.

PETERMAN, T., STONEBURNER, R. AND ALLEN, J. (1988). Risk of human immunodeficiency virus transmission from heterosexual adults with transfusion-associated infections. *Journal of American Medical Association* **259**, 55–58.

PETTIT, R.K., MCALLISTER, S.C. AND HAMER, T.A. (1999). Response of gonococcal clinical isolates to acidic conditions. *Journal of Medical Microbiology* **48**, 149–156.

POMERANTZ, R., DE LA MONTE, S., DONEGAN, S. *ET AL.* (1988). Human immunodeficiency virus (HIV) infection of the uterine cervix. *Annals of Internal Medicine* **108**, 321–327.

POPE, L.E., MARCELLETTI, J.F., KATZ, L.R. *ET AL.* (1998). The anti-herpes simplex virus activity of *n*-docosanol includes inhibition of the viral entry process. *Antiviral Research* **40**, 85–94.

POPOVIC, M., SARNGADHARAN, M.G., READ, E. AND GALLO, R.C. (1984). Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* **224**, 497–500.

PORTSMOUTH, S., STEBBING, J. AND GAZZARD, B. (2003). Current treatment of HIV infection. *Current Topics in Medicinal Chemistry* **3**, 1458–1466.

PRETI, G., HUGGINS, G.R. AND SILVEBERG, G.D. (1979). Alterations in the organic compounds of vaginal secretions caused by sexual arousal. *Fertility and Sterility* **32**, 47–54.

PUDNEY, J. AND ANDERSON, D.J. (1991). Orchitis and human immunodeficiency virus type 1-infected cells in reproductive tissues from men with the acquired immune deficiency syndrome. *American Journal of Pathology* **139**, 149–160.

RAGHUVANSHI, P., BAGGA, R., MALHOTRA, D., GOPALAN, S. AND TALWAR, G.P. (2001). Spermicidal and contraceptive properties of Praneem polyherbal pessary. *Indian Journal of Medical Research* **113**, 135–141.

RANKIN, J.C. AND JEANES, A. (1954). Evaluation of the periodate oxidation method for structural analysis of dextrans. *Journal of the American Chemical Society* **76**, 4435–4441.

RODDY, R.E., ZEKENG, L., RYAN, K.A., TAMOUFE, U., WEIR, S.S. AND WONG, E.L. (1998). A controlled trial of nonoxynol-9 film to reduce male-to-female transmission of sexually transmitted diseases. *New England Journal of Medicine* **339**, 504–510.

ROYCE, R.H., SENA, A., CATES, W., JR. AND COHEN, M.S. (1997). Sexual transmission of HIV. *New England Journal of Medicine* **336**, 1072–1078.

RUSCONI, S., MOONIS, M., MERRILL, D.P. *ET AL.* (1996). Naphthalene sulfonate polymers with CD-4 blocking and anti-human immunodeficiency virus type 1 activities. *Antimicrobial Agents and Chemotherapy* **40**, 234–236.

RUSTOMJEE, R. AND ABDOOL KARIM, Q. (2001). Microbicide research and development – where to? *HIV Clinical Trials* **2**, 185–192.

SARNGADHARAN, M.G., POPOVIC, M., BRUCH, L., SCHUPBACH, J. AND GALLO, R.C. (1984). Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. *Science* **224**, 506–508.

SCHAEFFER, D.J. AND KRYLOV, V.S. (2000). Anti-HIV activity of extracts and compounds from algae and cyanobacteria. *Ecotoxicology and Environmental Safety* **45**, 208–227.

SCHOLS, D., BABA, M., PAUWELS, R. AND DE CLERCQ, E. (1989). Flow cytometric method to demonstrate whether anti-HIV agents inhibit virion binding to $T4^+$ cells. *Journal of Acquired Immunodeficiency Syndromes* **2**, 10–15.

SCHOLS, D., PAUWELS, R., DESMYTER, J. AND DE CLERCQ, E. (1990). Dextran sulfate and other polyanionic anti-HIV compounds specifically interact with the viral gp120 glycoprotein expressed by T cells persistently infected with HIV-1. *Virology* **175**, 556–561.

SCHRIEBER, C.A., WAN, L.V., SUN, Y.T., LU, L., KREY, L.C. AND LEE-HUANG, S. (1999). The antiviral agents, MAP30 and GAP31, are not toxic to human spermatozoa and may be useful in preventing the sexual transmission of human immunodeficiency virus type 1. *Fertility and Sterility* **72**, 686–690.

SCHUPBACH, J., POPOVIC, M., GILDEN, R.V., GONDA, M.A., SARNGADHARAN, M.G. AND GALLO, R.C. (1984). Serological analysis of a subgroup of human T-lymphotropic retroviruses (HTLV-III) associated with AIDS. *Science* **224**, 503–505.

SCOLARO, M.J., GUNNILL, L.B., POPE, L.E., KHALIL, M.H., KATZ, D.H. AND BERG, J.E. (2001). The antiviral drug docosanol as a treatment for Kaposi's sarcoma lesions in HIV type 1-infected patients: a pilot clinical study. *AIDS Research and Human Retroviruses* **17**, 35–43.

SIMOES, J.A., CITRON, D.M., AROUTCHEVA, A. *ET AL.* (2002). Two novel vaginal microbicides (polystyrene sulfonate and cellulose sulfate) inhibit *Gardnerella vaginalis* and anaerobes commonly associated with bacterial vaginosis. *Antimicrobial Agents and Chemotherapy* **46**, 2692–2695.

SINGH, B., CUTLER, J.C. AND UTIDJIAN, H.M.D. (1972). Studies of the development of a vaginal preparation providing both prophylaxis against venereal disease and other genital infections and contraception: II. Effect *in vitro* of vaginal contraceptive and non-contraceptive preparations on *Treponema pallidum* and *Neisseria gonorrhoeae*. *British Journal of Venereal Disease* **48**, 57–64.

SPARLING, P.F. (1999). Biology of *Neisseria gonorrhoeae*. In: *Sexually transmitted diseases*. Eds. K.K. Holmes, P.F. Sparling, P.-A. Mardh *et al.*, pp 433–449. New York, USA: McGraw-Hill.

SPIRA, A., MARX, P., PATTERSON, J. *ET AL.* (1996). Cellular targets of infection and route of viral

dissemination after an intravaginal inoculation of simian immunodeficiency virus into rhesus macaques. *Journal of Experimental Medicine* **183**, 215–225.

STEPHENSON, J. (2000). Widely used spermicide may increase, not decrease, risk of HIV transmission. *Journal of the American Medical Association* **284**, 949.

STURN, A.W. AND ZANEN, H.C. (1984). Characteristics of *Haemophilus ducreyi* in culture. *Journal of Clinical Microbiology* **19**, 672–674.

TALWAR, G.P. (2001). Prevention of AIDS and sexually-transmitted infections. *Defence Science Journal* **51**, 409–414.

TALWAR, G.P., GARG, S., DHAR, V., CHABRA, R., GANJU, A. AND UPADHYAY, S.N. (1995). Praneem polyherbal cream and pessaries with dual properties of contraception and alleviation of genital infections. *Current Science* **68**, 437–440.

TALWAR, G.P., RAGHUVANSHI, P., MISHRA, R. *ET AL.* (2000). Polyherbal formulations with wide spectrum antimicrobial activity against reproductive tract infections and sexually transmitted pathogens. *American Journal of Reproductive Immunology* **43**, 144–151.

THE ECONOMICS OF MICROBICIDE DEVELOPMENT. A Report of the Pharmaco-Economics Working Group of the Rockefeller Foundation Microbicide Initiative. www.rockfound.org/Documents/488/rep3_economics.pdf

THOMPSON, K.A., MALAMUD, D. AND STOREY, B.T. (1996). Assessment of the anti-microbial agent C31G as a spermicide: comparison with nonoxynol-9. *Contraception* **53**, 313–318.

UNGCHUSAK, K., REHLE, T., THAMMAPORNPILAP, P., SPEIGELMAN, D., BRINKMANN, U. AND SIRAPRAPASIRI, T. (1996). Determinants of HIV infection among female commercial sex workers in northeastern Thailand: results from a longitudinal study. *Journal of Acquired Immune Deficiency Syndromes* **12**, 500–507.

VAN DAMME, L., WRIGHT, A., ROSENSTEIN, I. *ET AL.* (2000). A phase I study of a novel potential intravaginal microbicide, PRO 2000, in healthy sexually-inactive women. *Sexually Transmitted Infections* **76**, 126–130.

VAN DE WIJGERT, J.K., FULLEM, A., KELLY, C. *ET AL.* (2001). Phase I trial of the topical microbicide BufferGel: safety results from four international sites. *Journal of Acquired Immune Deficiency Syndromes* **26**, 21–27.

VEAZEY, R.S., SHATTOCK, R.J., POPE, M. *ET AL.* (2003). Prevention of virus transmission to macaque monkeys by a vaginally-applied monoclonal antibody to HIV-1 gp120. *Nature Medicine* **9**, 343–346.

VERNAZZA, P., ERON, J., COHEN, M., VANDER HORST, C., TROIAN, L. AND FISCUS, S. (1994). Detection of biologic characterization of infectious HIV-1 in semen of seropositive men. *AIDS* **8**, 1325–1329.

VERNAZZA, P., ERON, J. AND FISCUS, S. (1996). Sensitive method for the detection of infectious HIV in semen of seropositive individuals. *Journal of Virological Methods* **56**, 33–40.

VLIEGE, P., CLERC, T., PANNECOUQUE, C. *ET AL.* (2002). Synthesis of new covalently bound kapp-carrageenan-AZT conjugates with improved anti-HIV activities. *Journal of Medicinal Chemistry* **45**, 1275–1283.

WAGNER, G. (1979). Vaginal transudation. In: *The biology of the fluids of the female genital tract.* Eds. F.K. Beller and G.F.B. Shumacher, pp 25–34. New York, USA: Elsevier/North Holland.

WAGNER, G. AND LEVIN, R.J. (1980). Electrolytes in vaginal fluid during the menstrual cycle of coitally active and inactive women. *Journal of Reproduction and Fertility* **60**, 17–27.

WANG, Y.X., NEAMATI, N., JACOB, J. *ET AL.* (1999). Solution structure of anti-HIV-1 and anti-tumour protein MAP30: structural insights into its multiple functions. *Cell* **99**, 433–442.

WILKINSON, D., THOLANDI, M., RAMJEE, G. AND RUTHERFORD, G.W. (2002). Nonoxynol-9 spermicide for prevention of vaginally acquired HIV and other sexually transmitted infections: systematic review and meta-analysis of randomized controlled trials including more than 5000 women. *Lancet Infectious Diseases* **2**, 613–617.

WITVROUW, M. AND DE CLERCQ, E. (1997). Sulfated polysaccharides extracted from sea algae as potential antiviral drugs. *General Pharmacology* **29**, 497–511.

WITVROUW, M., SCHOLS, D., ANDREI, G. *ET AL.* (1991). Antiviral activity of low-MW dextran sulfate (derived from dextran MW 1000) compared to dextran sulfate samples of higher MW.

Antiviral Chemistry and Chemotherapy **2**, 171–179.

WITVROUW, M., FIKKERT, V., PLUYMERS, W. *ET AL.* (2000). Polyanionic (i.e. polysulfonate) dendrimers can inhibit the replication of human immunodeficiency virus by interfering with both virus adsorption and later steps (reverse transcriptase/integrase) in the virus replicative cycle. *Molecular Pharmacology* **58**, 1100–1108.

WOOLFSON, A.D., ELLIOTT, G.R.E., GILLIGAN, C.A. AND PASSMORE, C.M. (1999). Design of an intravaginal ring for the controlled delivery of 17β-estradiol as its 3-acetate ester. *Journal of Controlled Release* **61**, 319–328.

WOOLFSON, A.D., MALCOLM, R.K. AND GALLAGER, R. (2000). Drug delivery by the intravaginal route. *Critical Reviews in Therapeutic Drug Carrier Systems* **17**, 509–555.

WOOLFSON, A.D., MALCOLM, R.K. AND GALLAGHER, R.J. (2003). Design of a silicone reservoir intravaginal ring for the delivery of oxybutynin. *Journal of Controlled Release* **91**, 465–476.

WYAND, M.S., MANSON, K.H., MILLER, C.J. AND NEURATH, A.R. (1999). Effect of 3-hydroxyphthaloyl-β-lactoglobulin on vaginal transmission of simian immunodeficiency virus in rhesus monkeys. *Antimicrobial Agents and Chemotherapy* **43**, 978–980.

WYRICK, P.B., KNIGHT, K.T., GERBIG, D.G. *ET AL.* (1997). The microbicidal agent C31G inhibits *Chlamydia trachomatis* infectivity *in vitro*. *Antimicrobial Agents and Chemotherapy* **41**, 1335–1344.

YAMADA, T., OGAMO, A., SAITO, T., WATANABE, J., UCHIYAMA, H. AND NAKAGAWA, Y. (1997). Preparation and anti-HIV activity of low-molecular weight carrageenans and their sulfated derivatives. *Carbohydrate Polymers* **32**, 51–55.

YAMADA, T., OGAMO, A., SAITO, T., UCHIYAMA, H. AND NAKAGAWA, Y. (2000). Preparation of O-acylated low-molecular-weight carrageenans with potent anti-HIV activity and low anticoagulant effect. *Carbohydrate Polymers* **41**, 115–120.

YAMAMOTO, I., TAKAYAMA, K., HONMA, K., GONDA, T. AND MATSUZAKI, K. (1990). Synthesis, structure and antiviral activity of sulfates of cellulose and its branched derivatives. *Carbohydrate Polymers* **14**, 53–63.

YU, K. AND CHIEN, Y.W. (1982). Vaginal delivery and absorption of drugs. In: *Novel drug delivery systems: fundamentals, developmental concepts*, pp 153–185. New York, USA: Marcel Dekker.

ZACHAROPOULOS, V.R. AND PHILLIPS, D.M. (1997). Vaginal formulations of carrageenan protect mice from herpes simplex virus infection. *Clinical and Diagnostic Laboratory Immunology* **4**, 465–468.

ZANEVELD, L.J.D., ANDERSON, R.A. AND WALLER, D.P. (1996). Non-cytotoxic contraceptive antimicrobials and an acid-buffering formulation. North Carolina: Opportunities for Industrial Collaboration in Contraceptive Research. Family Health International, North Carolina, 90.

ZEITLIN, L., HOEN, T.E., ACHILLES, S.L. *ET AL.* (2001). Tests of Buffergel for contraception and prevention of sexually transmitted diseases in animal models. *Sexually Transmitted Diseases* **28**, 417–423.

ZHANG, H., DORNADULA, G., DEUMONT, M. *ET AL.* (1998). Human immunodeficiency virus type 1 in the semen of men receiving highly active antiretroviral therapy. *New England Journal of Medicine* **339**, 1803–1809.

ZHANG, Z., SCHULER, T., ZUPANCIC, M. *ET AL.* (1999). Sexual transmission and propagation of SIV and HIV in resting and activated $CD4^+$ T cells. *Science* **286**, 1353–1357.

ZUSSMAN, A., LARA, L., LARA, H.H., BENTWICH, Z. AND BORKOW, G. (2003). Blocking of cell-free and cell-associated HIV-1 transmission through human cervix organ culture with UC-781. *AIDS* **17**, 653–661.