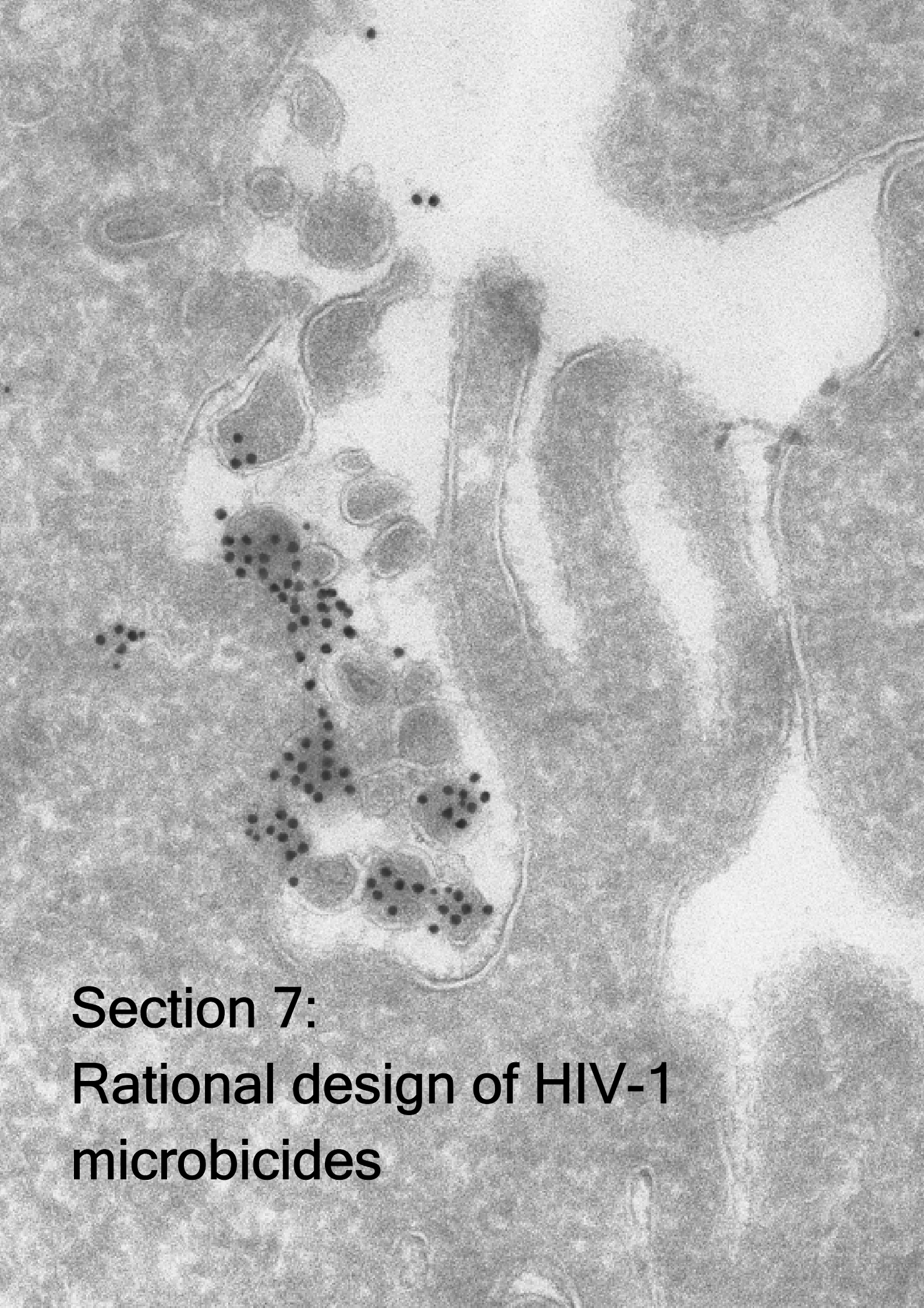

Section 7: Rational design of HIV-1 microbicides

Chapter 7.1

How to prevent HIV-1 transmission?

Bridge

In **Section 2 & 5** we have demonstrated that different mechanisms are involved in HIV-1 transmission. In **Section 6** we have described how different host factors influence HIV-1 transmission. In **Section 7,** we will describe the different methods to prevent HIV-1 transmission, with a focus on the development of microbicides. With the knowledge of the previous sections in mind, we will discuss the different targets for an HIV-1 microbicide and the considerations to warrant the safety. Furthermore, we will present data on the novel candidate microbicide C5A.

HIV-1 prevention: Awareness, condoms and vaccination

A major and still ongoing effort to prevent new HIV-1 infections is providing awareness to both the government and the global population on risks to acquire HIV-1, such as unprotected sex, use of contaminated needles and untested blood transfusions. Awareness has resulted in specific health laws, public policies providing clean needles and condoms, and behavioral changes. However, achieving global awareness is still a major goal towards prevention.

Methods to prevent new HIV-1 infections through sexual transmission must be effective against a broad variety of HIV-1 strains, cheap, easy to use and acceptable to both sexual partners. At present, safe sex through condom use is the only method that meets these criteria. Improvements must be made towards the strength of the condoms, price, availability and the general acceptance, especially among men.

In theory, a safe and inexpensive HIV-1 vaccine would be the most ideal and effective way to stop new viral infections[3]. However, despite intensive efforts, a successful HIV-1 vaccine is not in sight yet[5,6]. Reasons for this are the high mutation speed of the virus, the multiple viral variants present in the population, and the low immunogenicity of the viral envelope glycoprotein[6-8].

Microbicide

A microbicide is the name of a drug that is applied topically in the vagina, rectum, on the penis or condoms to prevent the transmission of sexual transmitted diseases (STDs), including HIV-1. Microbicides may be formulated as a cream or a sustained release device (e.g. vaginal ring). In the young adult population in sub-Saharan Africa HIV-1 infection is three times higher in women compared to men[9]. Men often refuse to use condoms, and a microbicide would place the women in control and enable them to protect themselves. In addition to being safe and effective, a microbicide should therefore be odourless, colourless and be able to use in a time frame of hours before or after sexual intercourse.

Table 7.1.1 Host targets for anti-HIV-1 microbicides

Target cell	Molecular targets for microbicides	"Anti"-targets
Epithelial cells	Syndecan-1 and -2	Epithelial barrier function
LCs	CD4/CCR5/unknown trans-receptor after activation	Langerin
DC-SIGN$^+$ DCs	CD4, CCR5, DC-SIGN, Syndecan-3, GalCer?	DC activation?
Macrophages	CD4, CCR5, mannose receptor, Syndecan-2 and -4	??
T cells	CD4, CCR5, CXCR4	??
Combination	CD4, CCR5, CXCR4, DC-SIGN, Mannose receptor, Syndecan-1-4	Langerin, epithelial barrier function, DC activation

Targets for anti-HIV-1 microbicides

Different mechanisms play a role in HIV-1 transmission and the receptors involved in these processes might be targets for microbicides (Table 7.1.1). However, also protective factors have been described, such as Langerin function and epithelial integrity. A candidate microbicide should preserve or even enhance these "anti-targets" (Table 7.1.1).

Microbicides might be specifically directed toward viral structures, to receptors on the target cells or their counter-structures on the virus particle. To date, an effective microbicide is not available. However, much effort has been done to develop efficient candidates. Different classes of microbicides have been

and are still being developed (Table 7.1.2). To this list we have added novel ideas that were raised by the data in **Section 2**, **5** and **6**. The syndecan family appears to be an important family of receptors for HIV-1 transmission. Syndecan-1 and -2 are involved in epithelial transcytosis[1], syndecan -2 and -4 in macrophage infection and syndecan-3 is involved in DC-mediated transmission. Antibodies directed against syndecan-3 did not block binding of HIV-1 to DCs, supporting a role for the heparan sulfate side chains. These side chains that are involved in the interaction of HIV-1 with the different members of the syndecan family or their counter-structures on the virus might be promising targets for microbicides. TNFα enhances transmission of HIV-1 by LCs and this might play a crucial role during co-infection. We therefore propose anti-TNFα as a candidate microbicide that could supplement other compounds. Langerin expression might be crucial for the protection against HIV-1. TGFβ enhances Langerin expression *in vitro*[11], and might therefore be used to increase the protective function of this lectin. Moreover, we have identified Lewis X as a specific block for DC-SIGN and not Langerin (Chapter 5.2). The ideal microbicide would be able to cope with this variety of HIV-1 infection mechanisms but also be able to block HIV-1 replication and release, once the virus is intracellular; this may require a combination of several active ingredients in one formulation.

Candidate microbicides increased susceptibility to HIV-1 in clinical trial

Different clinical trials are now performed to test the safety and efficacy of candidate microbicide (Table 7.1.2). The first clinically tested microbicides included detergents, acidic buffers, and polyanionic gels. Unfortunately, three out of six efficacy clinical trials were prematurely stopped after interim analysis. The results of the other trials are now pending[4]. C31G or Savvy was discontinued since the incidence of HIV-1 infection appeared too low among the trial applicants. Strikingly, the clinical trials on Nonoxynol-9 and cellulose sulfate were stopped since the drugs increased the susceptibility to acquire HIV-1. Nonoxynol-9 is a detergent that is widely used as a spermicidal agent, but also has microbicidal activity *in vitro*[2]. The detrimental effect of Nonoxynol-9 is now attributed to the disruption of the vaginal epithelial barrier and subsequent inflammation[2]. In **Section 6**, we have demonstrated that inflammation enhances HIV-1 transmission by LCs. In addition, we hypothesize that this treatment abrogated the protective Langerin function (**Section 4**). Cellulose sulfate is a polyanion, which blocks binding of HIV-1 by electrostatic binding to gp120. In contrast to Nonoxynol-9, no alterations in the epithelium and indications for inflammation have been observed with this compound. The only sign was a minor change in the vaginal flora, indicating that unknown/untested risk factors are involved in HIV-1 transmission[4,10,12].

How to warrant safety of a microbicide candidate?

Cellulose sulfate and Nonoxymol-9 enhanced the risk to acquire HIV-1 by known and unknown mechanisms. These results indicate a lack of efficacy of these compounds, since risk factors were still involved in HIV-1 transmission, similar to the normal situation. The devastating results from the Nonoxynol-9 and cellulose sulfate trials stress the need to further investigate the mechanisms underlying HIV-1 transmission and the risk factors involved. Prior to a trial, different *in vivo* and *in vitro* experiments were performed to test the safety of the drugs. Clearly, these experiments did not evaluate all the risk factors possibly involved and the efficacy of the compounds in the most reliable model. Based on our results we are planning to set up a method to test candidate microbicides for their effect on Langerin function and their effect on LC-mediated transmission.

Conclusion

The knowledge on HIV-1 transmission we have to date is limited, and factors that mediate transmission as well as protect against transmission are still being unravelled. Therefore we recommend targeting

either specific viral structures, such as polyanions, RT inhibitors or the novel candidate C5a, or the host factor CCR5, since population genetics have confirmed the importance of this receptor for HIV-1 transmission. However, due to the pressure of finding an effective microbicide, it is essential to investigate different candidates. The Nonoxynol-9 and cellulose sulfate trials emphasize the need to carefully assess the efficacy and the safety of a candidate microbicide before putting it into clinical trials.

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Table 7.1.2 [1,5,6,9] Candidate microbicides in different phases of development

Target	Drug	Mechanism	Phase of development
Non-specific	Detergent	Chemical barrier	Clinical trial (*)
	Surfactant	Denaturing viral proteins/Physical barrier	Clinical trial
Mucosal flora	Acidic buffer gel	Maintains vaginal pH acidic	Clinical trial
	Probiotic (Lactobacilli)	Maintains microflora	Clinical trial
	Anti-TNF-alpha	Blocks enhanced transmission in inflammation	No
Virus surface	Anionic polymer	Prevents binding of virus to cell surfaces (non-specific)	Clinical trial (*)
	gp120 mAb	Blocks gp120-CD4 binding	Preclinical
	soluble DC-SIGN/2G12/lectin-likes	Blocks gp120-DC-SIGN binding (Langerin?)	Preclinical
	gp41 mAb or small molecule	Inhibits fusion of X4 and R5 viruses	Preclinical
Viral replication	RT inhibitor	Blocks reverse transcription	Clinical trial
	Integrase inhibitor	Blocks viral fusion	No
HIV-1 receptors	CCR5 mAb, analogue, small molecule	Inhibits fusion of R5 viruses	Preclinical
	CD4 mAb or small molecule	Blocks CD4-gp120 interaction	No
	CXCR4 mAb, analogue, small molecule	Inhibits fusion X4 viruses	Preclinical
	DC-SIGN mAb, carbohydrate ligands, Lewis X	Blocks gp120-DC-SIGN interactions	Preclinical
	HSPG mAb, inhibitors	Blocks gp120-HSPG interaction	No
	Inducer of Langerin expression	Increases HIV-1 degradation	No