CHARACTERISATION OF THE REMNANT FORESKIN – IMPLICATIONS FOR HIV TRANSMISSION IN CIRCUMCISED MEN.

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ABSTRACT

Human Immunodeficiency Virus (HIV) prevention remains one of the world’s top public health and development priorities, and male circumcision is the only biomedical intervention that has achieved level 1 scientific evidence for effectiveness in HIV prevention. Three randomised controlled trials have provided strong evidence that adult male circumcision confers significant protection against HIV infection, with a reduction in the relative risk of at least fifty percent.

During surgical circumcision, a sleeve of preputial skin is removed and a cuff of skin around the base of the glans penis remains, forming the remnant foreskin. It is thought that the protective affect of circumcision can be attributable to the surgical removal of the inner foreskin epithelium, the main entry site of HIV into the penis.

Current accepted wisdom is that the inner foreskin epithelium is abundantly supplied with HIV-1 target cells, is poorly keratinised, at risk of microscopic tears, exposed to vaginal secretions during intercourse, has a higher degree of susceptibility to HIV infection when compared to the outer foreskin, and provides a moist environment that might sustain the viability of pathogens.

The aim of this study was to characterise the remnant foreskin (R), in comparison to the penile shaft skin (S) and the inner foreskin (I), and determine its role in the transmission of HIV. Tissue biopsies were obtained from the remnant foreskin and penile shaft skin of 10 circumcised men undergoing elective vasectomy and from the inner foreskin of 10 uncircumcised men undergoing elective circumcision. Biopsies were stained for Langerhans’ cells and keratin, and the number of Langerhans’ cells/mm$^2$ and the thickness of the epithelium and stratum corneum was measured at each site.

This study has shown that what was previously accepted wisdom regarding the keratin thickness of the inner foreskin is incorrect. Instead, our results revealed no significant difference in epithelial (RvS: p=0.38; IvS: p=0.53; RvI: p=0.82) or keratin (RvS: p=0.32; IvS: p=0.15; RvI: p=0.66) thickness between the three sites.
In keeping with current evidence, we found that the inner foreskin has a high density of Langerhans’ cells. We found that the remnant foreskin has a significantly smaller amount of Langerhans’ cell within its epithelial, in comparison to both the penile shaft skin and the inner foreskin. In fact, relative to the inner foreskin, there is an astonishing scarcity of Langerhans’ cells in the remnant foreskin. There was significantly fewer Langerhans’ cells in the remnant foreskin compared to the inner foreskin (p=0.00001) and penile shaft skin (p<0.01), and significantly more Langerhans’ cells in the inner foreskin than the penile shaft skin (p<0.02).

We believe that the reduced transmission of HIV seen in circumcised men is not because of a difference in keratin thickness between the inner foreskin and other penile skin, as has been previously accepted wisdom, but could be due to the surgical removal of HIV-1 target cells (Langerhans’ cells) in the inner foreskin and the subsequent development of the remnant foreskin, a tissue with a remarkable scarcity of Langerhans’ cells.
DECLARATION

This is to certify that
(i) the thesis comprises only my original work towards the Masters of Surgery,
(ii) due acknowledgement has been made in the text to all other material used,
(iii) the thesis is 19913 words in length, exclusive of figures, bibliographies and appendices.
ACKNOWLEDGEMENTS

I would like to thank the many people who helped me develop, implement and realise the aims of this study. I appreciate that many people have contributed a lot of their time in order to make this study possible and am grateful for the support and advice that I have received along the way. Especially, I would like to thank Melissa Yow, Damien Bolton, David Webb, James Turner, members of the Austin Health Urology Department, and staff from the Department of Zoology, the University of Melbourne.

Melissa Yow is a PhD student of the Department of Zoology, at the University of Melbourne. At the time of my study, she was concurrently conducting a study (“The use of topical oestrogen to keratinise the inner aspect of the human foreskin”) with the Austin Health Urology Department and Department of Zoology, the University of Melbourne, and shared the same supervisors, Associate Professor Damien Bolton and Dr Andrew Pask. We were able to use many of the same patients in both of our trials. As such, I was the clinician for her study, recruiting all of the patients required to participate in her trial, and she helped with me with many different aspects of my study. From the outset, Melissa organised ethics approval for both studies. After this was achieved, she helped construct my experimental plan, specifically developing my histology and immunohistochemistry experiments. Once I had recruited the patients to our trials, and I had obtained the tissue samples which were then transported to the Zoology laboratories at the University of Melbourne, Melissa embedded and sectioned all of the tissue samples. Melissa instructed me as to how to perform the Haematoxylin-Eosin stains, whilst she completed the immunohistochemical stains. Melissa taught me how to examine all of the sections under light-microscopy and perform the necessary analyses. Throughout this study, and after countless coffees and dinners, Melissa has also become a very close and supportive friend. Without Melissa, I wouldn’t have been able to complete this study and I owe her my sincerest gratitude.

Associate Professor Damien Bolton is the Director of Urology at the Austin Hospital, Melbourne, and has established a research program in Urology at the Austin Hospital with the University of Melbourne, Department of Surgery. With Damien’s support and encouragement, I was able to undertake this Masters of Surgery at the Austin Hospital.
and I am very grateful for his role as my supervisor. I would like to thank him specifically for his help recruiting some of the patients who participated in this trial. It was not an easy task, but it was certainly made easier with his help. Also, I would like to thank Damien for his enthusiasm and support throughout my research project, and for his mentorship throughout my Urology training.

Associate Professor David Webb is an Associate Professor of Surgery (Melbourne University) and a consultant Urologist at Austin Health. It was A/Prof Webb who originally asked me to become involved with the Austin Health Urology Research Department, and who suggested the topic for my Masters of Surgery. A/Prof Webb believed that the remnant foreskin may play a role in the transmission of HIV, and that it was an area of tissue that had not yet been described in the literature. It was A/Prof Webb who suggested that I investigate the role of the remnant foreskin in HIV transmission and I would like to thank him for this inspiration.

James Turner is the Urology Patient Liaison nurse at Austin Health and he spent countless hours trawling through the Austin Health Urology Waiting List looking for patients requiring circumcisions and vasectomies. Once found, James organised specific outpatient clinics for me at irregular times, random days and sometimes at very short notice to accommodate my availability. As the correct type of patient was difficult to find, not to mention difficult to recruit once found, this was a process that went on for over a year, and James did not complain once about the extra work and was always willing to help. I would like to thank James very much for all of his help, and patience, and stress that this study would not have eventuated without all of his assistance.

There were many other members of the Department of Urology at Austin Health that helped me throughout this study and I would like to thank all of them. Two other Urology Patient Liaison nurses, Aruna Morris and Michelyn El-Zein, were always willing to shuffle surgical lists in order to accommodate my trial patients’ surgery. I would like to thank all of the Urology consultants, registrars and fellows who performed the circumcisions and vasectomies and allowed me to obtain all necessary tissue samples. I would also like to thank the other Urology Research Fellows, in particular Dr Peter Wong, Dr Adee Ben-Davidson, Dr Bradley Newell and Dr Nathan Lawrentschuk, who
were always available to answer questions, provide guidance and a little extra motivation whenever it was needed.

This study was conducted in conjunction with members of the Department of Zoology, the University of Melbourne, and I would like to thank a number of people specifically. Associate Professor Andrew Pask provided guidance as a supervisor throughout my thesis. Professor Roger Short, like Associate Professor Webb, played an important part in the creation of my research topic. Dr Michael Magrath helped me perform my statistical analyses, and Mr Bruce Abaloz (Histologist, Department of Zoology, the University of Melbourne) allowed me to perform my experiments, under Melissa Yow’s and A/Prof Andrew Pask’s supervision, in his laboratory.

I would like to thank the Melbourne School of Graduate Research and the department of Medicine, Dentistry and Health Sciences (MDHS) for granting me the opportunity to undertake a Masters of Surgery. I would like to thank the MDHS Faculty Research Scholarship Sub-Committee for awarding me a Faculty Research Scholarship, specifically The Viola Edith Reid Bequest Scholarship (2008), which provided much needed financial support throughout my research year.

During my research year, my results were accepted for presentation at the American Urological Association (AUA), Chicago, 2009 meeting. I would like to thank the Urological Society of Australia and New Zealand (USANZ) for providing financial support to attend the 2009 AUA Meeting. I would also like to thank the USANZ for allowing me to defer the commencement of my urology clinical training in order to continue research in 2008.

My research has been extremely satisfying academically, has allowed me to travel overseas, and has re-enforced my keen interested in Urology and Clinical Research. Of course none of this would have been possible, or as rewarding, without the support, interest and love of my family. Thanks Hallamores. Lastly, I would like to thank Dougald Elmer. He wasn’t there at the beginning of my Masters, but he’s seen me closely through the thick of the project, the write up and the results. He’s read countless drafts, and listened to many presentations, frustrations and successes. Thank you.
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CHAPTER 1: HUMAN IMMUNODEFICIENCY VIRUS

Autoimmune Deficiency Syndrome (AIDS) remains a leading cause of mortality worldwide and the primary cause of death in Sub-Saharan Africa. In less than 15 years Human Immunodeficiency Virus (HIV) has reached the level of a pandemic and AIDS has been reported in over 190 countries.[1] In 2007 there were 33.2 million people living with HIV worldwide, 2.5 million new infections (68% in sub-Saharan Africa) and a total of 2.1 million AIDS deaths. 76% of these deaths occurred in sub-Saharan Africa alone.[2] In 2002, estimates suggested that an additional 45 million people will become infected in 126 low- and middle-income countries by 2010 unless prevention efforts are drastically expanded on a global scale. Unfortunately, current efforts are inadequate. In 2003, of all people at risk for HIV infection worldwide only 20% had access to prevention services, 24% to AIDS education and only 12% had access to voluntary HIV counselling and testing services.[3] Not only is there an urgency to improve education, prevention and treatment, but the future of the response to HIV/AIDS will also require research into, and the development of, new prevention tools that will be accessible worldwide, most importantly by the developing world.
DEFINITIONS AND CLASSIFICATIONS

Acquired immunodeficiency syndrome (AIDS) is due to the human immunodeficiency virus (HIV) of which there are two main variants, HIV-1 and HIV-2. HIV is a ribonucleic acid (RNA) retrovirus that integrates its genetic material into the host genome where it can remain latent for long periods of time. The virus is transmitted inside infected CD4+ T cells and macrophages, and is spread sexually, or through blood or blood products. CD4+ T cells are the key effector cells in the host’s immune response. With ongoing HIV replication within the CD4+ T cells, and eventual cell destruction, the host becomes profoundly immunosuppressed. The progressive decrease in CD4+ cells eventually leads to widespread immune dysfunction and a severely depressed cell-mediated immunity.[4] Once the absolute CD4 count falls below 200 cells/cu mm an individual becomes susceptible to opportunistic infections,[5] clinically recognised as AIDS.

HIV disease staging and classification systems are critical tools for tracking and monitoring the HIV epidemic and for providing information to the health care provider and patient about HIV disease stage and clinical management. Two major classification systems are currently in use: the U.S Centre for Disease Control and Prevention (CDC) classification system and the World Health Organization (WHO) Clinical Staging and Disease Classification System[6].

The CDC disease staging system (revised 1993) assesses the severity of HIV disease by CD4 cell counts and by the presence of specific HIV-related conditions, and is summarised in tables 1 to 3 below.[6]
Table 1: U.S Centre for Disease Control and Prevention (CDC) HIV Disease Staging System

<table>
<thead>
<tr>
<th>CD4 cells/µL Cell Categories</th>
<th>Clinical Categories</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A Asymptomatic, Acute HIV, or PGL</td>
</tr>
<tr>
<td>(1) ≥500</td>
<td>A1</td>
</tr>
<tr>
<td>(2) 200–499</td>
<td>A2</td>
</tr>
<tr>
<td>(3) &lt;200</td>
<td>A3</td>
</tr>
</tbody>
</table>
Table 2: CDC Classification System: Category B Symptomatic Conditions

| Category B symptomatic conditions are defined as symptomatic conditions occurring in an HIV-infected adolescent or adult that meet at least 1 of the following criteria: |
| a) They are attributed to HIV infection or indicate a defect in cell-mediated immunity. |
| b) They are considered to have a clinical course or management that is complicated by HIV infection. |

**Examples include, but are not limited to, the following:**

- Bacillary angiomatosis
- Oropharyngeal candidiasis (thrush)
- Vulvovaginal candidiasis, persistent or resistant
- Pelvic inflammatory disease (PID)
- Cervical dysplasia (moderate or severe)/cervical carcinoma in situ
- Hairy leukoplakia, oral
- Idiopathic thrombocytopenic purpura
- Constitutional symptoms, such as fever (>38.5°C) or diarrhea lasting >1 month
- Peripheral neuropathy
- Herpes zoster (shingles), involving ≥ 2 episodes or ≥ 1 dermatome
Table 3: CDC Classification System: Category C AIDS-Indicator Conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
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<tr>
<td>Bacterial pneumonia, recurrent</td>
<td>(≥ 2 episodes in 12 months)</td>
</tr>
<tr>
<td>Candidiasis of the bronchi, trachea, or lungs</td>
<td></td>
</tr>
<tr>
<td>Candidiasis, esophageal</td>
<td></td>
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<tr>
<td>Cervical carcinoma, invasive, confirmed by biopsy</td>
<td></td>
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<tr>
<td>Coccidioidomycosis, disseminated or extrapulmonary</td>
<td></td>
</tr>
<tr>
<td>Cryptococcosis, extrapulmonary</td>
<td></td>
</tr>
<tr>
<td>Cryptosporidiosis, chronic intestinal (&gt;1-month duration)</td>
<td></td>
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<tr>
<td>Cytomegalovirus disease (other than liver, spleen, or nodes)</td>
<td></td>
</tr>
<tr>
<td>Encephalopathy, HIV-related</td>
<td></td>
</tr>
<tr>
<td>Herpes simplex: chronic ulcers (&gt;1-month duration), or bronchitis,</td>
<td></td>
</tr>
<tr>
<td>pneumonitis, or oesophagitis</td>
<td></td>
</tr>
<tr>
<td>Histoplasmosis, disseminated or extrapulmonary</td>
<td></td>
</tr>
<tr>
<td>Isosporiasis, chronic intestinal (&gt;1-month duration)</td>
<td></td>
</tr>
<tr>
<td>Kaposi sarcoma</td>
<td></td>
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<tr>
<td>Lymphoma, Burkitt, immunoblastic, or primary central nervous system</td>
<td></td>
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<tr>
<td><em>Mycobacterium avium</em> complex (MAC) or <em>M kansasii</em>, disseminated or</td>
<td></td>
</tr>
<tr>
<td>extrapulmonary</td>
<td></td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em>, pulmonary or extrapulmonary</td>
<td></td>
</tr>
<tr>
<td><em>Mycobacterium</em>, other species or unidentified species, disseminated or</td>
<td></td>
</tr>
<tr>
<td>extrapulmonary</td>
<td></td>
</tr>
<tr>
<td>Pneumocystis <em>jiroveci</em> (formerly <em>carinii</em>) pneumonia (PCP)</td>
<td></td>
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<tr>
<td>Progressive multifocal leukoencephalopathy (PML)</td>
<td></td>
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<tr>
<td>Salmonella septicaemia, recurrent (non-typhoid)</td>
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<tr>
<td>Toxoplasmosis of brain</td>
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<tr>
<td>Wasting syndrome due to HIV (involuntary weight loss &gt;10% of baseline</td>
<td></td>
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<tr>
<td>body weight) associated with either chronic diarrhea (≥ 2 loose stools per</td>
<td></td>
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<tr>
<td>day ≥ 1 month) or chronic weakness and documented fever ≥ 1 month</td>
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In contrast, the WHO clinical Staging and Disease Classification System (revised 2005) classifies HIV disease on the basis of clinical manifestations. The World Health Organization (WHO) has adopted a simplified definition of AIDS in adults (1985 Bangui definition), based on the recognition of at least two major clinical signs in combination with at least one minor sign. In early 1994, taking into account better access to laboratory diagnostic methods, a positive serologic test for HIV-1 and/or HIV-2 and a broader spectrum of clinical manifestations of HIV such as tuberculosis and pneumonia were added to this definition.[1] The WHO clinical Staging and Disease Classification System is summarised in table 4.[6]
Table 4: World Health Organisation (WHO) Clinical Staging of HIV/AIDS for Adults and Adolescents (Interim Definitions)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Symptoms</th>
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| Primary HIV Infection  | Asymptomatic  
                      | Acute retroviral syndrome                                                |
| Clinical Stage 1       | Asymptomatic  
                      | Persistent generalised lymphadenopathy                                   |
| Clinical Stage 2       | Moderate unexplained weight loss (<10% of presumed or measured body weight)  
                      | Recurrent respiratory infections  
                      | Herpes zoster  
                      | Minor mucocutaneous manifestations |
| Clinical Stage 3       | Presumptive diagnosis based on clinical signs or simple investigations  
                      | -Severe weight loss (>10%)  
                      | -Unexplained chronic diarrhoea > 1 month  
                      | -Unexplained fever for > 1 month  
                      | -Oral candidiasis  
                      | -Oral hairy leukoplakia  
                      | -Pulmonary TB within 2 years  
                      | -Severe presumed bacterial infections  
                      | -Acute necrotising ulcerative stomatitis, gingivitis or periodontitis |
| Clinical Stage 4       | Presumptive diagnosis based on clinical signs or simple investigations  
|                        | Conditions for which confirmatory diagnostic testing is necessary  
                      | -Unexplained anaemia  
                      | -Neutropaenia  
                      | -Thrombocytopaenia |

Conditions for which confirmatory diagnostic testing is necessary

- Cryptococcosis, extrapulmonary
- HIV wasting syndrome (see Table 2)
  - Pneumocystic jiroveci pneumonia
  - Recurrent severe or radiologic bacterial pneumonia
  - Chronic HSV infection > 1 month
  - Oesophageal candidiasis
  - Extrapulmonary TB
  - Kaposi sarcoma
  - CNS toxoplasmosis
  - HIV encephalopathy

- Disseminated nonTB *Mycobacteria* infection
  - Progressive multifocal leukoencephalopathy
  - *Candida* of trachea, bronchi or lungs
  - Cryptosporidiosis
  - Isosporiasis
  - Visceral HSV, CMV
  - Any disseminated mycosis
  - Recurrent non-typhoid Salmonella septicaemia
  - Lymphoma (cerebral or B-cell non-Hodgkin)
  - Invasive cervical carcinoma
  - Visceral leishmaniasis
EPIDEMIOLOGY

The global epidemiologic pattern of HIV has changed dramatically over the last 25 years. Originally HIV was confined primarily to North America, Western Europe and parts of sub-Saharan Africa. Now it has spread throughout the world, with major epidemic foci in all continents and resembles the “classic” infectious disease, disproportionately affecting those most socially and economically vulnerable.[3]

The United Nations Program on HIV/AIDS (UNAIDS) and WHO estimate that more than 33 million cumulative AIDS cases in adults and children have occurred worldwide. Two broad pandemic patterns are seen: the epidemics sustained in the general populations of many sub-Saharan African countries, especially in the southern part of the continent; and the epidemics in virtually all regions outside of sub-Saharan Africa, where HIV disproportionately affects injecting drug users, men who have sex with men, and sex workers. Since 1981, more than 25 million people have died of AIDS,[1] and during 2007 alone, HIV infection and AIDS-associated illnesses killed an estimated 2 million people, including 270,000 children and left 11.4 million AIDS orphans. At the end of 2007 women accounted for 50% of all adults living with HIV worldwide, and for 59% in sub-Saharan Africa.[7]

In July 2007, a UNAIDS/WHO report found that the global percentage of people living with HIV has stabilised since 2000, however the overall number of people living with HIV has increased as a result of the ongoing number of new infections each year and wider use of antiretroviral therapeutics. Sub-Saharan Africa remains most heavily affected by HIV, accounting for 72% of all AIDS deaths. Out of all HIV infected people worldwide, 67% of adult cases, and 90% of infected children are from sub-Saharan Africa.[7]

There has been some success in the fight against HIV, with the rate of new HIV infections stabilising, or declining, in several countries. Behavioural trends and significant declines in HIV prevalence among young pregnant women in urban and rural areas of 5 countries (Botswana, Cote d’Ivoire, Kenya, Malawi, Zimbabwe) suggest that prevention efforts are having an impact in several of the most affected countries. Unfortunately the number of new infections continues to increase in other African
countries and outside of Africa infections are on the rise in a number of countries.[8]

The latest statistics on the world epidemic of AIDS & HIV were published by UNAIDS/WHO in July 2008, and refer to the end of 2007.[7]

Table 5: AIDS and HIV: Global Statistics[7]

<table>
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<tr>
<th>2007 statistics</th>
<th>Estimate</th>
<th>Range</th>
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<tr>
<td>People living with HIV/AIDS</td>
<td>33.0 million</td>
<td>30.3-36.1 million</td>
</tr>
<tr>
<td>Adults living with HIV/AIDS</td>
<td>30.8 million</td>
<td>28.2-34.0 million</td>
</tr>
<tr>
<td>Women living with HIV/AIDS</td>
<td>15.5 million</td>
<td>14.2-16.9 million</td>
</tr>
<tr>
<td>Children living with HIV/AIDS</td>
<td>2.0 million</td>
<td>1.9-2.3 million</td>
</tr>
<tr>
<td>People newly infected with HIV</td>
<td>2.7 million</td>
<td>2.2-3.2 million</td>
</tr>
<tr>
<td>Children newly infected with HIV</td>
<td>0.37 million</td>
<td>0.33-0.41 million</td>
</tr>
<tr>
<td>AIDS deaths in 2007</td>
<td>2.0 million</td>
<td>1.8-2.3 million</td>
</tr>
<tr>
<td>Child AIDS deaths in 2007</td>
<td>0.27 million</td>
<td>0.25-0.29 million</td>
</tr>
</tbody>
</table>
Table 6: AIDS and HIV: Regional statistics[7]

<table>
<thead>
<tr>
<th>Region</th>
<th>Living with HIV/AIDS</th>
<th>New infections</th>
<th>Adult (15-49yo) prevalence</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-Saharan Africa</td>
<td>22.0 million</td>
<td>1.9 million</td>
<td>5.0%</td>
<td>1.5 million</td>
</tr>
<tr>
<td>North Africa &amp; Middle East</td>
<td>380,000</td>
<td>40,000</td>
<td>0.3%</td>
<td>27,000</td>
</tr>
<tr>
<td>Asia</td>
<td>5 million</td>
<td>380,000</td>
<td>0.3%</td>
<td>380,000</td>
</tr>
<tr>
<td>Oceania</td>
<td>74,000</td>
<td>13,000</td>
<td>0.4%</td>
<td>1,000</td>
</tr>
<tr>
<td>Latin America</td>
<td>1.7 million</td>
<td>140,000</td>
<td>0.5%</td>
<td>63,000</td>
</tr>
<tr>
<td>Caribbean</td>
<td>230,000</td>
<td>20,000</td>
<td>1.1%</td>
<td>14,000</td>
</tr>
<tr>
<td>Eastern Europe &amp; Central Asia</td>
<td>1.5 million</td>
<td>110,000</td>
<td>0.8%</td>
<td>58,000</td>
</tr>
<tr>
<td>North America, Western &amp; Central Europe</td>
<td>2.0 million</td>
<td>81,000</td>
<td>0.4%</td>
<td>31,000</td>
</tr>
<tr>
<td>Global Total</td>
<td>33.0 million</td>
<td>2.7 million</td>
<td>0.8%</td>
<td>2.0 million</td>
</tr>
</tbody>
</table>
HIV TRANSMISSION

HIV is spread by contact with infected blood or blood products. The most common modes of infection are sexual transmission at the genital or colonic mucosa, exposure to other infected fluids such as blood or blood products, transmission from mother to infant, and occasionally, accidental occupational exposure[9]. During sexual contact, the virus can cross the mucosal barrier of the vagina, vulva, penis, and rectum [10] or through micro-breaks or disruptions of the genital, anorectal, oral or parental epithelium or mucosa. In the developing world, HIV transmission during unprotected heterosexual intercourse accounts for more than 85% of new HIV infections, whereas in the industrialised world HIV predominantly affects injecting drug users, men who have sex with men, and sex workers. Occupational risks to healthcare workers occur mainly through accidental needlestick injuries or mucosal splash with contaminated blood. As of 2007, there were 57 documented cases of HIV seroconversion secondary to occupational exposure within the US.[11]

The risk of infection during intercourse can vary depending on the infectiousness of the source partner and also the susceptibility of the recipient partner. Infectiousness of the transmitter is a composite result of viral load in genital (or rectal) fluids, the tropism of the virus, and the infectivity of that virus. After deposition of the virus on the recipient mucosa, the outcome is affected by properties of the virus: its retention of infectivity while traversing the mucus and extracellular matrix, its affinity for entry receptors, the fusogenicity of its envelope protein, and the efficiency of its interaction with dendritic cells (DCs) that can promote virus spread to, and amplification in, CD4+ T cells, locally and possibly at distant sites[12].

Biologic factors that increase the probability of HIV transmission from an infected person to a susceptible individual include a high viral load, as seen in primary infection or advanced-stage disease, concurrent sexually transmitted infections (STIs), anal intercourse, genital trauma, menstruation, lack of male circumcision[1] and cervical ectopy[13]. STIs make recipients more susceptible by breaking down the mucosal barrier in the case of genital ulcer disease and by increasing the number of susceptible cells in the mucosa through inflammation[12]. A cross-sectional study conducted by Auvert et al in 2001 to identify risk factors for HIV infection in four urban populations in
sub-Saharan with different levels of HIV infection found that in men, current or recent (within the past 12 months) infection with herpes simplex virus (HSV-2), syphilis or genital ulceration increased the prevalence of HIV infection. In women, HSV-2 infection, syphilis, gonorrhoea or trichomoniasis made them more likely to have HIV infection.[14] A systematic review of 68 studies conducted between 1986 to 2006 found that the number of sexual partners, a history of paid sex, and infection with HSV-2 or other STIs each showed significant associations with HIV infection and are as important to HIV transmission in advanced HIV epidemics as in early epidemics.[15]

A greater percentage of women are HIV positive or face disproportionate risk for infection. This is partly due to female physiological characteristics, such as the large amount of mucosal surface area that is exposed to seminal fluid [10], but also because of gender inequalities, such as violence against women and economic vulnerability limiting access to health care resources. High-risk behaviours such as substance abuse, unprotected sex with multiple partners and sex workers, coupled with social norms that perpetuate lack of sexual knowledge among women, stigmatise condom use, and reinforce the subordination of women, place both sexes at increased risk for infection[3].

There is a risk of transmitting HIV from a pregnant mother to the foetus or newborn during pregnancy, during delivery, or by breast feeding. Although the exact ways the virus is transmitted are unknown, scientists think it may happen when the mother's blood enters foetal circulation or by mucosal exposure to the virus during labour and delivery[16]. Documented rates of transmission of HIV-1 from mother to child vary from 13% to 48%. Reasons for such variations probably involve multiple factors such as immunologic status and viral load of the mother, maternal vitamin A deficiency, ingestion of HIV-infected maternal blood or amniotic fluid by the newborn at the time of delivery, and ingestion of infected breast milk[1].

A summary of factors that can influence the spread of HIV is given in Table 7.
Table 7: Factors Influencing the Spread of HIV.[3]

<table>
<thead>
<tr>
<th>Factors</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexual behaviour</td>
<td>Number of sexual partners</td>
</tr>
<tr>
<td></td>
<td>Age of sexual debut</td>
</tr>
<tr>
<td></td>
<td>Rates of casual and commercial sex</td>
</tr>
<tr>
<td></td>
<td>Behaviour of partner</td>
</tr>
<tr>
<td></td>
<td>Anal intercourse</td>
</tr>
<tr>
<td></td>
<td>Condom use</td>
</tr>
<tr>
<td>High risk behaviours</td>
<td>Substance abuse</td>
</tr>
<tr>
<td></td>
<td>Unprotected sex with multiple partners</td>
</tr>
<tr>
<td></td>
<td>Violence</td>
</tr>
<tr>
<td>Demographics</td>
<td>Migration and rapid urbanisation</td>
</tr>
<tr>
<td></td>
<td>Male to female ratio influencing sex worker contacts by men</td>
</tr>
<tr>
<td></td>
<td>Health care: resources, education, regulation</td>
</tr>
<tr>
<td></td>
<td>Poverty</td>
</tr>
<tr>
<td>Social context</td>
<td>Gender inequalities</td>
</tr>
<tr>
<td></td>
<td>Stigma and discrimination</td>
</tr>
<tr>
<td></td>
<td>Prevention and care programs</td>
</tr>
<tr>
<td></td>
<td>National responses: politically and financially</td>
</tr>
</tbody>
</table>
IMMUNOPATHOGENESIS

HIV entry, dissemination and life cycle

HIV is a retrovirus, an enveloped virus that stores its genetic material in the form of ribonucleic acid (RNA). The immediate precursor of HIV-1 is the simian immunodeficiency virus of chimpanzees (SIV<sub>cpz</sub>)<sup>17</sup> and it is believed that the retroviral HIV infection of humans arose through primate-to-human species-jumping events (zoonoses), occurring in Central and West Africa. These events most likely occurred at multiple times, with the more recent attaining major epidemic significance<sup>9</sup>.

HIV belongs to a subtype of retroviruses known as lentiviruses or “slow” viruses. The course of infection with these viruses is characterised by a long interval between the initial infection and the onset of serious symptoms<sup>18</sup>. Like all viruses, HIV can replicate only inside cells and uses a replication strategy to transcribe the viral RNA into linear double-stranded deoxyribonucleic acid (DNA) with subsequent integration into the host genome. The characteristic enzyme used for this process, reverse transcriptase, is an RNA-dependent DNA polymerase that reverses the flow of genetic information<sup>9</sup>.

During the course of HIV-1 infection, the error-prone viral polymerase generates genetically diverse quasispecies, including the strains designated R5 and X4, which use the CCR5 and CXCR4 coreceptors, respectively, to infect cells. Mainly R5 strains are transmitted, whereas X4 strains emerge later and are associated with a rapid progression to AIDS. Epithelial cells selectively capture R5 HIV-1 and then transfer infection to CCR5-expressing target cells underneath the epithelia. These target cells include T cells, dendritic cells (DC) and macrophages. Evidence indicates that in vivo, both intraepithelial and submucosal DCs and CD4+ T lymphocytes are the predominant cell populations first targeted by SIV and HIV-1<sup>18</sup>.

Transmission of HIV-1 begins with the attachment of the virus to the host CD4+ T cell, the key effector cells in the host’s immune response. The HIV-1 envelope glycoproteins (gp120 and gp41) sequentially bind CD4 and the chemokine coreceptors CCR5<sup>19</sup> and CXCR4<sup>20</sup>, leading to a membrane fusion reaction between the viral envelope and the plasma membrane of the target cell<sup>21</sup>. The HIV virion then enters the cell and releases
its RNA. Once inside the cell, reverse transcriptase reverse transcribes a single-strand viral RNA molecule into viral DNA. The viral DNA copy is then degraded into a smaller functional piece that can then be inserted into the genome of the host during the process of integration[22]. Here HIV integrase, in conjunction with host cellular cofactors, splices the viral DNA into the host’s DNA[23]. Integration of viral DNA establishes a linear copy of the viral genome in the genome of the cell, and replication of the virus occurs along with replication of the cell. Synthesis of new viral RNA genomes and proteins is accomplished in a highly regulated manner utilising host cell enzymes. Integration is generally for the life of the cell and, with the cell and its progeny, for the life of the organism[9]. In addition, the viral replication process is prone to error and produces new mutations each day[24] and over time the diversity within the viral genome increases, enabling escape from the host’s immune response.

Upon successful addition of viral DNA to the host genome of an activated cell, the virus can replicate and go on to make viral proteins. If the host CD4+ T cell is not activated, it is possible for the virus to persist in a latent stage within the T cell for many years[10]. A number of cellular and viral factors are required to activate viral promoters and facilitate virus production. External factors, including inflammatory cytokines, coinfection with other agents, and cellular activation, may enhance viral replication by inducing viral genome expression or suppressing the innate immunity and allowing a high level of viral expression[25].

The activated cell makes viral proteins by copying DNA back to RNA via a process called transcription. Using cellular enzymes, such as RNA polymerase II, transcription of the provirus is initiated. The viral messenger RNA (mRNA) and genomic RNA transcripts, processed by cellular machinery, are then spliced by HIV protease, capped, polyadenylated, and transported to the cytoplasm for translation into viral proteins. Assembly of mature viral particles, virions, occurs at the cell membrane where they characteristically bud from the cell surface into the surrounding media, completing the life cycle of the virus[9]. A single cell can make thousands of infectious particles of HIV, either chronically, over weeks, or as a single burst resulting in cell death[10].
As mentioned earlier, dendritic cells are also prime targets for HIV-1, expressing CCR5, dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN) and other C-type lectin receptors (CLRs). Their role in the initiation of HIV infection likely includes capturing virions at sites of entry and then carrying them to the paracortical regions of lymphoid organs[26]. Dendritic cells capture HIV-1 through the C-type lectin receptors (CLRs) and are productively infected by a CCR5-dependent mechanism. Captured virus can be internalised and then rapidly transmitted to nearby CD4+ T cells [18]. The DC-SIGN present on dendritic cells can also facilitate the initial contact of dendritic cells with resting T cells[27] and binds the HIV-1 envelope glycoprotein. The DC-SIGN can capture HIV, retaining the infectious virus on their surface for extended periods of time, and transports it to lymph nodes without promoting viral entry or replication. The dendritic cell then either presents the HIV to activated T cells[28, 29], or to resting CD4+ T cells that become activated through their interaction with the dendritic cells[26]. Within the lymphoid tissue, the virus can then replicate in the absence of HIV-specific immune responses and subsequently spread to other organs and peripheral tissues.
Dendritic cells thus greatly facilitate HIV infection of T cells and play an important role in the amplification and dissemination of the infection.

Figure 2 is an overview of the sexual transmission of HIV to women and acute HIV infection. Shown are the major stages and events in transmission. R5 viruses from a viral quasispecies of R5 and X4 viruses are selectively transmitted. After crossing the cervicovaginal mucosal barrier, DCs, CD4+ T cells and macrophages in the underlying submucosa are infected. Infection is subsequently propagated and disseminated, thereby establishing the lymphatic tissue reservoir that spreads infection to other organs and peripheral tissues. Innate and adaptive host defences (left column) are directed at the different stages to prevent transmission and contain infection[18].
Host defences

The host response to HIV infection and disease is complex and poorly understood. Research of long-term nonprogressors and exposed yet uninfected persons have helped to elucidate the mechanisms by which some persons have slow rates of disease progression or are protected from HIV acquisition[30]. The cellular immune response plays a vital role in HIV pathogenesis and current evidence suggests an important role of cytotoxic T cells and T-helper cells in controlling viraemia, slowing disease progression, and perhaps in preventing establishment of infection. CD8+ T cells are an important component of the immune system-mediated restriction of HIV replication. With help from HIV-specific CD4+ lymphocytes (T helper cells), the HIV-specific CD8+ T cells recognise, and kill, HIV infected cells that present viral peptides associated with HLA class 1 molecules. Cytotoxic T-cell lymphocytes (CTLs) are specialised CD8+ T-cell lymphocytes that kill target cells expressing foreign antigen. Naïve CTLs that recognise the HIV antigens are selectively activated, proliferate and become functional killer cells[4]. Within the first few months of HIV infection, MHC-1 restricted, HIV-specific, CD8+ CTL responses are found in the peripheral blood, and are also detected during the chronic phases of infection, in the majority of HIV-infected individuals[26].

The role of cell-mediated immunity in preventing transmission and slowing disease progression remains unclear. The combination of low viral load, persistent CTL activity, and lack of disease progression is consistent with the hypothesis that CTLs have a protective effect, but the degree to which CTLs contribute to lack of progression is uncertain. In primary infection, development of an HIV-specific CTL response is temporally correlated with control of viraemia. Recent studies have shown an inverse correlation between the frequency of HIV-specific CTL activity and plasma viral load at all stages of HIV infection[4]. A study of seronegative Kenyan commercial sex workers, however, found that seroconversion eventually occurred in several women despite pre-existing HIV-1 specific CTL responses and absence of viral escape mutations. These women all described a reduction in work activity thereby decreasing their level of exposure, suggesting that ongoing antigenic exposure may be necessary for continued protection[31].
The relationship between virus-specific T-helper and cytotoxic T-lymphocyte (CTL) responses to HIV virus and their role in the host’s cellular immune response has not been clearly defined. A study examining functional HIV-1-specific memory CTL precursor frequencies and T helper proliferative responses demonstrated that T-helper proliferative responses were positively correlated with levels of CTL precursors and negatively correlated with levels of plasma HIV-1 RNA (viral load). The study also found that levels of CTL precursors seemed to depend on the presence of T-helper function[32]. HIV-specific CTLs and HIV-specific T-helper cells both seem to play a complimentary role in controlling disease progression, and persistence of functional CTLs may depend partially on preservation of the T-helper response[4].

Despite the presence of detectable activated CTLs in the peripheral circulation, HIV retains the ability to persistently and actively replicate, for reasons not fully understood. Whilst the peripheral blood contains only 2% of the total CD4+ T cell population in the body, typically effector memory cells in transit, the vast majority of CD4+ T cells reside in the secondary lymphoid tissues (e.g. lymph nodes and mucosal lymphatic tissues). During both primary infection and throughout the course of the disease, the impact of HIV replication is most profound on the CD4+ T cell population residing within these secondary compartments. By the time the patient presents with symptoms of HIV seroconversion approximately 50% of the population of the CD4+ T cell is already depleted[33].

Humoral immunity also affects outcome, although research suggests that it is not fully protective. A combination of cellular and humoral immunity, on the other hand, is thought to be crucial in the host’s immune response. The fact that viral replication persists despite the appearance of antibodies to HIV within 2 to 3 weeks after infection provides evidence that humoral immunity is unlikely to play a major role in preventing or controlling infection[4].

There are two likely explanations for the ongoing viral replication in the presence of antibody formation. Firstly, effective neutralising antibodies are usually not formed until after HIV infection is well established, and broader-acting neutralising antibodies do not occur until later into the course of infection[34, 35]. Secondly, HIV has remarkable variability (many mutations occur during the process of reverse transcriptase) and high
rates of virus turnover, contributing to phenotype diversity, and resulting in altered cell tropism and immune escape\[36-38\]. Antibody-dependent cell-mediated cytolysis\[39\] and antibody activated complement-mediated lysis of virions\[40\] have also been linked with temporary control of viraemia during acute infection, although the extent of these responses are uncertain. Some studies have found significantly higher titres of such antibodies in nonprogressors than in rapid progressors\[41, 42\]. Different studies, on the other hand, have not shown a difference in antibody levels\[43-45\].

The role of other local factors, such as mucosal immunity, in preventing HIV transmission and slowing disease remains unclear. Dendritic cells are believed to play a critical role in the initial mucosal infection and further amplification and dissemination of the HIV-1, as discussed earlier. Recent studies have shown that epithelial Langerhans cells in the genital mucosa are the first subset of dendritic cells to encounter HIV-1, and that they may in fact have a protective function in intact mucosa by scavenging invading HIV-1 and preventing Langerhan cell infection and viral dissemination. There is evidence that Langerin, a C-type lectin on Langerhans cells that captures HIV-1 similar to the DC-SIGN of dendritic cells, can internalise HIV-1 (at low virus concentrations) into Birbeck granules with subsequent degradation of HIV-1, inhibiting transmission \[46\].

The presence of ulcerative and non-ulcerative sexually transmitted infections, such as herpes simplex virus-2 and syphilis, at the time of inoculation is known to increase the risk of HIV seroconversion most likely due to inflammation, ulceration, and breakdown in local barriers \[14\]. Concomitant infection may also increase the risk of HIV transmission and disease progression secondary to nonspecific systemic inflammation and release of cytokines that produce systemic immune activation. These factors may affect the outcome by recruiting inflammatory cells to the site of transmission, encouraging HIV-1 uptake and also by up-regulating HIV replication within the infected host cells \[4\].

A number of genetic host factors have been found to affect susceptibility to infection with HIV or the rate of progression to disease once infection is established. There has been extensive research into mutant chemokine receptors or ligands that are known to be necessary co-receptors for HIV entry into CD4+ cells. Examples of such mutations
include CCR5-Delta32, CCR2-V64I, stromal cell-derived factor-1 3'alpha, and CCR5 promoter polymorphisms, or certain HLA types, all of which have been shown to affect susceptibility to infection and subsequent clinical course [4, 30]. Similarly, other cytokines and soluble factors, which can be stimulatory or inhibitory or both, help to determine the balance of HIV replication within the host [30] and further highlight the complex host response to HIV exposure and infection.
Immune dysfunction

The course of HIV varies widely; the median time from infection to AIDS is 8-10 years, however 5-10% of those infected will become long-term nonprogressors, whilst another 10% will progress rapidly, developing AIDS within 5 years. There are multiple factors that influence disease progression, and these factors may also play a possible role in an individual’s initial risk of HIV acquisition. These include characteristics unique to the virus itself, such as virulence, and individual factors including the host’s genetics, immune responses and cytokine milieu.

Following successful transmission of HIV into the host, acute or primary HIV infection occurs within days to weeks with the establishment of a vigorous immune response. This period is characterised by a high viraemia and the recruitment of a large number of activated CD4+ T cells (that inadvertently support HIV replication). Throughout this time the patient may be asymptomatic or they may develop an acute retroviral syndrome, displaying non specific symptoms, such as fever, fatigue, pharyngitis, rash, lymphadenopathy or aseptic meningitis. The host’s immune response, over the next few weeks to months, partially controls viral replication and a hypothesised viral ‘set point’ is reached, where there is a balance between production and destruction of virions. Studies have shown that the ‘set point’ is inversely correlated to disease progression.[47] After the viraemia reaches its peak, the CD4 cell count acutely decreases, returning to near normal levels.

During the latent stage, a variable period of time (usually years) during which viral replication persists, the host remains largely asymptomatic whilst billions of virions and CD4 cells are produced and destroyed daily, despite vigorous HIV-specific cell-mediated and humoral immune responses. This process, known as viral escape, is multifactorial and there have been many mechanisms described that may play a role. These include: antigenic variation[4]; downregulation of MHC molecules on the surface of HIV-infected cells[47]; disappearance of initially expanded HIV-specific CD8 cells through clonal exhaustion[48]; persistence of CD4 infected cells in immune privileged sites (CNS, eye, testis) avoiding exposure to effector immune cells; lymph-node follicular dendritic cells can trap HIV; and the presence of a latent reservoir of infected resting
memory T cells that do not express antigen therefore are not recognised by effector immune cells.

Once the absolute CD4 count falls below 200 cells/cu mm[5] progression to disease occurs and the individual becomes susceptible to opportunistic infections. With decreasing CD4 cells counts, viraemia steadily increases. Ultimately profound immunosuppression and dysfunction across the entire immune system occurs, summarised in table 8 below[26].

Table 8: Immune Dysfunction Caused by HIV Infection

<table>
<thead>
<tr>
<th>System</th>
<th>Disruption</th>
<th>Increased activity</th>
<th>Decreased activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoid tissue</td>
<td>Architecture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>Redistribution</td>
<td>Destruction Apoptosis Lymphocyte turnover Autoimmune phenomena Bystander phenomena</td>
<td>Production</td>
</tr>
<tr>
<td>CD8+ T cells</td>
<td>Numbers Functions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-lymphocytes</td>
<td>Activation</td>
<td>TNF-α, IL-6 and cytokine secretion</td>
<td>Antigenic response</td>
</tr>
<tr>
<td>Natural Killer Cells</td>
<td>Defective killing of target cells</td>
<td>Viral suppression</td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Function</td>
<td>Candida infections</td>
<td>Nonoxidative killing</td>
</tr>
<tr>
<td>Monocyte-macrophages</td>
<td>Viral reservoir</td>
<td>CD4+ T cell dysfunction Host defence against intracellular pathogens</td>
<td></td>
</tr>
<tr>
<td>Dendritic cells</td>
<td></td>
<td>CD4+ T cell infection Viral dissemination</td>
<td></td>
</tr>
</tbody>
</table>
HIV PREVENTION

Recently the Lancet published a 6 paper series on the prevention of HIV. The following section is a summary of these articles.

History of HIV prevention

HIV prevention remains one of the world’s top public health and development priorities. The initial characterisation of the disease as gay-related immunodeficiency disease (GRID) created a strong worldwide stigma. It was associated with marginalised populations, sexual transmission and death, intensifying the discrimination of infected, and at risk, individuals and creating denial worldwide. As a consequence, spread of HIV during the early years of the pandemic progressed essentially unabated, despite significant knowledge on the transmission on HIV. Most established health, development, social services, and faith organisations took some years to address the sensitive social factors influencing HIV transmission (sexual behaviour, drug use, gender inequalities, community structures and systems), mistakenly underestimating the pandemic’s rapid spread. It took the people affected by AIDS and community groups to initiate the early responses to HIV/AIDS, promoting awareness, condom use, and sexual education. Their success stemmed from confrontation of the stigma, discrimination, and denial associated with the disease.[49]

It was not until 1987 that WHO finally launched the Special Programme on AIDS to “direct and coordinate the global response to the pandemic”. [50] Within 3 years, the Global Programme of AIDS (GPA) became the largest programme in WHO’s history, formulating a global HIV/AIDS strategy and providing a practical framework to develop common policies to confront the pandemic. GPA supported a broad range of prevention strategies and widely promoted condom use, sex education in schools, simplified treatment of other sexually transmitted infections, and needle exchange programmes.[49]

Around the year 2000, there were a number of significant developments that triggered resurgence on the importance of tackling HIV prevention: the availability of effective treatment and the recognition that HIV/AIDS had both development and security implications. The threat to global security with the spread of HIV into Russia, China and
India prompted concern that HIV/AIDS could destabilise global political and economic systems. The World Bank, recognising the potential threat HIV posed to development, increased its HIV/AIDS-related commitments from $500 million in 1998 to $4 billion today, with most of these funds being allocated to Sub-Saharan Africa. The objective of the 13th international AIDS conference in Durban, South Africa, 2000, was to raise the global awareness of Africa’s AIDS-related mortality and the number of children being orphaned by HIV/AIDS, stressing the need for accessible, affordable antiretroviral drugs. In a move largely to reduce the numbers of AIDS-orphans, a number of politically powerful religious groups embraced the need for global treatment.[49]

With the increased political and financial support, prevention successes have been reported in a number of countries after significant changes in sexual behaviour. The number of new HIV infections occurring in Kenya, Cambodia, Zambia, India, Zimbabwe, India and Haiti have decreased, providing evidence that barriers to scaled-up prevention efforts can be overcome. Common themes in their response to HIV prevention were seen and a number of key messages have been learnt from their successes: it is important to address sensitive social factors surrounding HIV prevention, such as sexual behaviour, drug use, and gender inequalities, oppose stigma and minimise discrimination. These national-level prevention successes have been associated with strong government leadership and community activism, with the concerted application of combination prevention (behaviour, structural, biomedical) in a country/county-specific context. A global movement for HIV prevention that supports a combination of behavioural, biomedical, and structural approaches is now required.[49]
Behavioural approaches to HIV prevention

Behavioural change has been responsible for the HIV prevention successes to date and strategies to modify risk behaviours need to remain a main priority for HIV prevention. A 70% decrease in HIV prevalence has been seen in Uganda, and was linked to a 60% reduction in sex with non-primary partners, a 2-year delay in onset of first sex, and increases in condom use.[51] Similar actions were stimulated by local leadership from parliament, district councils, and regional AIDS coordinators in the Mbeya region of Tanzania. A decrease of 7% in HIV prevalence (20% to 13%) was seen over 10 years, its success brought about by one regional plan, enhanced surveillance for planning and assessment, and improved laboratories for testing.[52] From these two successes, three important lessons were learnt. Firstly, to reduce HIV transmission, a radical behavioural change is needed in a sufficiently large number of people who are potentially at risk. Secondly, a variety of communication channels is necessary to disseminate simple and clear messages about several risk reduction and health-seeking options. Thirdly, to help motivate behavioural change, members of the local community who are most affected by HIV should be involved in the design, production and dissemination of the HIV prevention message.[53]

There are multiple factors that contribute to increasing high-risk behaviours such as unprotected sexual intercourse and needle sharing, and can be as simple as reproduction necessitating unprotected intercourse. There are, however, many important and complex issues that underlie a persons’ choices and behaviours, such as those that are psychological (desire, pleasure, self-esteem, love, habit), physical (coercion and force), perceived or real pressures (peer, physical or psychological dependence, obligation, gender roles, custom, culture), and access to HIV prevention, information and treatment. Substance use (alcohol and illicit drugs) also plays a significant role in HIV transmission in many places and in many groups of people.[53]

For successful reductions in HIV transmission, behavioural strategy goals should target several behavioural changes and avoid emphasis on one to the exclusion of others. An important aspect of behavioural prevention involves increasing knowledge to all people, especially those at high risk, aiming to reduce stigma and encourage disclosure of HIV serostatus. People who are unaware of their serostatus are very likely to transmit a high
proportion of infections, and evidence from all countries shows that individuals who know their serostatus reduce high-risk behaviour and take precautions to protect their partners. A good prevention programme should therefore provide HIV prevention information, enable early recognition of STI or HIV symptoms, teach and reinforce harm reduction strategies, and provide up to date information about biomedical interventions, such as male circumcision (both its benefits and limitations), STI treatment, and the importance of adherence to antiretroviral medications. To achieve this, a behavioural strategy should increase the access to services such as counselling, HIV/STI testing and treatment, methadone maintenance, and antenatal and reproductive health services. In Rwanda and Zambia [54, 55], voluntary counselling and testing for couples has shown benefits including reduction of HIV transmission, STIs, and unintended pregnancies between couples. Other objectives of behavioural prevention aim to delay the onset of first intercourse, reduce the number of sexual partners, increase condom use and sales thereby increasing the number of sex acts that are protected, and decrease the sharing of contaminated injection equipment and substance use.[53]

Multilevel approaches have to be taken to effect behavioural change, targeting populations both uninfected and infected with HIV. For the best results, behavioural strategy programmes should be directed at different societal groups and applied in the appropriate context. These groups include couples, families, social networks, sexual networks, institutions and entire communities. Coates et al outlined three primary approaches to use peer groups and networks as agents of change: peer education, recruiting influential community leaders and instigating network-based intervention. Peer education has been shown to be especially effective in increasing condom use and reducing STIs in high-risk groups in sub-Saharan Africa and Asia, and in secondary-school students (aged 13 to 19 years old) and rural populations. By involving influential community leaders, there can be a greater acceptance and diffusion of information, influencing behavioural change. Community involvement and leadership plays a role in developing momentum, exposure, and repetition, is responsible for delivering effective, theory-based HIV prevention messages, and initiating and sustaining risk reduction conversations. Network-based interventions involve gaining access to social networks through identifying key individuals. These people are trained as peer educators who play an important role in disseminating HIV risk reduction messages throughout their networks and then assessing their effects.[53] In Eastern Europe, these interventions
have been used successfully to reduce sharing of contaminated injecting equipment between injecting drug users, and reducing unprotected sex. [56, 57]
Biomedical approaches to HIV prevention

Despite major advances in our understanding of HIV over the last 20 years, no vaccine or topical prophylaxis will be available in the foreseeable future. Existing prevention strategies, however, can be effective in reducing the risk of HIV exposure and now exist to prevent every mode of HIV transmission – sexual, blood borne (including through injecting drug use and in health care settings), and mother-to-child. Male condoms remain the gold standard for sexually transmitted HIV prevention and recently male circumcision has been recommended as a potentially valuable technology for HIV risk reduction in men. Antiviral medicines have an increasing role in HIV prevention strategies – including prevention of mother-to-child transmission, post-exposure prophylaxis, experimental regimens for pre-exposure prophylaxis, and probably secondary prevention benefits from therapeutic administration of antiretroviral medicines.[8] In order to optimise effects, maintain adherence and avoid sexual disinhibition (which could offset any positive effects brought about by an intervention), biomedical interventions should be inextricably implemented together with other behavioural and structural prevention strategies and be introduced, sustained and scaled-up within a combination framework.

A Cochrane review estimated the effectiveness of male latex condoms for prevention of HIV transmission as 85%,[58] and studies have shown that when used consistently, their effectiveness can be as high as 95%. For a number of reasons (sociocultural, economic, female subordination and gender inequality), a female’s ability to promote and enforce condom use is limited. To overcome these barriers, female-initiated methods need to be effective, easy to use, cheap, and non-toxic. A randomised control trial comparing the use of female and male condoms in women who were both supplied with either female or male condoms and counselled about their appropriate use, found that there was no difference in STI rates (gonorrhoea, chlamydia, syphilis, trichomoniasis).[59] Despite these findings, the uptake of female condoms remains poor due to mechanical difficulties and cost. Another female-initiated physical barrier, the diaphragm, has an unknown role in protection from HIV transmission, however it may be a potential option for women unable, or unwilling, to use male or female condoms. Currently, there are studies assessing its possible role in the delivery of topical antimicrobial and antiretroviral
products, with the assumption that this combination could be more effective than the antimicrobial alone.[60]

Male circumcision is the only biomedical intervention that has achieved level 1 scientific evidence for effectiveness. Male circumcision is traditionally practiced for religious, cultural, and medical reasons, and its prevalence within a population can vary significantly by geographical location, by religious affiliation, and by socioeconomic classification, and can be less than 5%, to more that 80%.[60] Three randomised controlled trials have provided strong evidence that adult male circumcision confers significant protection against HIV infection. The summary ratio for the three trials was 0.42 (95% CI 0.31 – 0.57), as were the results obtained from observational studies.[61] This translates to a protective effect of 58%, which is thought to be attributable to the surgical removal of the inner foreskin epithelium, the main entry site of HIV into the penis. Current accepted wisdom is that the inner foreskin epithelium is abundantly supplied with HIV-1 target cells, is poorly keratinised, at risk of microscopic tears, exposed to vaginal secretions during intercourse, has a higher degree of susceptibility to HIV infection when compared to the outer foreskin, and provides a moist environment that might sustain the viability of pathogens.

In 2007, following the results of the three randomised controlled trials, WHO/UNAIDS recommended that male circumcision be implemented as an additional HIV risk-reduction strategy in areas with hyperendemic and generalised HIV epidemics and a low prevalence of male circumcision. Whilst such an intervention could potentially avert millions of new infections over the next ten years, there are a number of challenges that must not be overlooked. Although surgical complications are rare, and relatively minor, relatively high rates are seen after unsterile cultural circumcisions. Risk compensation in the form of increased high-risk sexual behaviour was seen in the South African trial (not in Kenyan or Ugandan trials) and there is the potential for increased HIV transmission if sex is resumed before complete wound healing occurs after surgical circumcision. It is extremely important, therefore, that surgically safe circumcisions are substituted for the traditional circumcision, and that they are performed together with intensive counselling about the ongoing need for risk-reduction behaviours (condom use) and abstention until complete wound healing.
Unfortunately, the decreased HIV transmission from females to males (during heterosexual intercourse) attributed to male circumcision was not reproduced in studies looking at HIV transmission in men who have sex with men. This is thought to be due to the higher number of micro tears that occur during anal sex and higher viral loads in rectal and anal secretions.[60]

Treatment of STIs is an important public health priority and evidence suggests that concurrent STIs facilitate HIV transmission, most likely as a consequence of HIV shedding in the genital secretions and breaches in the epithelium. Genital ulcer diseases, syphilis, chancroid, and genital herpes, have been shown to have the largest effect.[60] A meta-analysis found that prevalent HSV-2 infection was associated with a three-fold greater risk of heterosexually acquired HIV infection in the general population.[62] The effect of treating curable STIs (on decreasing HIV transmission), such as chancroid, syphilis, gonorrhoea, chlamydia, and trichomoniasis, depends on the stage of the HIV epidemic and decreases with time as the epidemic becomes established. Conversely, the attributable fraction of new HIV infections associated with HSV-2 increased with time.[60] It is believed that HSV-2 suppressive treatment of coinfected individuals might reduce HIV transmission, based on two studies of valaciclovir that demonstrated reduced shedding of HIV in genital secretions.[63, 64] The introduction of such a treatment at a population level, however, is probably unfeasible as it requires high levels of adherence and considerable funding, especially as the prevalence of HSV-2 infection is high in many low-resource settings.[60]

Since the 1990s, antiretroviral prophylaxis has played a significant role in prevention of mother-to-child (MTC) HIV transmission and works via three mechanisms of action: reducing the mother’s infectiousness by lowering maternal viral load, and providing pre-exposure and post-exposure prophylaxis to the infant, before and after birth respectively. The provision of chemoprophylaxis should occur in the framework of a comprehensive maternal health service that provides additional counselling and materials for contraception, a cost-effective strategy to further reduce MTC transmission. Research is now assessing the use of antiretroviral chemoprophylaxis for prevention of HIV transmission in other high risk populations, such as sex workers and men who have sex with men. There are a number of issues regarding the widespread use of antiretroviral agents for prevention, however, which include viral resistance, long-term toxic effects or
side effects, possibility of drug sharing or black markets in resource-constrained settings, and the ethics of recommending antiretroviral drugs for prevention in areas with potentially insufficient resources and coverage to provide such health care.[60]

Antimicrobial products could represent a future avenue of HIV prevention through the application of a microbicide into the vagina or rectum before sex. Currently, however, none have been proven to protect against vaginal or rectal HIV acquisition. At present, there are three major research directions focusing on antimicrobials for HIV prevention. They are looking into the use of antiretroviral compounds that specifically inhibit HIV replication, assessing long-acting dispersal methods, and using combination products composed of several different compounds with different mechanisms of action. No HIV vaccine of even moderate effectiveness is yet available, and most experts predict that its creation is at least twenty years away.[65, 66] The development of an HIV vaccine is extremely difficult given the extent of the genetic diversity of HIV and its capacity to escape the effects of neutralising antibodies. Some are optimistic, however, and a vaccine might be possible given that some HIV-infected individuals produce broadly neutralising antibodies and infusion of high-dose neutralising antibodies in non-human primates has been proven to protect against infection with primate lentiviruses.[67, 68]
Structural approaches to HIV prevention

Structural factors, such as physical, social, cultural, organisational, community, economic, legal, or policy aspects of the environment, directly affect HIV risk and vulnerability and have the potential to impede or facilitate efforts to avoid HIV infection. With 90% of the world’s HIV infections occurring in developing countries, it is obvious that social, economic, and political structures drive risk behaviours and shape vulnerability. Addressing such factors is therefore extremely important when trying to change the behaviours and circumstances that put individuals at risk of infection. Structural approaches to HIV prevention seek to change social, economic, political or environmental factors. Many of these factors, however, are well-established and difficult to change, like gender or income inequality and social marginalisation.[69]

The eight United Nations Millennium Development Goals (MDG) offer a framework for promoting structural approaches in HIV prevention. In 2002, the UN Secretary-General commissioned the Millenium Project to develop a concrete action plan for the world to achieve the MDG and to reverse the immense poverty, hunger and diseases affecting billions of people. The MDG address poverty and inequity while promoting basic human rights and environmental sustainability, and its goals range from halving extreme poverty by 2015 to combating HIV/AIDS, malaria and other diseases. Goal six seeks to achieve universal HIV/AIDS treatment by 2010 and halt and reverse the spread of HIV/AIDS by 2015. Most countries, however, are struggling to meet these targets.[70]

Sweat and Denison categorised the different levels of structural factors that affect an individual’s HIV risk (the probability that they will contract HIV) and vulnerability (the societal context that affects their ability to control health outcomes). Superstructural factors, such as economic development and national cultural attitude, affect a nations’ HIV risk. Structural factors, like laws and policies, affect a segment of the population, whilst environmental factors, such as living conditions and available opportunities, affect the conditions and resources of individuals. Individual factors, in turn, affect how these environmental factors are experienced.[71]

Essentially, structural approaches address factors that affect individual behaviour, rather than target the behaviour itself. They can be implemented as single policies or
programmes that are directed at changing the living conditions. Multiple structural actions can be employed simultaneously or communities can generate social or political changes that endeavour to produce change in a wider population.[69] The intervention with microfinance for AIDS & gender equity (IMAGE) study sought to reduce gender-based HIV vulnerabilities and is an example of a successful programme that used a structural approach to induce significant changes. The three main issues that were addressed were women’s financial dependence on men, female knowledge about HIV (treatment and prevention), and violence against women. IMAGE partnered with a local microfinance institution to enable women to pursue microenterprises, while offering participants HIV education and counselling, facilitating opportunities to discuss and mobilise local action against gender-based violence. In doing so, IMAGE was able to significantly reduce levels of domestic violence and improve living conditions in the form of enhanced household wellbeing, social capital, and empowerment.[72]
Combination HIV prevention: the way forward

Whilst HIV prevalence has declined substantially in a growing number of countries and regions – including Zimbabwe, Cote d’Ivoire, Burkina Faso, Thailand, Cambodia, southern India, and urban Haiti and Kenya – worldwide the current degree of HIV prevention and treatment is insufficient and the epidemic continues to intensify.[73] In order to achieve the maximum reduction in HIV risk, vulnerability and transmission at an individual and population level, sustained progress in HIV prevention requires structural approaches that support, promote and facilitate behavioural and biomedical prevention strategies. Combination prevention (behavioural, structural, and biomedical) is the best hope for successful prevention[49], requires locally contextualised approaches and needs to appreciate that the HIV/AIDS epidemic is highly dynamic. Knowledge of a country-specific nature of the epidemic as well as the community and country context is necessary, and there should be a focus on targeting where the next 1000 HIV infections are likely to come from in any given context.[73]

To maximise the effect of national HIV prevention programmes, interventions need to be improved and funding needs to be optimised. Prevention programmes need to effectively reach people at high risk of contracting HIV. To achieve this, prevention programmes need to focus on appropriate targeting, selection and delivery of the interventions. In order to select suitable interventions and target the correct population, a comprehensive understanding of the epidemiology of the virus, local and population infection trends, and the basis of high risk human behaviours in the specific population is necessary. Behaviours are usually an individual choice, however that decision is clearly affected by peers, family, community, and context.

Even the best designed programme cannot effectively prevent HIV infection if implemented poorly. A scarcity of information can make it difficult to design and implement large scale interventions, and potential challenges should be anticipated. There may be a limited capacity to measure the success, or pitfalls, of the prevention programmes, however this should not distract from the importance of scaling up national prevention programmes. Structural factors, such as political, legal, cultural, and social barriers, can hinder implementation of targeted plans and expected short comings need to be accounted for. Bad politics, including the absence of leadership, is one of the main
obstacles to effective HIV prevention. Laws, such as anti-sodomy, criminalisation of prostitution and barriers to supplying uncontaminated injecting equipment, can hinder HIV prevention and potentiate the spread of the virus. A focus should be placed on alleviating such obstacles through increasing HIV knowledge, encouraging community involvement in the planning process, prioritising the implementation of appropriate interventions and monitoring and sustaining the implemented strategies.[74]

Initiation of a nationwide emergency response has 3 major policy implications, as described by Piot et al: HIV prevention must be an integral part of a country’s development plan; multiple sectors in government and civil society must be actively engaged; and the effort must be effectively led at the highest level of the state. Piot et al described four core challenges to effective combination prevention: inadequacy of attempts to tackle sexual transmission; unwillingness to be frank with young people; difficulties of dealing rationally with drug use; and failure to eliminate mother-to-child HIV transmission.[73]

With 85% of worldwide HIV transmission attributable to heterosexual intercourse it is of utmost importance that decreasing heterosexual transmission be a priority and efforts should be directed at couples, as well as individuals. This should include promotion of HIV testing, reducing the negative consequences of a positive status (stigmatisation and violence), and finding effective strategies to promote risk-reduction. Effective HIV treatment reduces the risk of HIV transmission and should be readily accessible, and affordable, to sero-discordant couples wishing to conceive. Currently, less than 70% of countries with generalised epidemics have school-based HIV/AIDS education programmes, despite sex education having been shown to encourage delay in sexual debut and increase rates of protected sex. All young people need to have the information they need to prevent HIV infection and learn safe behaviour before they become sexually active.[73]

Intravenous drugs not only increase HIV transmission through the use of contaminated equipment, but alcohol and drug use plays a role in increasing high-risk behaviours and sustaining subcultures of risk. Dealing rationally with drug has significant barriers, largely due to the issue of governments attempting to ensure harm minimisation by intravenous drug users (IVDU), whilst at the same time reducing the supply and demand
of drugs. A number of harm reduction approaches in IVDU have been shown to reduce HIV transmission within the injecting drug community, strategies which have variable support and availability depending on the individual country’s resources and policies. These include needle and syringe programmes, opioid substitute therapy, voluntary counselling and testing, antiretroviral therapy, prevention of STIs, condom programming for users and their sexual partners, targeted information provision, hepatitis diagnosis and treatment, and tuberculosis prevention, diagnosis, and treatment.[74]

Despite the potential to eliminate the transmission of HIV from mother-to-child with the appropriate treatment, only Kenya and South Africa have programmes that reach at least half of their HIV-positive pregnant women. The rest of Sub-Saharan Africa, which contains more than 75% of the world’s HIV-positive pregnant women, is failing to provide accessible or affordable treatment to these women. Successful programmes need a strong government commitment that coordinates, supports and manages one national plan. All women should have access to comprehensive services, including: HIV testing and counselling in antenatal care settings; family-centred HIV/AIDS care; maternal, newborn, and child health; and sexual and reproductive health care.[74]

The challenge for HIV prevention today is to sustain a momentum for effective, complex, combination efforts over the long haul. Comprehensive HIV prevention should urgently include programmes that address the key drivers of the epidemic in the region, in particular those that change societal norms and create safer sexual environments – for example working towards the elimination of sexual coercion and violence – and those that reduce the vulnerability of communities and individuals, such as food security programmes or adjustments to labour migration to minimise family disruption. Importantly, HIV prevention activities must go hand in hand with HIV treatment and strategies to mitigate effects on individuals, households, and communities.[74]
CHAPTER 2: MALE CIRCUMCISION

HISTORY AND EPIDEMIOLOGY OF MALE CIRCUMCISION

Male circumcision is one of the oldest and most commonly practiced procedures worldwide, predating recorded history. Anthropologist Sir Grafton Elliot Smith suggested that circumcision originated over 15000 years ago in ancient Egypt[75] and there are records of male circumcision from at least 6000 years ago, with discoveries of Egyptian mummies and wall carvings during the 19th century.[76]

Figure 3: Ancient Egyptian Relief from Ankhmahor, Saqqara, Egypt (2345-2182 BC), representing Adult Circumcision Ceremony, reproduced from http://en.wikipedia.org/wiki/Image:Egypt_circ.jpg.[77, 78]

Traditionally, male circumcision is practiced for religious and cultural reasons (non-therapeutic male circumcision), and medical reasons (therapeutic male circumcision). It is believed that early circumcision was performed for hygienic reasons among the elite classes of Egyptians and Aztecs. [79] Historically, curing masturbation was the most
common medical indication for circumcision, however during the 19th century male circumcision was described as a treatment for ailments such as epilepsy, paralysis, hernias and insanity and quickly grew to include headache, strabismus, rectal prolapse, hydrocephalus, clubfoot, alcoholism, asthma, enuresis, and gout.[76] In several African and Oceanic societies, male circumcision is a pubertal rite of passage.[80] Ritual circumcision may also be seen as a mark of cultural identity or practiced as a ceremonial sacrifice to the gods.[81]

Today, the most frequent medical reason for male circumcision is phimosis – a stricture of the foreskin that narrows the opening and prevents it from being retracted to uncover the glans. Other less common medical indications for circumcision are otherwise untreatable paraphimosis (in which the foreskin is trapped behind the corona and forms a tight band of constricting tissue, causing swelling of the glans and foreskin), balanposthitis (an acute or chronic inflammation of the mucosal surface of the foreskin) and balanitis xerotica obliterans (a chronic sclerosis and atrophic process of the glans penis and foreskin – a risk factor for penile cancer and the only absolute indication for circumcision).[77] Studies have also provided evidence for preventative indications for male circumcisions. It has been shown that circumcised men are at significantly lower risk of urinary tract infections, HIV, syphilis and chancroid,[77, 82-84] and that women have a reduced risk of cervical cancer if their partner is circumcised.[85] The following Table 9 summarises the systematic reviews and randomized controlled trials of the association of male circumcision with penile infections.[77]

<table>
<thead>
<tr>
<th>Infection</th>
<th>Type of study/review</th>
<th>No. of studies</th>
<th>Relative risk (RR) or odds ratio (OR) (95% CI)</th>
<th>Strength of evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary tract infections (115)</td>
<td>Randomized controlled trial</td>
<td>1</td>
<td>OR = 0.13 (0.01–2.02)</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Systematic review and meta-analysis</td>
<td>12</td>
<td>OR = 0.13 (0.08–0.20)</td>
<td>++</td>
</tr>
<tr>
<td>Chancroid (103)</td>
<td>Systematic review</td>
<td>7</td>
<td>RRs from 0.12 to 1.11(^b)</td>
<td>++</td>
</tr>
<tr>
<td>Syphilis (100)</td>
<td>Systematic review and meta-analysis</td>
<td>14</td>
<td>RR = 0.67 (0.54–0.83)</td>
<td>++</td>
</tr>
<tr>
<td>HIV (130–134)</td>
<td>Randomized controlled trial</td>
<td>3</td>
<td>RRs from 0.40 to 0.52</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Systematic review and meta-analysis of observational data</td>
<td>15</td>
<td>RR = 0.52 (0.40–0.68)</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Systematic review of observational data</td>
<td>19</td>
<td>RRs from 0.12 to 1.25(^c)</td>
<td>++</td>
</tr>
<tr>
<td>HSV-2 (102)</td>
<td>Systematic review and meta-analysis of observational data</td>
<td>7</td>
<td>RR = 0.86 (0.77–1.01)</td>
<td>++</td>
</tr>
<tr>
<td>HPV (135)</td>
<td>Systematic review and meta-analysis of observational data</td>
<td>8</td>
<td>OR = 0.57 (0.39–0.82)</td>
<td>++</td>
</tr>
</tbody>
</table>

a. For meta-analyses of HIV infections in adults, only studies with adjusted RRs are included, as crude RRs are likely to be misleading due to potential confounding with behaviour and other factors. The meta-analyses of chancroid, syphilis and HSV-2 include best estimates of effect, which are the adjusted RR if it was available, otherwise the crude RR.

b. Protective effect in 6 out of 7 studies, of which 4 were significantly protective.

c. Protective effect in 18 out of 19 studies, of which 14 were significantly protective.
Modern day circumcision also had violent beginnings. The bible describes David bringing King Saul 200 foreskins of his vanquished foes to prove his worthiness (I Samuel 18)[76]. There are reports from centuries ago of Indians and Somalians, who overthrew British soldiers and Colonial explorers respectively, forcibly circumcising their captives as “trophies of victory”. A warrior in the Moghul Empire reportedly “rose in rank according to the number of foreskins he brought in from the field”. It was through fear, and later through a perceived health and moral advantage, that the British Governor of India and Council of Madras ordered all cadets circumcised. During the Turkish invasion of Armenia in 1915, all Armenian men were forcibly circumcised, and in Nazi Germany, circumcision status determined whether a Jew was deported or not. [76]

To this day male circumcision, with its important individual and social implications, remains a hotly contested practice. The prevalence of males circumcised within a population can vary significantly by geographical location, by religious affiliation, and by socioeconomic classification, and can be less than 5%, to more that 80%.[60] The British Royalty began circumcising male heirs during the 19th century, creating both a trend and a social status difference. By the start of world war two, approximately 80% of upper class males in the United Kingdom were circumcised, in comparison to only 50% of working class men.[80] With the introduction of a nationalised healthcare system in 1948, and the omission of publicly funded non-therapeutic male circumcision, the United Kingdom rates dropped dramatically, with European rates also dropping in 1949. In the early 1970s, the Australian and Canadian rates fell following the Australian Paediatrics Association and Canadian Paediatric Society recommending against routine circumcision.[76] In 2007, the Federal Prohibition of Genital Mutilation Act of 2007 bill was submitted in the USA to enforce protection from male circumcision, or genital mutilation, citing evidence of physical and psychological damage.[80]

Common determinants of male circumcision are ethnicity, perceived health and sexual benefits, and the desire to conform to social norms. Currently, the estimated rates of male circumcision globally is approximately 30%, with an estimated two thirds of these men being Muslims (living mainly in Asia, the Middle East and North Africa), and 0.8% are Jewish. In several countries, the prevalence of non-religious circumcision has undergone rapid increases and decreases, reflecting changing perceptions of health and
sexual benefits, and cultural mixing. The estimated number of males aged 15 years or older circumcised for non-religious reasons, by country, is summarised in Table 10 below.[77]

THE PREPUCE

During development, the hindgut and the allantois (a diverticulum from the endoderm of the yolk sac) meet in a common cavity at the caudal end of the embryo, forming the cloaca, which is bounded distally by the cloacal membrane. The cloaca is divided into two, as the urorectal septum grows downwards from the dorsal wall of the allantois to meet the cloacal membrane: at the front, the urogenital sinus and urogenital membrane; and at the back the anorectal canal and the anal membrane. The urogenital sinus (derived from endoderm) has three unequal-sized parts, and it is the lowest of phallic part of the sinus that becomes the dorsal part of the penis and penile urethra. At the front of the urogenital membrane (which breaks down) is a midline mesodermal swelling, the genital tubercle, which becomes the glans penis. Leading back from the tubercle on either side are the urogenital folds that unite at the back to form the midline raphe of the scrotum, and at the front unite from the scrotum forwards as the ventral part of the penis and penile urethra.[86] Embryologically, the glans penis has developed by four to six weeks’ gestation and the skin of the body of the penis begins growing forward at about eight weeks’ gestation and eventually covers the glans. Initially there is no separation of the epithelium between the glans and the foreskin. The fused mucosa of the glans penis and the inner lining of the prepuce separates gradually over years, as a spontaneous biological process.[87] Complete separation of the foreskin from the glans creates the preputial space, however separation of the epithelial layers may be only partially complete by birth, and may not be fully retractable until several years after birth. In approximately 90% of uncircumcised males, the foreskin is retractable by 5 years of age.[88]

The skin of the penis is hairless and is prolonged forward in a fold, the prepuce, which folds inwards upon itself to attach to the coronal sulcus of the glans penis (its slightly projecting lower margin) and cover some or all of the rest of the glans. The prepuce is a specialized, junctional mucocutaneous tissue which marks the boundary between mucosa and skin and is composed of muscle, nerves, blood vessels, dermis, and mucosa.[87] The dorsal artery of the penis supplies the skin and glans. Venous return from the penis is partly by of veins that accompany the arteries and join the internal pudendal veins, but mostly the deep dorsal vein which pierces the suspensory ligament, passes above the perineal membrane and enters the vesicoprostatic venous plexus. The superficial dorsal vein (beneath the skin in the midline of the prepuce) drains the dorsal skin of the penis.
and divides to join the superficial external pudendal and great saphenous veins. Lymphatics from the penile skin pass to join the superficial inguinal lymph nodes, but the glans drains to the deep inguinal nodes. The skin of the penis and prepuce is supplied by the pudendal nerves via the posterior scrotal and dorsal nerves; the later supply the glans. The dermatome mainly involved is S2. The sympathetic nerves (necessary for the initial stages of ejaculation) are derived from the L1 segment of the spinal cord, and the pelvic splanchnic nerves (S2, 3) provide the parasympathetic supply to the cavernous tissue of all three corpora and allow increased blood flow for erection.[86]

The unique innervation of the prepuce establishes its function as an erogenous tissue. The glans penis has primarily protopathic sensitivity and is primarily innervated by free nerve endings. Protopathic sensitivity refers to cruder, poorly localized feelings, including pain, some temperature sensations and certain perceptions of mechanical contact. In contrast, the ridged band of the male prepuce at the mucocutaneous junction has a high concentration of encapsulated somatosensory receptors. The innervation difference between the protopathic sensitivity of the glans penis and the corpuscular receptor-rich ridged band of the prepuce is part of the normal complement of penile erogenous tissue. [87]

The male prepuce is composed of five layers, as demonstrated in Figure 4 below. The mucosal epithelium of the male prepuce is the same as the squamous mucosal epithelium that covers the glans penis. The lamina propria is very vascular and has loose collagen, and the underlying dermis of the prepuce consists of connective tissue, blood vessels, nerve trunks, Meissner corpuscles within the papillae, and scattered sebaceous glands. The elastic tissue of the prepuce dermis, along with the dartos muscle and frenulum, tether the prepuce and help return it to its anatomically correct position after deployment during erection or after manual retraction. The outer epithelium of the prepuce consists of stratified squamous cells, that are keratinized, and melanocytes within the basal layers. Langerhans’ cells and Merkel cells are also present. As described previously, the Langerhans’ cells are the first line of the body’s immune defence system and are required for normal immune function.
Figure 4; Male Prepuce with Five Layers, reproduced from *The prepuce*, Cold, C.J. and J.R. Taylor, BJU Int, 1999.

Mucosa (M), lamina propria (LP), dartos muscle (D), dermis (DE) and glabrous outer epithelium (E).[87]
SURGICAL REMOVAL OF THE FORESKIN

Different techniques are employed to remove the prepuce, or perform male circumcision. At first, Jewish faith only required removal of the tip of the foreskin. Throughout history, however, their practice has progressed from originally removing the only the tip, to then include tearing of the frenulum, to the current practice of complete removal of the foreskin.[80]

In adults, circumcision can be done under general anaesthesia or with local anaesthesia, by blocking the dorsal nerves at the base of the penis and circumferentially infiltrating the superficial layers of the penile base. In men and older boys, the favoured surgical technique is a sleeve circumcision. The foreskin is retracted and any adhesions between the inner mucosal layer and the glans are separated. With the foreskin in its retracted position (backward to the limb of the coronal sulcus), a marking pen outlines an incision, leaving a small preputial cuff. This mark should go straight across the base of the frenulum.[89] The foreskin is stretched, and to facilitate haemostasis, is gently compressed with a straight clamp at the marking. At this level the foreskin is sharply divided with straight scissors.[90]

Alternatively, two Halsted mosquito haemostats are placed at the 2 o’clock and 10 o’clock positions. With fine curved scissors, a dorsal slit is made down to the level of the coronal sulcus.[90] This incision is made and carried through the dartos fascia to the superficial lamina of Buck’s fascia. The foreskin is reduced, and a second incision is marked, following the outlines of the coronal margin and the V of the frenulum on the ventral side. The frenulum usually retracts into a V.[89] Alternatively; the ventral skin can be incised to the level of the frenulum, dividing the foreskin into two halves that can then be circumferentially excised at the level of the coronal sulcus. Bleeding vessels are ligated with 3-0 absorbable sutures as the incision is deepened and the skin edge mobilized. The use of electrocautery should be avoided because it runs the risk of deep coagulation of vessels in the base of the penis.[90] In older boys and adults, the vessels are more substantial and not easily sealed by compression, no matter how vigorous. Thus, circumcision clamps can be ineffective and are not recommended even though larger sizes are available.
After the sleeve of preputial skin has been removed, haemostasis is obtained and the skin edges are re-approximated.[89] The skin is aligned to the inner mucosa, ensuring that no twisting has occurred, and these two layers are sutured together with interrupted 3-0 absorbable sutures (such as plain catgut). A 2- to 4-mm cuff of tissue should remain around the coronal sulcus – the remnant foreskin. Any persistent bleeding can be addressed by placing interrupted simple sutures between the mucosa and the skin. A non-adherent dressing is placed around the wound.[90] Figure 5 summaries the important steps in a surgical circumcision.


A: With a fine-curved scissors, a dorsal slit is made down to the level of the coronal sulcus.
B: The frenular vessel is ligated with 3-0 absorbable suture.
C: The skin is aligned to the mucosa, and these two layers are sutured together with interrupted 3-0 absorbable suture such as plain catgut.[90]
Circumcision may lead to complications which range from minor to severe, however complications should be uncommon. The rates of complications reported in several large case series are low, from 0.2% to 0.6%,[91] however published rates range from as low as 0.06%[92] to as high as 55%[93]. Most patients develop some hyperesthesia of the glans, which resolves. A haematoma is probably the most common immediate complication, as well as easily controllable bleeding [94, 95] and local infection. Some patients notice minor cosmetic imperfections that are functionally insignificant. One of the most distressing problems that is seen, however, is a patient who complains that the surgeon has removed too much skin. To avoid this, a circumcision should be done precisely, and the incisions should first be marked with the skin lying undistorted on the shaft.[89] Other rare complications include amputation of the glans, [94-96] acute renal failure, [97] life-threatening sepsis and, rarely, death.[94, 95]
MALE CIRCUMCISION SIGNIFICANTLY REDUCES THE RISK OF HIV ACQUISITION

Since the 1980s, the protective effect of circumcision against HIV has been confirmed by more than 30 studies.[98] It was observed that male circumcision practices vary throughout Africa, and that the HIV rates varied accordingly.[98] Multiple cross-sectional, prospective, and ecologic (population-level) studies have identified a lack of male circumcision as a risk factor for HIV infection.[99, 100] From these studies, it was noticed that in countries in West Africa, where male circumcision is common, the prevalence of HIV was well below those of countries in eastern and southern Africa with low circumcision rates[98, 101, 102], and it was identified that circumcised men have a lower prevalence of HIV infection than uncircumcised men[21].

In 2001, Auvert et al conducted a study to identify factors that could explain differences in rate of spread of HIV between different regions in sub-Saharan Africa. The study took place in two cities with a relatively low HIV prevalence (Cotonou, Benin and Yaounde, Cameroon), and two cities with a high HIV prevalence (Kisumu, Kenya and Ndola, Zambia). They found that concurrent sexually transmitted infections, especially syphilis and HSV-2, male circumcision, condom use and certain sexual practices (such as anal intercourse, “dry sex” and rate of partner change) positively influenced the transmission of HIV. They also concluded that the efficiency of HIV transmission as mediated by biological factors, specifically concurrent sexually transmitted infections and male circumcision status, outweighed differences in sexual behaviour in explaining the variation in the rate of spread of HIV between the four cities, and suggested that substantial reductions in HIV incidence and prevalence may be achieved by reducing the prevalence of HSV-2 infection, and by circumcising all men in the high HIV prevalence cities.[14]

Subsequently, three randomised controlled trials have provided strong evidence that adult male circumcision confers significant protection against HIV infection.[103-105] Auvert et al conducted a randomised control trial in South Africa, where 3,274 uncircumcised men were randomised to a control or an intervention group. Immediately after randomisation, male circumcision was offered to the intervention group, and to the
control group at the end of the follow-up. The trial was stopped early at 18 months, however, when it was found during early analysis that there were only 20 new HIV infections in the intervention group, in contrast to 49 in the control group. These results corresponded to a relative risk of 0.40, or a protection of 60%, and when controlling for behavioural factors, condom use and health-seeking behaviour, the protection was 61%. Bailey et al conducted a randomised controlled trial of 2784 men in Kisumu, Kenya, and discovered the relative risk of HIV infection in circumcised men was 0.47, corresponding to a reduction in the risk of acquiring an HIV infection of 53%. In Rakai, Uganda, Gray et al enrolled 4996 uncircumcised, HIV-negative men into a randomised trial that found a protection of 51%. The summary ratio for the three trials was 0.42 (95% CI 0.31 – 0.57), as were the results obtained from a meta-analysis of observational studies which also found an adjusted relative risk of 0.42. In 2007, following the results of the three randomised controlled trials, the joint United Nations program on AIDS and the World Health Organisation (WHO/UNAIDS) recommended that male circumcision be implemented as an additional HIV risk-reduction strategy in areas with hyperendemic and generalised HIV epidemics and a low prevalence of male circumcision.

Currently, male circumcision is the only biomedical prevention intervention that has achieved level 1 scientific evidence for effectiveness. It is hypothesised that the foreskin promotes the initial survival and eventual transmission of HIV and a number of other viral and bacterial pathogens. It has been shown that the inner foreskin epithelium is poorly keratinised and is abundantly supplied with the HIV-1 target cells, Langerhans’ cells, that have been observed to play an important role in the initial transmission of HIV-1. The moist nature of the area between glans and foreskin invites viral replication, and micro tears, or tiny abrasions of the inner surface of the foreskin during intercourse can provide a portal of entry for HIV, thereby putting uncircumcised men at a much higher risk of infections than if they had been circumcised.
Figure 6: The Main Entry Sites of HIV into the Penis, reproduced from *Potential HIV-1 target cells in the human penis*, McCoombe, S.G. and R.V. Short, Aids, 2006. Diagram of the uncircumcised penis in flaccid (a) and erect (b) state. The main entry sites of HIV into the penis are shown by the red lines. When the penis is erect the inner foreskin covers a large portion of the shaft, exposing the HIV entry sites.
CHAPTER 3: THE REMNANT FORESKIN

JUSTIFICATION FOR THE RESEARCH

To date, there has been no investigation characterising the epithelium of the remnant foreskin. This project will generate an evidence base for how HIV is transmitted in a circumcised male.

HYPOTHESIS

The reduced acquisition of HIV in circumcised men is attributed to surgical removal of the inner foreskin. This protection is incomplete, partly due to the presence of a “remnant foreskin”.
BACKGROUND

The protection incurred by male circumcision is thought to be attributable to the surgical removal of the inner foreskin epithelium, the main entry site of HIV into the penis. Current accepted wisdom is that the inner foreskin epithelium is abundantly supplied with HIV-1 target cells, is poorly keratinised,[101] at risk of microscopic tears,[102] exposed to vaginal secretions during intercourse, has a higher degree of susceptibility to HIV infection when compared to the outer foreskin,[109] and provides a moist environment that might sustain the viability of pathogens[103]. Dendritic cells within the epithelium of the inner foreskin are believed to play a critical role in the initial mucosal infection and further amplification and dissemination of HIV-1.

During the course of HIV-1 infection, the error-prone viral polymerase of HIV generates genetically diverse quasispecies, including the strains designated R5 and X4, which use the CCR5 and CXCR4 coreceptors, respectively, to infect cells. Mainly R5 strains are transmitted, whereas X4 strains emerge later and are associated with a rapid progression to AIDS. Epithelial cells selectively capture R5 HIV-1 and then transfer infection to CCR5-expressing target cells underneath the epithelium. These target cells include T cells, dendritic cells and macrophages. Evidence indicates that in vivo, both intraepithelial and submucosal dendritic cells and CD4+ T lymphocytes are the predominant cell populations first targeted by HIV-1.[18]

Recent studies have shown that epithelial Langerhans cells in the genital mucosa are the first subset of dendritic cells to encounter HIV-1[101] and may have a variety of functions in HIV-1 transmission. Studies have shown that Langerhans’ cells can become infected by HIV-1, facilitating HIV-1 infection of the host via transmission of HIV-1 to T cells.[110, 111] Other studies have shown that immature Langerhans’ cells may also prevent HIV-1 infection by clearing invading HIV-1 through the C-type lectin langerin, a receptor that captures, then internalises HIV-1 into Birbeck granules, in turn leading to virus degradation. Langerin, however, may be inhibited by antibodies or high viral loads which then allows Langerhans’ cell infection with HIV-1. Langerhans’ cells capture virions by means of a c-type lectin, Langerin, virions are internalised and subsequently degraded. However, the availability of Langerin is finite and when saturated, in the case
of a large viral load, this virucidal mechanism becomes compromised. Under these conditions, Langerhans’ cells facilitate the transport of virus to the afferent lymphatics where subsequent systemic infection is established. This suggests that Langerhans’ cells may be protective against HIV-1 infection at low viral concentrations but at a high viral concentration the langerin is saturated, inhibiting its protective function and allowing transmission to T cells.[46, 112, 113]

The remnant foreskin is an area of tissue that has been poorly described histologically, and we were interested to see whether the remnant foreskin adopted properties similar to the inner foreskin or the penile shaft skin and what influence this could have on the transmission of HIV through the penis. We defined the remnant foreskin as the tissue between the circumcision scar (proximally) and the coronal sulcus (distally), as illustrated by D in Figure 7 below.[89]
Figure 7: Surgical Circumcision: Creation of the Remnant Foreskin, reproduced from *Campbell’s Textbook of Urology, 9th edition*, Jordan, S., 2008.[89]

A, Appearance before dorsal slit.

B, Incision in the dorsal aspect of the foreskin, which has been drawn over the glans. The broken line indicates the point of incision for removal of the foreskin.

C, Suture in the frenulum.

D, Closure (and formation of the remnant foreskin).
AIM

The purpose of this study was to characterise the remnant foreskin. It is postulated that male circumcision provides significant, but incomplete, protection against HIV infection. We wanted to know if the presence of a remnant foreskin could account for the ongoing transmission of the virus into the penis or does the formation of a remnant foreskin help reduce HIV transmission after circumcision? We wanted to determine the structure and composition of the remnant foreskin epithelium in circumcised men, and compare this to the epithelium of the inner foreskin and penile shaft. We also wanted to determine, and compare, the distribution of Langerhans’ cells in the remnant foreskin to the inner foreskin and penile shaft skin.

The questions we posed were:

1. What is the epithelial structure of the remnant foreskin? Does it possess properties of the inner foreskin or eventually develop a structure similar to penile shaft skin?

2. Is there any difference in Langerhans’ cell distribution across the penile epithelium, and if so, could this difference explain the decreased rates of HIV transmission occurring in circumcised men? We compared Langerhans’ cell density between the following sites:
   - Remnant foreskin
   - Inner foreskin
   - Penile shaft skin

3. Given that male circumcision is only partially protective against HIV acquisition, could the remnant foreskin be a site for ongoing HIV transmission, or does the removal of the inner foreskin, with the subsequent formation of a remnant foreskin, account for the protection achieved by circumcision?
METHODS

The study, “Characterisation of the remnant foreskin – implications for HIV transmission in circumcised men”, (Project Number H2008/03197) was approved by the Austin Hospital Human Research Ethics Committee for the period 4th of June 2008 until the 4th of June 2011 (see Appendix 1). Written and informed consent was obtained from all men who participated in this trial.

Tissue

All consenting patients who participated in this trial were recruited from the Austin and Repatriation Medical Centre (ARMC, Heidelberg, Australia) Urology preadmission clinics. To characterise the remnant foreskin, we required biopsies from the remnant foreskin of men who had been previously circumcised in childhood. All of the men involved were on the ARMC Urology elective surgery waiting lists booked for alternative urological procedures (vasectomies, excision of a scrotal lipoma and excision of scrotal cysts). Biopsies of the penile shaft skin were also taken from the men who supplied the remnant foreskin biopsies. To compare these samples to the inner foreskin, we required biopsies from the inner foreskin of uncircumcised men. These men were also recruited from the ARMC Urology elective surgery lists awaiting circumcisions.

10 uncircumcised men, mean age 37.3 (22-58), who were planned for elective circumcision at the ARMC were recruited to our trial. At their preadmission clinic, after consent was obtained, 2mm punch biopsies were taken from their inner foreskin. With the patient lying supine, the foreskin was retracted and the inner foreskin and surrounding area was sterilised using a chlorhexidine antiseptic skin wash. 0.5ml of local anaesthetic (1% lignocaine) was infiltrated into the inner foreskin. After anaesthesia was confirmed, a 2mm punch biopsy was taken from the ventral aspect of their inner foreskin, in the midline approximately half way between the coronal sulcus and the most distal portion of the inner foreskin mucosa. All biopsies were taken from approximately the same position in all patients. The biopsy was immediately fixed in formalin after sampling, and transported to the laboratory on ice for further processing. Any bleeding at the biopsy site was controlled at the time using local pressure. Patients
were advised to administer pressure if there was any further bleeding and to notify myself if there were any complications. No complications were reported.

10 men, mean age 34.8 (23-41), undergoing elective vasectomy at the ARMC, were recruited to our trial. All men had been previously circumcised in childhood. At the time of their vasectomy, after consent had been obtained, 4mm punch biopsies were taken of their remnant foreskin and their penile shaft skin. After the patient’s vasectomy had been completed (without complication), additional cetrimide skin antiseptic preparation was administered to the remnant foreskin, penile shaft skin and the surrounding region. If the patient was under general anaesthetic, no further local anaesthetic was administered. If the patient had received local anaesthesia with a penile block (2% lignocaine) and sedation, a further 1ml of local anaesthesia (2% lignocaine) was infiltrated into the remnant foreskin and penile shaft skin where the planned biopsies were to be taken. A 4mm punch biopsy was taken from the dorsal aspect of the remnant foreskin, approximately 1 cm from the midline, and a 4mm punch biopsy was taken from the dorsal aspect of the penile shaft, near the midline approximately 2 cm from the coronal sulcus (Figure 2). All biopsies were taken from approximately the same position in all patients. All biopsies were immediately fixed in formalin after sampling, and transported on ice to the laboratory for further processing. Bleeding was controlled with a single skin suture (3.0 monocryl) and patients were told to notify myself if there were any complications. No complications were reported.

All biopsies were immediately fixed in formalin after sampling. They were then embedded in paraffin prior to sectioning and staining. All samples were sectioned at 8 μm.
Figure 8: Biopsy sites, reproduced from www.blumenthalclinic.com.au.[114]
Table 11: Tissue Samples. Each Patient was Assigned a Code for Confidentiality Purposes. This Table Details the Tissue Sample Code, Patient Age, Operation Performed, Biopsy Site and Date of Collection.

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<td>Remnant foreskin</td>
<td>27/06/2008</td>
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<td>30/06/2008</td>
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<td>Inner foreskin</td>
<td>18/09/2008</td>
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Keratin and structural staining

All biopsies were immediately fixed in formalin and transported to the laboratory on ice, where they were then fixed in ethanol. After washing, tissue blocks were transversely oriented and embedded in paraffin for histological analysis. Embedded tissues were sectioned at 8 μm using a microtome and then placed on electrostatically charged microscopic slides. Tissue sections were stained specifically for keratin using the Haematoxylin and Eosin stain. Stained 8 μm sections were examined under light microscopy at 20 x magnification. We analysed 3 sections of each shaft and remnant biopsy, and 4 sections of each inner foreskin biopsy. Digital images were obtained using a confocal microscope. A randomly offset grid was superimposed onto the digitalised section using ImageJ, and measurements of the stratum corneum (or keratin layer) and the epithelium were taken where the grid intersected the epithelium. 10 measurements of the keratin and epithelial layers were taken from each. In total, 2000 measurements were taken.

Table 12: Tissue Sections

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<td>Inner</td>
<td>10</td>
<td>4</td>
<td>SC = 10 Ep = 10</td>
<td>800</td>
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2000
Figure 9: An Example of a Haematoxylin-Eosin (H&E) Stain of a Section from a Remnant Foreskin Biopsy.

The white arrows indicate the measurements that were taken of the stratum corneum (SC) and the epithelium (Ep).
Immunohistochemistry (IHC): Langerhans’ cell staining

All biopsies were immediately fixed in formalin, subsequently fixed in ethanol at the laboratory and then embedded in paraffin (transversely oriented). Embedded tissues were all sectioned at 8 μm using a microtome and sections were placed on electrostatically charged microscopic slides.

Langerhans’ cell density was quantified using an anti-CD1a antibody, staining immature Langerhans cells. The slides of embedded tissues, sectioned at 8 μm, were dewaxed and rehydrated, antigens retrieved with sodium citrate, and endogenous peroxidise activity quenched. Sections were blocked with 10% normal goat serum, and incubated for 1 hour. Primary antibody (anti-CD1a, Immunotech, Marseille, Cat. No. 1590) was used with a negative IgG control, and incubated overnight at 4°C. Secondary antibody (goat anti-mouse – 1:500 dilution) was then added to the sections and incubated for 1 hour at room temperature. This was followed by ABC reagent that was added to the sections for 30 minutes at room temperature. AEC (chromogen/substrate; Dako) was added to the sections and incubated for a further 30 minutes at room temperature. All sections were counterstained with haematoxylin.

Sections were examined under light microscopy at 20 x magnification and digital images were obtained using a confocal microscope. We analysed 6 sections of each biopsy (10 remnant foreskin biopsies, 10 inner foreskin biopsies and 10 penile shaft skin biopsies). A total of 180 sections were examined. The number of Langerhans’ cell bodies were counted in one frame of each section (0.58mm$^2$), and values were converted into cells per square millimetre (cells/mm$^2$).
Figure 10: An Example of a Section from a Remnant Foreskin Biopsy, Staining Immature Langerhans’ Cells.
The white arrows indicate the Langerhans’ cell bodies.
Statistical analyses

Two-tailed, unpaired t tests were used to determine differences between epithelial thickness, keratin (or stratum corneum) thickness, and Langerhans’ cell distribution in the various penile epithelia tested (inner foreskin, remnant foreskin, penile shaft skin).
RESULTS

Epithelial thickness

10 biopsies were obtained from the remnant foreskin and penile shaft of 10 circumcised men, and 10 biopsies were obtained from the inner foreskin of 10 uncircumcised men. This study reported to epithelial thickness for all 3 biopsy sites. We found that the epithelial thickness of the remnant foreskin, inner foreskin and penile shaft skin were all similar. The mean thickness of the remnant foreskin (R) was 115.9μm (SE, 8.6μm), the mean thickness of the inner foreskin (I) was 112.9μm (SE, 9.6μm), and the mean thickness of the penile shaft skin (S) epithelium was 104.1μm (SE, 9.7μm). When a two-sample t test was applied to these values, we found that there was no significant difference in epithelial thickness between the remnant foreskin, inner foreskin or penile shaft skin (two-sample t test: R vs S, p = 0.38; S vs I, p = 0.53; R vs I, p = 0.82).

Figure 11: Epithelial Thickness of the Remnant Foreskin, Inner Foreskin and Penile Shaft Skin.
Stratum corneum thickness

In this study the same 10 biopsies that were obtained from the remnant foreskin and penile shaft of 10 circumcised men, and the 10 biopsies obtained from the inner foreskin of 10 uncircumcised men, were examined to determine the thickness of the stratum corneum, or keratin layer, for all 3 biopsy sites. Again we found that the stratum corneum thickness of the remnant foreskin, inner foreskin and penile shaft skin were all similar. The mean thickness of the remnant foreskin (R) stratum corneum was 14.7µm (SE, 1.1µm), the mean thickness of the inner foreskin (I) stratum corneum was 15.4µm (SE, 1.2µm), and the mean thickness of the penile shaft skin (S) stratum corneum was 13.2µm (SE, 0.8µm). When applying a two-sample t test to these values, there was no significant difference in keratin thickness found between the remnant foreskin, inner foreskin, or penile shaft skin (two-sample t test: R vs S, p = 0.32; S vs I, p = 0.15; R vs I, p = 0.66).

Figure 12: Stratum Corneum Thickness of the Remnant Foreskin, Inner Foreskin and Penile Shaft Skin.
Figure 13: Typical H&E Stains of the Remnant Foreskin, Penile Shaft Skin and Inner Foreskin, Demonstrating their Similarities.
Langerhans’ cell distribution

Using an anti-CD1a antibody, the distribution and density of immature Langerhans’ cells in the remnant foreskin, inner foreskin and penile shaft skin were analysed. Langerhans’ cells were distributed throughout the squamous epithelium and were observed at high densities in the inner foreskin epithelium (31.1 cells/mm$^2$, SE: 2.9 cells/mm$^2$). A lower density of Langerhans’ cells was observed in the penile shaft skin epithelium (mean 19.2 cells/mm$^2$, SE: 3.6 cells/mm$^2$). In comparison to both the inner foreskin epithelium and the penile shaft skin epithelium, there were scant Langerhans’ cells seen within the remnant foreskin epithelium (mean 10.1 cells/mm$^2$, SE: 1.7 cells/mm$^2$). We found that there was a significantly higher density of Langerhans’ cells within the inner foreskin than both the penile shaft (p < 0.02) and the remnant foreskin (p = 0.00001). The remnant foreskin, in fact, had a significantly lower number of Langerhans’ cells than the penile shaft skin as well (p < 0.01). Two-sample t test: R vs S, p < 0.01; S vs I, p < 0.02; R vs I, p = 0.00001.

Figure 14: Langerhans’ Cells/mm$^2$ of the Remnant Foreskin, Inner Foreskin and Penile Shaft Skin.

![Bar chart showing Langerhans’ cells/mm$^2$ for Remnant, Inner, and Shaft skin with cell counts of 10.1, 31.3, and 19.2 respectively.](chart)
Figure 15: Typical Immunohistochemistry Stains of the Remnant Foreskin, Penile Shaft Skin and Inner Foreskin, Demonstrating the Scarcity of Langerhans’ Cells within the Remnant Foreskin, in Comparison to both the Penile Shaft Skin and the Inner Foreskin.
DISCUSSION

Novel observations from this series of experiments provide possible biological explanations to the protection against HIV acquisition seen in circumcised men. Firstly, the inner foreskin (which is removed during surgical circumcision) contains a high density of Langerhans’ cells. Recent studies have shown that epithelial Langerhans’ cells in the genital mucosa are the first subset of dendritic cells to encounter HIV-1. The roles of the Langerhans’ cells, however, are varied. At low HIV load, they may have a protective function. Within the intact mucosa, the Langerhans’ cell scavenge invading HIV-1, internalise the virus into Birbeck granules via Langerin, a C-type lectin on the surface of the Langerhans’ cell, with subsequent degradation of the HIV-1, preventing Langerhans’ cell infection, transmission and viral dissemination.[46] At higher HIV loads, or in the presence of antibodies, Langerin, however, may be inhibited which then allows Langerhans’ cell infection with HIV-1, facilitating HIV-1 infection of the host via transmission of HIV-1 to T cells.[110, 111] This suggests that Langerhans’ cells may be protective against HIV-1 infection at low viral concentrations but at a high viral concentration the langerin is saturated, inhibiting its protective function and allowing transmission to T cells.[46, 112, 113] By removing the inner foreskin, and its Langerhans’ cells, this site of HIV-1 entry into the penis is eliminated.

Secondly, the remnant foreskin contains very few Langerhans’ cells. In fact, in comparison to the inner foreskin, the remnant foreskin is relatively devoid of these HIV-1 target cells. As described previously, we defined the remnant foreskin as the tissue between the circumcision scar (proximally) and the coronal sulcus (distally). The remnant foreskin is formed after circumcision by the approximation of the remaining inner foreskin mucosa to the penile shaft skin. The remnant foreskin will undergo a process of healing, with complete wound healing taking between an average of 4 to 6 weeks. We believe that after undergoing healing, the remnant foreskin will sustain a variable amount of mechanical “wear and tear” that contributes to the development of the permanent remnant foreskin structure. Despite these variables, we found that there was very little difference in the structure of the 10 different remnant foreskins that were analysed. The size of this area of tissue on each individual, however, was inconsistent and depends on the amount of foreskin that was
initially removed at circumcision and the amount of inner foreskin left that was sutured to the penile shaft skin.

We were surprised to find that the epithelial and stratum corneum structure at all sites were similar, and that there was no significant difference in the epithelial, or keratin, thickness between the inner foreskin, the remnant foreskin or the penile shaft skin. We found that the degree of keratinisation of the inner foreskin did not differ significantly from either the penile shaft skin epithelial keratinisation or the thickness of the remnant foreskin keratin layer. It has previously been accepted wisdom that the inner foreskin of the penis was poorly keratinised and that this structural difference could enable HIV-1 to readily interact with the underlying target cells, enhancing HIV-1 transmission. It has been postulated that the inner foreskin could be the primary site of HIV-1 infection because it is poorly keratinised. We do not believe this to be the case. Alternatively, we believe that the increased HIV-1 acquisition seen in uncircumcised men is more likely to be attributed to higher density of Langerhans’ cells within the inner foreskin, in comparison to the remnant foreskin and penile shaft skin, and that the degree of keratinisation does not play a significant role in increasing the vulnerability of the inner foreskin.

Current accepted wisdom is that the inner foreskin epithelium is abundantly supplied with HIV-1 target cells, is poorly keratinised, at risk of microscopic tears, exposed to vaginal secretions during intercourse, has a higher degree of susceptibility to HIV infection when compared to the outer foreskin, and provides a moist environment that might sustain the viability of pathogens. This study has shown that what was previously accepted wisdom regarding the keratin thickness of the inner foreskin is incorrect. We found that the epithelial and keratin thickness of the inner foreskin did not differ significantly from either the remnant foreskin or the penile shaft skin. In keeping with current evidence, we found that the inner foreskin has a high density of Langerhans’ cells, which play an important role in HIV transmission in the penis. Remarkably, we found that the remnant foreskin, the small area of tissue formed by the approximation of the inner and outer foreskin at surgical circumcision, has a significantly smaller amount of Langerhans’ cell within its epithelial, in comparison to both the penile shaft skin and the inner foreskin. In fact, relative to the inner foreskin, there is an astonishing scarcity of Langerhans’ cells in the remnant foreskin.
The purpose of this study was to characterise the remnant foreskin, and there were a number of questions that we asked in order to determine the properties of the remnant foreskin and its role in HIV transmission. Firstly we wanted to determine the epithelial structure of the remnant foreskin. We found that the epithelial structure of the remnant foreskin was similar to both the inner foreskin and the penile shaft skin. Specifically, we found that the thickness of the epithelium and keratin at all sites was similar. These similarities suggest that after circumcision, the epithelium of the remnant foreskin is structurally similar to the penile shaft skin. The fact that it is also structurally similar to the inner foreskin implies that there is little structural change, if any, in the epithelium after circumcision.

Our second question asked whether there was a difference in Langerhans’ cell distribution across the penile epithelium, and if so, could this difference explain the decreased rates of HIV transmission occurring in circumcised men? As described, we found significantly different densities in Langerhans’ cell distribution at all sites. Importantly, the inner foreskin contained a high density of Langerhans’ cells, whilst the remnant foreskin had very few. Certainly by removing the inner foreskin and its Langerhans’ cells, and replacing this tissue with an epithelium that has very few Langerhans’ cells, as seen in the remnant foreskin, the transmission of HIV through the penis must be reduced.

Given that male circumcision is only partially protective against HIV acquisition, we also wanted to assess whether the remnant foreskin could be a site for ongoing HIV transmission after circumcision. We suggest that not only does male circumcision play a protective role in decreasing HIV transmission through the penis by physically removing the inner foreskin, which has a high density of Langerhans’ cells, but this inner foreskin tissue is replaced by a remnant foreskin that has such a low density of Langerhans’ cells that the transmission of HIV through this tissue, potentially, is greatly reduced. The fact that there are Langerhans’ cells present in the epithelium of the penile shaft, albeit significantly less than in the inner foreskin, provides a possible explanation for the ongoing transmission of HIV after circumcision. We now believe that the removal of the inner foreskin, with the subsequent formation of a remnant foreskin, could play a very important role in reducing the transmission of HIV.
through penis and could be a key biological factor in the protection achieved by circumcision.

HIV prevention remains one of the world’s top public health and development priorities, and male circumcision is the only biomedical prevention intervention that has achieved level 1 scientific evidence for effectiveness in HIV prevention. Traditionally, male circumcision is practiced for various religious, cultural, and medical reasons. The prevalence of males circumcised within a population can vary significantly by geographical location, by religious affiliation, and by socioeconomic classification, and can be less than 5%, to more that 80%.[60] Recently, three randomised controlled trials have provided strong evidence that adult male circumcision confers significant protection against HIV infection. The summary ratio for the three trials was 0.42 (95% CI 0.31 – 0.57), as were the results obtained from observational studies.[61] This translates to a protective effect of 58%, which is thought to be attributable to the surgical removal of the inner foreskin epithelium, the main entry site of HIV into the penis.

In 2007, following the results of the three randomised controlled trials, WHO/UNAIDS recommended that male circumcision be implemented as an additional HIV risk-reduction strategy in areas with hyper-endemic and generalised HIV epidemics and a low prevalence of male circumcision. Whilst such an intervention could potentially avert millions of new infections over the next ten years, there are a number of challenges that must not be overlooked. Although surgical complications are rare, and relatively minor, relatively high rates are seen after unsterile cultural circumcisions. Risk compensation in the form of increased high-risk sexual behaviour was seen in the South African trial (not in Kenya or Uganda) and there is the potential for increased HIV transmission if sex is resumed before complete wound healing occurs after surgical circumcision. It is extremely important, therefore, that surgically safe circumcisions are substituted for the traditional circumcision, and that they are performed together with intensive counselling about the ongoing need for risk-reduction behaviours (condom use) and abstention until complete wound healing. In order to optimise effects, maintain adherence and avoid sexual disinhibition (which could offset any positive effects brought about by an intervention), biomedical interventions should be inextricably
implemented together with other behavioural and structural prevention strategies and be introduced, sustained and scaled-up within a combination framework.
CHAPTER 4: SUMMARY

HIV is a leading cause of mortality worldwide and the primary cause of death in Sub-Saharan Africa. HIV/AIDS remains one of the world’s top public health and development priorities. Not only is there an urgency to improve education, prevention and treatment, but the future of the response to HIV/AIDS will also require research into, and the development of, new prevention tools that will be accessible worldwide, most importantly by the developing world. Male circumcision is the only biomedical prevention intervention that has achieved level 1 scientific evidence for effectiveness in HIV prevention. A meta-analysis found that circumcision confers a relative risk of 0.44, with a number needed to treat of 72,[106] therefore provides a degree of protection equivalent to a high efficacy vaccine. In 2007, following the results of three randomised controlled trials, WHO/UNAIDS recommended that male circumcision be implemented as an additional HIV risk-reduction strategy in areas with hyperendemic and generalised HIV epidemics and a low prevalence of male circumcision.

Our study provides biological evidence to help explain the protection incurred by male circumcision. Whilst the epithelial structure of the inner foreskin is similar to the remnant foreskin and the penile shaft skin, the distribution of Langerhans’ cells, importantly HIV target cells, is significantly different. The reduced transmission of HIV in circumcised men could be explained by the removal of the high density of Langerhans’ cells within the inner foreskin, and the subsequent formation of a remnant foreskin that has very few Langerhans’ cells. As the protection incurred by circumcision is incomplete, the ongoing HIV transmission could be explained in part by the remaining Langerhans’ cells within the penile shaft skin (significantly higher levels than in the remnant foreskin).

Whilst our study characterised the epithelial structure and Langerhans’ cell distribution across different regions of the penile skin, there are other factors that impact HIV transmission that were not looked at. Importantly, we did not have time to determine the density of other HIV target cells, such as CD4+ cells or macrophages. Nor did the scope of this study allow for direct analysis of HIV
transmission across these areas of penile epithelium. We have therefore drawn our conclusions with regards to HIV transmission (via Langerhans’ cells) from accepted wisdom published in the literature.

It is important that future research is directed to determine, and compare, the lymphocyte profile of the inner foreskin, remnant foreskin and penile shaft epithelium with other HIV-target cells, such as CD4+ cells and macrophages, and see if there is also such a significant difference in densities between the inner foreskin and the remnant foreskin. It will be fascinating to see if there is in fact a scarcity of all HIV target cells in the remnant foreskin, in contrast to an abundance of target cells in the inner foreskin, and the implications this will have on circumcision for HIV prevention.

A concurrent study being investigated by researchers at Austin Health and in the Department of Zoology, the University of Melbourne, is “The use of topical oestrogen to keratinise the inner aspect of the human foreskin”. An association between the use of topical oestrogen, subsequent keratinisation of the vaginal epithelium, and the reduction of susceptibility to HIV infection has been demonstrated by a study conducted on ovariectomised female macaques given an intravaginal inoculum of Simian Immunodeficiency Virus (SIV).[115] It is hypothesised that oestrogen topically applied to the epithelium of the inner foreskin may similarly cause keratinisation with a subsequent reduction in HIV transmission. Pending further investigation, this novel intervention could potentially be used as an alternative to, or in conjunction with, surgical circumcision. Topical oestrogen, to keratinise the inner foreskin and reduce HIV transmission, could be implemented within existing comprehensive HIV prevention programs to reduce the sexual acquisition risk of HIV in males, particularly within at-risk populations.
REFERENCES

89. Jordan, S., *Campbell's Textbook of Urology*, 9th edition, 2008(Figure 33-17).
Human Research Ethics Committee
Research Ethics Unit
Level 8 HSB – Room 8322
Austin Hospital

TO: A/Prof Damien Bolton
Urology
Austin Campus

PROJECT: Characterisation of the remnant foreskin - implications for HIV transmission in circumcised men

PROTOCOL NO: 
PROJECT NO: H2008/03197

FROM: Jill Davis Research Ethics Unit Manager

DATE: 4 June 2008


Approval Period: 4 June 2008 – 4 June 2011

Further to my letter dated 28 April 2008 concerning the above detailed project, I am writing to acknowledge that your response to the issues raised by the Human Research Ethics Committee at their meeting on 17 April 2008 is satisfactory. This project now has full ethical approval for a period of three years from the date of this letter.

For trials involving radiation to volunteers, the research must be added to the Austin Health Research with Human Volunteer’s licence issued by the Department of Human Services – Radiation Safety Section prior to commencement. The HREC must be notified when the research has been added to the licence.

It is now your responsibility to ensure that all people associated with this particular project are made aware of what has actually been approved. Any changes to the original application will require a submission of a protocol amendment to the Committee for consideration as this approval only relates to the original application as detailed above.

The Committee has requested me to make arrangement for progress reports to be submitted by the Investigator to the Committee at the end of twelve (12) months, or
sooner if the project is completed within twelve (12) months. Should your study not commence twelve (12) months from the date of this letter this approval will lapse. A resubmission to the Human Research Ethics Committee would then be necessary before you could commence.

The Committee wishes to be informed immediately of any untoward effects experienced by any participant in the trial where those effects in degree or nature were not anticipated by the researchers.

DETAILS OF ETHICS COMMITTEE:

It is the policy of the Committee not to release personal details of its members. However I can confirm that at the meeting at which the above project was considered, the Committee fulfilled the requirements of the National Health and Medical Research Council in that it contained men and women encompassing different age groups and included people in the following categories:

- Chairperson
- Lay Man
- Lay Woman
- Minister of Religion
- Lawyer
- Person with Research Experience
- Person with Counselling Experience

Additional members include:
- Nurse Administrator
- Surgeon
- Pharmacologist
- Pharmacist

I confirm that the Principal Investigator or Co-Investigators were not involved in the approval of this project. I further confirm that all relevant documentation relating to this study is kept on the premises of Austin Health for more than three years.

The Committee is organised and operates according to the Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95), annotated with TGA comments; and The National Statement on Ethical Conduct in Human Research (NHMRC The National Statement) and the applicable laws and regulations; and the Health Privacy Principles in The Health Records Act 2001. This hospital is registered under the United States DHHS Federal Wide Assurance number 00001363

PLEASE NOTE: The Committee requests that the Research Ethics Unit (ethics@austin.org.au) is informed of the actual starting date of the study as soon as the study commences. A written notice (e-mail, fax or letter) is considered the appropriate format for notification.

Jill Davis
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Author/s:
Hallamore, Sandra Leigh

Title:
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