

5. Sexually transmitted diseases

Syphilis, gonorrhoea and chancroid are generally considered as the main sexually transmitted diseases (STDs), but a wide variety of pathogens also are sexually transmissible, including Herpes simplex virus type 2 (HSV-2), *Chlamydia trachomatis*, human papillomaviruses (HPV), human T lymphotropic virus type 1 (HTLV-1), human immunodeficiency virus (HIV), and hepatitis B virus (HBV). Women, particularly adolescents, are disproportionately vulnerable to STDs, many of which remain asymptomatic, favouring spread of infection. The risk of acquiring an STD is highest in urban areas, in low socioeconomic groups, in the young, and in association with illicit drug use and prostitution. In the USA, as an example, rates of gonorrhoea and syphilis are greater for African Americans and Hispanics than in the non-Hispanic white American population, and African American and Hispanic women suffer a greater share of severe complications of these diseases, such as pelvic inflammatory disease (PID) caused by bacterial infections, and cervical cancer caused by HPV infection.

Apart from the HIV epidemic, STDs cause significant morbidity and contribute greatly to increasing health-care costs. Several common STDs adversely affect pregnancy, causing spontaneous abortions, stillbirths, preterm delivery and postpartum endometritis. Neonatal infections include gonococcal conjunctivitis which may lead to blindness, chlamydial pneumonia which may lead to chronic respiratory disease, and herpes encephalitis. Genital infection with HPV is causally associated with cervical cancer, the most common cause of cancer-related death in women throughout the world. For this reason, HPV will be discussed in Chapter 8 (*see 8.3.*).

A consensus has emerged that the prevention and control of STDs require a global initiative, the success of which will largely depend on the development of safe and effective vaccines. Currently, except for hepatitis B infection, no such vaccines exist.

5.1. *Chlamydia trachomatis*

5.1.1. Disease burden

More cases of STD are caused by *Chlamydia trachomatis* than by any other bacterial pathogen, making *C. trachomatis* infections an enormous public health problem throughout the world. In both men and women, silent, asymptomatic infection is common. The bacterium is transmitted from one partner to another by sexual intercourse. In men, *C. trachomatis* is the commonest cause of non-gonococcal (non-specific) urethritis. Conjunctivitis (that does not progress to blindness) and joint inflammation may occur. Men with asymptomatic infection serve as carriers of the disease, spreading the infection while only rarely suffering long-term health problems. Women, in contrast, are at high risk of severe complications of infection. Acute infection with *Chlamydia* can result in acute salpingitis and PID, whose long-term consequences include chronic pain, ectopic pregnancy and infertility. Contamination of the hands with genital discharge may lead to a conjunctival infection following contact with the eyes. Babies born to mothers with infection of their genital tract frequently present with chlamydial eye infection within a week of birth (chlamydial “*ophthalmia neonatorum*”), and may subsequently develop pneumonia. Various studies have estimated that there are four to five million new cases of chlamydial infection each year in the USA alone. Among urban adolescent females, the incidence rate can be as

high as 30%. The annual costs of treating and caring for patients with PID might be as high as US\$10 billion.

Worldwide, the most important disease caused by *C. trachomatis* is trachoma that affects the inner upper eyelid and cornea and is one of the commonest infectious causes of blindness. The disease starts as an inflammatory infection of the eyelid and evolves to trachomatous trichiasis (at least one eyelash rubbing on the eyeball, or loss of interned eyelashes) and blindness due to corneal opacity. In some parts of the developing world, over 90% of the population is infected. Despite long-standing control efforts, it is estimated that more than 500 million people still are at high risk of infection, over 140 million persons are infected and about 6 million are blind in Africa, the Middle East, Central and South-East Asia, and countries in Latin America. The disease is particularly prevalent and severe in rural populations living in poor and arid areas of the world where people have limited access to water and personal hygiene is difficult. Visual loss from trachoma often starts in middle life and is 2–3 times more common in women. It is therefore a major cause of disability in affected communities, attacking the economically important middle-aged female population. Trachoma is a communicable disease of families, with repeated reinfection occurring among family members.

5.1.2. Bacteriology

C. trachomatis is a small obligate intracellular bacterium found in two forms: the elementary body (EB) and the reticulate body (RB). The infectious form is the EB which lies outside cells. After attachment, EBs penetrate into their host cells where they reorganize into metabolically active and replicative RBs that accumulate by division in a large cytoplasmic inclusion. RBs then reorganize into infectious and spore-like EBs, which are released by host cell lysis or extrusion. The genus *Chlamydia* includes three species: *C. trachomatis*, an exclusively human pathogen; *C. psittaci*, which infects a variety of animals and can cause pneumonia or psittacosis in humans; and *C. pneumoniae*, a relatively common cause of LRIs in humans. Based on the type of disease produced, *C. trachomatis* has been divided into biovars, including: the lymphogranuloma (LGV) biovar, associated with *lymphogranuloma venereum*, an inguinal lymphadenopathy; and the trachoma biovar, associated with human conjunctival or urogenital columnar epithelium infections. *C. trachomatis* is further divided into serotypes (or serovars), some of which produce almost exclusively ocular trachoma in endemic countries, whereas others are associated with both ocular trachoma and genital-tract infections. The whole nucleotide sequence of the genome of *C. trachomatis* (serovar D) has been determined. *Chlamydiae* are sensitive to a number of antibiotics including erythromycin and tetracyclins. Chemotherapeutic intervention thus consists of topical (tetracyclin) or systemic (azithromycin) treatment with antibiotics. Other interventions consist of surgery of the eyelid. Global elimination of trachoma as a disease of public health importance has been targeted by WHO for 2020.

5.1.3. Vaccines

A safe vaccine administered prior to adolescence that is effective through childbearing age would have a significant impact on the spread of the disease. The lack of a suitable animal model and the difficulties in genetic manipulation of the bacterium have hampered progress in the field.

Antex Biologics has developed a subunit vaccine candidate (TRACVAX) which has been tested in a randomized Phase I trial designed to assess the safety and immunogenicity of the candidate vaccine.

Identification of potential vaccine antigens is today an active area of research which is greatly helped by the availability of the complete *C. trachomatis* genome sequence, allowing for the identification and testing of candidate proteins based on their similarity to proteins important in protective immunity against other bacterial pathogens.

5.2. Gonorrhoea

5.2.1. Disease burden

Gonorrhoea is caused by *Neisseria gonorrhoeae*. It usually is characterized by purulent genital discharge, urethritis and dysuria. Infection also can be asymptomatic, especially in women. Asymptomatic carriers are more likely to transmit the disease than people with overt infections. Similarly, anorectal and pharyngeal infections, which are not uncommon in women and men who have sex with men, frequently remain asymptomatic but constitute a potential source of transmission. Global estimated incidence of gonorrhoea is 62 million infected people annually. Complications of the disease include epididymitis in men and PID in women, with subsequent risk of infertility and ectopic pregnancy. In about 1% of cases, the gonococcus becomes invasive and a bacteraemia develops, leading to disseminated gonococcal infection characterized by skin rash and asymmetrical septic polyarthrititis. The most common manifestation of gonorrhoea in the newborn is purulent conjunctival infection (gonococcal *ophthalmia neonatorum*), which constitutes a medical emergency because blindness may rapidly ensue. The incidence of the disease has been greatly reduced by routine prophylactic administration of 1% silver nitrate eyedrops.

5.2.2. Bacteriology

N. gonorrhoeae is a gram-negative diplococcus. Specific serological reactions serve to distinguish gonococci from other species of *Neisseria* and permit serogrouping of gonococcal strains. The gonococcal liposaccharide (LPS) consists of branched oligosaccharide chains whose antigenic heterogeneity constitutes the basis of interstrain differences. The bacterial envelope is traversed by long pili constituted of repeated peptide subunits (pilin) that are characterized by both antigenic and phase variations. Antigenic variations result from chromosomal rearrangements altering the expression of any one of several silent pilin genes. Phase variation (*pi* + to *pil*-) occurs when the rearrangement involves a defective pilin gene. The predominant protein in the gonococcal outer membrane is termed protein I. This protein, which exists in two allelic forms, PIA and PIB, forms anion-selective transmembrane channels through the outer membrane and thus functions as a porin (POR protein). It is possible to divide gonococci into at least 24 PIA serovars and 32 PIB serovars on the basis of antigenic determinants on protein I. The complete nucleotide sequence of *N. gonorrhoeae* has been determined.

The life cycle of the bacterium was studied using a variety of cell culture systems. These studies have shown that the bacterium not only adheres to the epithelial cells but also penetrates and transits across the epithelial layer and exits into the subepithelial space where the symptoms of the disease are actually elicited.

5.2.3. Vaccines

The lack of a suitable animal model and the considerable antigenic variability of the bacterium have hampered the development of a vaccine for gonorrhoeal disease. Attachment of gonococci to mucosal cells is mediated in part by the pili, and it was found that rabbit antibody to pili reduces attachment of the bacteria to mammalian cells. Pilin was therefore chosen as the most likely vaccine candidate and tested for efficacy in military recruits and in volunteers challenged urethrally. This approach was met with some success, but protection

was strain-limited, due the high rates of antigenic variation of pili. Porin also was studied as a vaccine antigen but the induced anti-porin antibodies were not bactericidal.

Identification of potential vaccine antigens will hopefully be helped by the availability of the complete genome sequence, allowing the search of candidate proteins with similarity to proteins important in immunity to other bacterial pathogens.

5.3. Herpes simplex type 2

5.3.1. Disease burden

Herpes simplex virus type 2 (HSV-2) is the cause of genital herpes. The hallmark of herpesvirus infections is the establishment of a lifelong, latent infection that can reactivate to cause one or more rounds of disease. Latent HSV-2 infection occurs primarily in neurons found in the sacral root ganglia. The clinical spectrum of HSV-2 includes primary infection, characterized by the appearance of vesicles on the vulva or the penis that soon break to leave shallow, painful ulcerating lesions. The ulcers heal in 2–3 weeks, although healing may be very slow in immunocompromised patients. Primary infection is then followed by recurrent episodes of clinical disease (4–5 per year). The proportion of symptomatic infections is estimated to be between 13% and 37%, and probably higher in HIV positive individuals. Subclinical infection may be associated with infectious viral shedding. The virus is transmitted in genital secretions. Transmission of HSV-2 to newborns at the time of delivery may lead to devastating systemic infection with encephalitis. The risk of neonatal herpes fortunately is low among HIV-negative pregnant women living in industrialized countries (less than 3%), but few data are available on neonatal herpes in developing countries.

Genital herpes is one of the most common ulcerating diseases of the genital mucosa. It is estimated that in the USA, for example, from 40 to 60 million people are HSV-2-infected, with an incidence of 1–2 million infections and 600 000–800 000 clinical cases per year. Prevalence in the 30–40 year-old population is about 30%. Overall prevalence is higher in women compared with men, especially among the young. The same independent factors of HSV-2 infection were identified in both genders: older age, higher lifetime number of sexual partners, positive HIV serology and positive syphilis serology. Prevalence in developing countries can vary from 2–74% according to the country, age, gender, or urban versus rural areas. Rates up to 40% have been reported among women 15–19 years of age in rural Costa Rica, Kenya (Kisumu) and Mexico (Mexico-City). A study conducted on truck drivers in Bangladesh showed a high prevalence of HSV-2 (25.8%), compared to syphilis (5.7%), gonorrhoea (2.1%), and chlamydia (0.8%).

There is now ample evidence that HSV-2 infection is a major cofactor of HIV infection. In developed countries, where acquisition of HSV-1 in childhood has decreased, HSV-2 seroprevalence has increased, suggesting a possible protective effect of HSV-1 against HSV-2 acquisition. Although HSV-1 does not actually seem to modify the risk of HSV-2 acquisition, it appears to increase the proportion of asymptomatic seroconverters.

5.3.2. Virology

HSV-2, together with HSV-1 and the varicella-zoster virus (chickenpox), belongs to the subfamily *Alphaherpesvirinae* in the family *Herpesviridae*. These are large, complex enveloped viruses with an outer lipid envelope studded with at least 10 viral glycoproteins, an intermediate tegument layer comprising at least 15 viral proteins, and an icosahedral nucleocapsid containing the double-stranded DNA genome. The genome is organized into a 126-kb long and a 26-kb short region of double-stranded DNA bracketed by inverted repeat sequences that readily allow isomerization or recombination of the two regions. The genome

comprises some 84 open reading frames. These have been divided into immediate-early genes, whose transcription depends on a virally-encoded activating protein, VP16, and which encode the viral α proteins; the early genes, which are turned on by the α proteins and whose products (β proteins) are involved in DNA replication; and the late genes, the products of which (γ proteins) are virion structural proteins and proteins needed for virus particle assembly and egress. Some of the viral envelope glycoproteins (gD) are antigenically related to those of HSV-1, whereas most are type-specific (particularly gG1 and gG2). Numerous viral gene products, which are dispensable for virus growth in vitro, can be considered as virulence genes that are involved in preventing apoptosis in the infected host cell, blocking the induction of interferons, or downregulating the presentation of viral antigens in the context of class I histocompatibility antigens (HLAs).

When the latent state is established in the neural ganglia, transcription is severely restricted such that a single transcript is produced from the latency-associated transcript (LAT) promoter, and only a few viral proteins are made. At intervals, changes in neuronal physiology induced by trauma, hormones, stress or immune suppression, render the neurones permissive to virus replication, resulting in full transcription of the genome and a burst of progeny virions.

5.3.3. Vaccines

The prospect for developing a vaccine against HSV-2 that could provide sterilizing immunity is thought to be unrealistic. The goals of the vaccines under development are rather to prevent the establishment of latent infection by blocking access of the virus to sensory ganglia, to reduce the severity of the symptoms, and/or to reduce the frequency of recurrences. The correlates of protective immunity against HSV-2 are not entirely understood. Passive maternal antibody seems important in preventing infection of the newborn and CD4⁺ Th1 T-cells appear to be crucial to the immune response. IFN- γ secretion and CD8⁺ CTL may also play a major role, particularly in the prevention of recurrences.

HSV-2 subunit vaccines were developed based on the use of viral envelope glycoproteins.

- A two-component gB2 and gD2 recombinant glycoproteins subunit vaccine formulated in MF59 adjuvant was developed by Chiron. The 2-component vaccine induced high antibody titres and showed 26% efficacy in women for a period of six months but protection did not persist and male volunteers were not protected.
- GSK developed a single component gD2 vaccine formulated in AS04 adjuvant (alum + monophosphoryl lipid A). The gD2 vaccine induced good Th1 immunity in mice, including high IFN- γ secretion, and provided good protection against vaginal HSV-2 challenge in female guinea pigs. The vaccine was tested in two large, double-blind, controlled Phase III trials on volunteers with a partner with genital herpes disease. In the first study, 847 subjects were selected as seronegative for both HSV-1 and HSV-2, whereas in the second study the 2491 selected subjects were selected only on the basis of HSV-2 seronegativity. The vaccine was 73% efficacious against genital herpes *disease* in doubly seronegative women. Trends towards protection against *infection* were also observed, but the figures were not statistically significant (less than 48% efficacy). Most unexpectedly, however, the vaccine was not effective in women previously seropositive for HSV-1 and in men, regardless of their HSV seropositivity status. This suggests that HSV-1 immunity is protective against HSV-2, but no satisfactory explanation is available of why subunit vaccines seem to provide only gender-specific protection. Further Phase III efficacy trials of the gD2 vaccine (Herpevac) are in progress in collaboration with the NIH, involving about 7500 persons from 18 to 30 years of age,

double HSV-1/HSV-2 seronegative women. A vaccine that protects women could be expected to decrease the rate of neonatal HSV infection and have an impact on the epidemic spread of genital herpes. Lack of efficacy of vaccines in HSV-1 infected individuals would however render the vaccine of little use in developing countries, where HSV-1 infection is ubiquitous.

- A novel, live attenuated HSV-2 candidate vaccine has been developed by Xenova/GSK using a replication-impaired virus mutant that lack the gene of the essential glycoprotein gH (ICP8 gene mutation) as a disabled infectious single cycle (DISC) virus vaccine. The vaccine was tested in Phase II trials in the USA as a therapeutic vaccine in HSV-2 seropositive symptomatic patients. It was well tolerated and induced neutralizing antibodies and CTL in 83% of the vaccinees, but no difference in time to recurrence and no difference in virus shedding were observed as compared with controls. The development of the DISC vaccine has been refocused towards its use as a prophylactic vaccine.
- Another live, replication-impaired vaccine is currently under development by Avant Immunotherapeutics. Other viral mutants that are defective for replication and impaired for establishment of latency, such as mutant dl5–29, are at a preclinical stage of development.
- A live attenuated vaccine based on a replication-competent ICP10 mutant of HSV-2 developed by AuRix is in Phase II clinical study.

DNA vaccine formulations have shown incomplete efficacy in animal models. Similarly, whole inactivated virus vaccines did not show efficacy and their development has been stopped.

5.4. HIV/AIDS

5.4.1. Disease burden

The acquired immunodeficiency syndrome (AIDS) emerged in the human population in the summer of 1981. There now is convincing evidence that its agent, the human immunodeficiency virus (HIV), probably crossed the simian-human species barrier before the middle of the 19th century. At the end of 2004, the number of adults and children living with HIV/AIDS was estimated by WHO/UNAIDS to have reached 39 million worldwide. An estimated 4.8 million people (including 600 000 children less than 15 years of age) becomes infected each year, 95% of whom live in developing countries, and an annual 2.9 million people die of the disease. Today, HIV/AIDS is the leading cause of death in sub-Saharan Africa and the fourth biggest killer in the world. The number of HIV infections is equally distributed between men and women, but infection rates in young women in today's Africa are close to three times higher than those among young men, reflecting the degree to which gender inequities are driving the epidemic, as many women in developing countries lack socio-economic independence, education and access to health information and services, and have difficulty avoiding exposure to the virus.

sub-Saharan Africa remains the hardest-hit region in the world, with at least 25 million infected people, accounting for 70% of the people living with HIV/AIDS and 77% of AIDS deaths worldwide. The overwhelming majority of HIV transmission in the region stems from sexual behavior. In some African countries, overall prevalence in the adult population can be greater than 10%, with figures reaching up to 38.8% in some areas. Among the most severely hit countries are South Africa, with more than 5.6 million infected people, together with Bostwana, Mozambique, Tanzania and Zimbabwe. Highest infection rates are found among

commercial sex workers, truck drivers and seasonal migrant workers. Sub-Saharan Africa also is home to an estimated 500 000 infants who contracted HIV each year before the onset of prevention of vertical transmission by use of antiretroviral drugs: transmission from mother-to-child can occur in utero, at birth or as a result of breastfeeding. In addition, sub-Saharan Africa faces numerous wars and civil conflicts, producing large numbers of refugees who are at heightened risk of contracting HIV. A remarkable success story in the fight against AIDS was undertaken in Uganda, which was facing a severe HIV epidemic in the mid 1980s. Through voluntary HIV counselling, expanded treatment of STDs, awareness campaigns and community mobilization encouraging delayed initiation of sexual activity, monogamy and use of condoms, the level of infection declined significantly since 1992 – from nearly 30% to 11.2% in prenatal settings in Kampala and from 13% to 5.9% in clinics outside major urban areas.

The estimated number of people living today with HIV in Asia and the Pacific Region is more than seven million, but the accuracy of the figure is questionable, in view of the fast pace at which the epidemic is expanding. It has been projected that the region will contribute 40% of all new infections by the end of the decade, with China reaching 10 million infected persons, from an official 800 000 today, and India 20–25 million, from 5.1 million today. Increasing sex trade, use of illicit drugs, and rates of sexually transmitted infections contribute to an increased vulnerability in the region. Injection drug use and heterosexual intercourse are the primary modes of transmission, although improper blood donation practices in China and unsafe injection practices in health-care settings in India and surrounding countries have resulted in hundreds of thousands of infections. Substantial transmission also occurs in men who have sex with men, with prevalence rates of 14–20% reported in male homosexual communities in Cambodia, India and Thailand. Gender inequities play a major role in the epidemic as young girls are frequently steered toward sex work by their families.

The estimated number of adults and children living with HIV in Latin America and the Caribbean at the end of 2003 was two million. While in some countries HIV infections remain concentrated mainly in men who have sex with men and injecting drug users, others are experiencing increasing rates of heterosexual transmission.

The Eastern European countries continue to experience one of the sharpest increases in the number of new HIV infections, most of which occur among injecting drug users. The number of people with HIV/AIDS in the region is estimated to be 1.3 million.

In industrialized countries, highly active antiretroviral treatment (HAART) has considerably reduced disease progression to AIDS and transformed HIV/AIDS from a deadly disease to a somewhat manageable chronic disease. However, successes in treatment and care are not being matched by progress in prevention. Each year, some 75 000 individuals become infected with HIV in industrialized countries, where an estimated 1.6 million people are living with HIV/AIDS (1 million in North America alone), and where new evidence of rising HIV infection rates is emerging, particularly in marginalized communities.

5.4.2. Virology

The human immunodeficiency virus (HIV), together with the simian, the feline, and the bovine immunodeficiency viruses (SIV, FIV, and BIV, respectively), the Visna virus of sheep, the caprine arthritis-encephalitis virus (CAEV) and the equine infectious anaemia virus (EIAV), belongs to the genus *Lentivirus* in the family *Retroviridae*. These enveloped RNA viruses produce characteristically slow, progressive infections. Their replication depends on the presence of an active reverse transcriptase responsible for the transformation of the RNA genome into a DNA copy that integrates into the host cell chromosome in the

form of a provirus. The provirus is eventually transcribed into a set of mRNAs that encode the viral proteins and into progeny genomic RNA. The genome of HIV is a single-stranded positive sense RNA molecule, 9.5 kb in length, which encodes the typical retrovirus proteins Gag (further cleaved into Matrix, Capsid and Nucleocapsid proteins), Pol (itself cleaved into Protease, Reverse Transcriptase and Integrase) and Env (a 160 kD glycoprotein eventually cleaved into a gp120 external subunit and a gp41 transmembrane subunit that form together trimeric spikes on the surface of the virion). In addition, the genome encodes a variety of nonstructural proteins, such as regulatory proteins Tat and Rev and accessory proteins Nef, Vif, Vpr and Vpu. The gp120 subunit binds the CD4 receptor and CCR-5 or CXCR-4 co-receptors on the surface of target cells, whereas gp41, which anchors the spikes in the viral envelope and maintains their trimeric organization, plays a major role in fusion of the virus and cell membranes. Neutralizing human monoclonal antibodies have allowed the identification of several neutralization epitopes on gp120 that overlap the receptor or co-receptor binding sites, but they appear to be little accessible to the cognate antibodies due to hindrance by the many glycosylation motifs on the molecule as well as by the presence of hypervariable loops that act as antigenic decoys. Fusion-blocking antibodies also have been described, with corresponding epitopes located at the base of the gp41 ectodomain. Contrary to laboratory-adapted virus strains (“X4” strains) against which protection in chimpanzees could readily be obtained with neutralizing antibodies, field virus isolates (“R5” virus strains) have turned out to be extremely difficult to neutralize, casting doubt on the feasibility of a vaccine to elicit protection against infection by induction of neutralizing antibodies alone.

Two types of HIV have been described: HIV-1 and HIV-2, the latter being less virulent and geographically limited to West Africa. HIV-1 is phylogenetically close to SIVcpz, a commensal virus in chimpanzees, whereas HIV-2 is closely related to SIVmac, the agent of simian AIDS, and to SIVsm, a commensal virus in sooty mangabey monkeys. HIV-1 is further divided into three groups, M, N, and O. The vast majority of the HIV-1 strains responsible for the global pandemic belong to group M. These have further been classified in 10 subtypes, also known as clades, which have been designated by letters from A to K. HIV-1 subtype B predominates in industrialized countries as well as in Latin America and the Caribbean. Subtypes A and D are more common in Central Africa. Subtype C accounts for the majority of infections in southern Africa, parts of Eastern Africa and India. Interclade recombinant strains are relatively common and have been designated “circulating recombinant forms” (CRF). Major CRFs are CRF_AG, prevalent in western Africa, CRF_AE, which predominates in south-eastern Asia, and CRF_BC, prevalent in China. Amino acid sequence of the viral envelope glycoprotein shows 25–35% divergence between clades and up to 20% divergence within any given clade, which constitutes a formidable challenge to vaccine development.

5.4.3. Vaccines

The development of a safe and effective vaccine is hampered by the high genetic variability of HIV, the lack of knowledge of immune correlates of protection, the absence of relevant and predictive animal models, and the complexity of the implementation of efficacy trials, especially in developing countries. The first Phase I trial of an HIV vaccine was conducted in the USA in 1987. Since then, more than 30 candidate vaccines have been tested in over 80 Phase I/II clinical trials, involving more than 10 000 healthy human volunteers. Two Phase III trials have been carried to completion and a third one is in progress. Most of the effort to develop and evaluate HIV vaccines is borne by the NIH, CDC and WRAIR in the USA and by ANRS in France, with strong help from the International AIDS Vaccine Initiative (IAVI) in New York, the European Union, initiatives in WHO and UNAIDS, and the recent commitment of the Bill and Melinda Gates Foundation for a Global Enterprise. The HIV Vaccine Trial Network (HVTN) established by NIAID in 2000, with 25 clinical

sites in four continents, represents a major resource for clinical HIV vaccine research. The European Union has created the European and Developing Countries Clinical Trials Partnership (EDCTP) with the aim of helping developing countries build up their capacity in testing the efficacy of new drugs, microbicides, and vaccines.

In the absence of identified correlates of immune protection, multiple vaccine concepts are being explored in parallel.

5.4.3.1. *Live attenuated vaccines*

The observation that *nef*-deleted mutants of SIV could elicit protection against challenge with pathogenic SIV in rhesus macaques served as a model in favour of a live attenuated HIV vaccine approach. The SIV Δnef mutants, however, establish a lifelong, persistent low grade infection that does not protect the vaccinated monkeys against superinfection with wild-type virus, although the animals seem to be protected against subsequent disease. In addition, the attenuated virus still may cause AIDS when administered orally to infant monkeys. Additional deletions or mutations can further attenuate the virus but at the expense of its protective efficacy. Mostly because of safety concerns, this approach was therefore not pursued.

5.4.3.2. *Subunit vaccines*

A subunit HIV vaccine was developed based on monomeric gp120 added with alum (VaxGen). The vaccine was tested in two double-blind, controlled Phase III efficacy trials, one on 5000 volunteers at risk (mostly men who had sex with men) in the USA, with sites in Canada and in the Netherlands, using a mixture of two subtype B gp120s as the immunogen, the other on 2500 volunteers in Thailand (mostly drug users), using a mixture of a subtype E (CRF_AE) and a subtype B gp120s. None of these studies showed a statistically significant reduction of HIV infection in the vaccinees in spite of biannual booster immunizations. A reduction of the number of HIV infections was observed in certain ethnic subgroups in the first study, correlating with a higher level of anti-gp120 antibody, but the numbers were too small to provide statistical confidence. The same subtype E/B gp120 vaccine is planned to be used for booster immunizations in a prime-boost Phase III trial which was launched in late 2003 in Thailand in collaboration between the Ministry of Health of Thailand, WRAIR, Sanofi-Pasteur and VaxGen, and uses for priming a recombinant canarypox virus (ALVAC) that expresses CRF_AE gp120 and subtype B Gag, Pol and Nef antigens. The trial will enrol 16 000 heterosexual volunteers and is expected to last four years.

Other approaches aimed at eliciting HIV neutralizing antibodies are at an early clinical stage. These include the use of:

- trimeric gp140 molecules (gp120 + the ectodomain of gp41) with a deletion of the hypervariable V2 loop in order to expose the neutralization epitopes overlapping the CD4-binding site;
- oligomeric gp140 molecules covalently coupled to synthetic mimics of the CD4 receptor that should expose neutralization epitopes overlapping the coreceptor (CCR5 or CXCR4)-binding site;
- gp120/gp41 trimers internally stabilized by disulfide bond formation (SOS proteins) which should elicit both neutralizing and fusion-blocking antibodies.

Induction of fusion-blocking antibodies by immunization with recombinant oligomeric gp41 molecules is a promising new approach that still is at an early preclinical stage of development.

5.4.3.3. *Live recombinant vaccines*

Rather than attempting to elicit a neutralizing antibody response, recent HIV vaccine approaches have aimed to elicit a T-cell response, especially a CD8+ CTL response, whose role in control of virus load and evolution of disease has been well documented in the monkey model. In addition to perforin-based cellular cytotoxicity, CD8+ T-cells secrete antiviral cytokines (IFN- γ), still unidentified antiviral factors (CAF) and virus entry-blocking β -chemokines that have been correlated with protection against SIV infection in the monkey model, as well as associated to asymptomatic HIV-1 infection in humans and slower disease progression in HIV-2-infected patients. Vaccines that stimulate the T-cell arm of the immune response are however not expected to protect against infection, but rather to control its course and reduce viral loads, thus preventing or at least delaying the occurrence of symptoms. Reduction of viral loads in vaccinated but HIV-infected individuals also would hopefully result in lowering the probability of virus transmission to their partners.

Several prime-boost strategies involving priming with a DNA vaccine followed by boosting with a live recombinant vector-based vaccine have been tested in monkeys against challenge with a lethal dose of simian-human immunodeficiency virus (SHIV) that causes AIDS-like illness in the animals. These strategies resulted in reduction in virus load and provided protection against disease and death in the vaccinated animals. The same approach was however less successful in protection against SIV challenge.

A number of these vaccines also have been tested in Phase I/II trials in humans, including plasmid DNA and poxvirus vectors (MVA, fowlpox or canarypox viruses) expressing a variety of HIV antigens, such as Gag, Env, Pol and Nef.

Replication-defective adenovirus type 5 (Ad5) represents another promising vector: a recombinant Ad5-*gag/pol/nef* vaccine has entered Phase II trials on some 1200 men and 400 women at high risk who will be followed for 3 years after 3 immunizations at 0, 4 and 26 weeks (Merck).

The list of other vectors is long. It includes, among others:

- BCG (NIH Japan)
- *Salmonella* (IAVI/Institute for Human Virology, University of Maryland)
- Venezuelan equine encephalitis virus (VEEV; Alphavax)
- adenovirus-associated virus (AAV; IAVI/ Targeted Genetics)
- Sendai virus (NIH Japan)
- vesicular stomatitis virus (VSV; Yale University/Wyeth)
- Newcastle Disease Virus (NDV; Mount Sinai, New York, and Kyoto University, Japan)
- measles virus (Pasteur Institute/GSK).

The immunogenicity of the DNA and poxvirus vector vaccines in humans has usually been relatively weak, with generally less than 35% of the vaccinees scoring positive at any time point, as determined by IFN- γ ELISPOT assays. This was emphasized by the very disappointing results of a recent DNA prime-MVA boost Phase I study in Kenya which showed that the promising immunogenicity data obtained in monkeys with clade A HIV DNA and MVA constructs could not be repeated in human volunteers.

The best results so far, in terms of the percentage of human responders, level of T-cell responses and duration of immune responses have been obtained with the Ad5 vector. However, Ad5-based recombinant vaccines are confronted with the problem of a frequent pre-existing anti-vector immunity in the human population, especially in developing countries, which dampens the immune response to the HIV transgene. This has prompted the development of less prevalent human adenovirus serotypes (Ad35, Ad11, or Ad24) as nonreplicative vectors. Like Ad5, these vectors readily multiply to large yields in PRC-6 cells in fermenters. Nonreplicative chimpanzee adenovirus vectors (AdC68, AdC6 and AdC7) also are being developed. Combinations of different vectors in mixed modality prime-boost regimens will likely be developed in the future.

In rhesus monkeys, responses arising from an Ad5 priming-poxvirus (MVA or ALVAC) boosting regimen were significantly greater than those elicited by homologous regimens with the individual vectors, but this was not observed in human volunteers (Merck and Sanofi-Pasteur).

5.4.3.4. Other vaccinal approaches

Induction of persistent HIV Gag-specific CD8⁺ CTL responses was attempted in a Phase I trial involving immunization with a fusion protein comprising the HIV p24Gag protein and detoxified *Bacillus anthracis* lethal factor (*see 7.1.2.*) to target the antigen to antigen presenting-cells (Avant Therapeutics and WRAIR).

Multi-epitopic combinations of peptides, fusion proteins and long lipopeptides also are at an early stage of clinical development, either alone or in prime-boost combinations with live vector-based recombinant vaccines. Lipopeptides whose sequence corresponds to that of CTL epitopes-rich regions in the Gag and Nef viral proteins are in Phase II trials in the USA and in France (NIAID/ANRS).

Finally, a number of candidate vaccines that target nonstructural viral proteins such as Tat, Rev, Vif and Nef are being developed using viral vectors such as MVA or Ad5 (bioMérieux/Transgene), fowlpoxvirus (AVC), DNA (Vical, Istituto superiore dei Sanita/Parexel) recombinant proteins (FIT Biotech, Institute for Human Virology) or fusion proteins (GSK), or polyepitopic peptides (Wyeth/Duke University, Epimmune). Some of these candidate vaccines will be tested as therapeutic vaccines in patients as a complement to antiretroviral therapy. Tat has been shown to act as a viral toxin and to promote apoptosis of uninfected bystander T-cells and secretion of Th2 cytokines.

The population-wide effects of partially effective vaccines that do not prevent infection but only can reduce viral loads are largely unknown. Mathematical models predict that the factor with the greatest impact on reducing infections and deaths will be the degree of virus load reduction. A 90% reduction in viral load, which is a reasonable expectation with current candidate vaccines under development, would significantly reduce HIV mortality within 20 years after introduction of the vaccine.

The development of a safe, effective, and affordable HIV vaccine remains a formidable scientific and public health challenge at the dawn of this century.

