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6. Sexually Transmitted Bacterial Infections

6.1 Overview

6.1.1 Introduction

More than 30 different bacteria, (including *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Treponema pallidum*), viruses (such as HIV, HBV, HPV, and HSV-2) parasites (*Trichomonas vaginalis*) and fungi (*Candida albicans*) are spread by sexual contact and cause sexually transmitted infections (STIs), a significant public health problem in both industrialized and developing countries. Nearly a million people acquire a STI, including HIV, everyday [1]. Each year an estimated 340 million new cases of curable STIs occur worldwide, with the largest proportion in the region of South and South East Asia, followed by subSaharan Africa and Latin America and the Caribbean [2]. The highest rates of sexual infections occur among the 20-24 years olds, followed by the 15-19 years olds. In developing countries, STIs and their complications are one of the top five reasons that adults seek medicare [1] [2] [3].

STIs are responsible for significant human suffering and carry significant economic costs [4]. Many STIs are entirely attributable to unsafe sex. Disease burden linked to unsafe sex amounted in 2004 to 70 million disability-adjusted life years (DALYs) worldwide, of which 52 million were accounted for by developing countries. Unsafe sex ranked second among the 10 leading risk factor causes of DALYs worldwide, and third among the leading causes of DALYs in developing countries [5]. Strategies to control STIs, such as abstinence, screening programs and condoms, have had limited success. Vaccines are an attractive addition to the STI prevention tools as they could provide durable protection and offer the additional advantage of potential protection of the nonimmunized through the induction of herd immunity [6]. To the exception of the Human Papillomavirus Virus (HPV) vaccine, however, much work remains to be done on vaccines against prevalent STIs.

This chapter will deal with vaccines against bacterial STIs. Vaccines against viral STIs, such as AIDS (HIV), papillomavirus infections (HPV), and Herpes simplex virus type 2 (HSV-2) infection will be the subject of the following chapter.

6.2.2 Bacterial STIs

Bacterial infections of the genital tract, including chlamydia (*Chlamydia trachomatis*), gonorrhoea (*Neisseria gonorrhoeae*), chancroid (*Haemophilus ducreyi*), and syphilis (*Treponema pallidum*), cause significant morbidity worldwide. Each is capable of causing infections repeatedly, suggesting lack of broad effective immunity following a first infection, a major concern about the feasibility of developing effective vaccines [7]. Currently, indeed, there is no vaccine against any of the bacterial STIs.

Further advances in STI prevention and control have been hampered by our limited knowledge of the natural history of untreated genital infections in humans and the difficulty of collecting accurate disease burden figures. This is also due to the fact that many bacterial STIs remain asymptomatic, favoring the further spread of infection to sex partners. This is for example particularly true of *C trachomatis* infections [8].

Women, particularly adolescents, are disproportionately vulnerable to a variety of STIs with potentially devastating reproductive consequences, including pelvic inflammatory disease (PID) and ectopic pregnancy. Syphilis prevalence in pregnant women in Africa, for example, ranges from 4% to 15% [1]. Untreated gonococcal and chlamydia infections in females will result in PID in up to 40% of cases, and one in four of such cases will result in infertility [1]. Several common bacterial STIs adversely affect pregnancy, causing spontaneous abortions, stillbirths, preterm delivery and postpartum endometritis. In addition, some can also affect neonates, as for example gonococcal conjunctivitis, which leads to blindness, or chlamydial pneumonia, which may lead to chronic respiratory disease. Up to 4000 newborn babies become blind every year because of eye- infection linked to untreated maternal gonococcal or chlamydial infection [1].

The importance of STIs has been highlighted by appreciation of their role in facilitating transmission of the human immunodeficiency virus (HIV), either through the formation of genital ulcers that provide an easy portal of entry (or exit) to the virus [9] [10] or through the creation of a local inflammatory response leading to microulcerations and increased local accumulation of activated T cells and macrophages [11] [12] [13].

6.2 Chancroid

6.2.1 Overview

Chancroid is an ulcerating infection of stratified squamous epithelia caused by *Haemophilus ducreyi*, a Gram-negative, unencapsulated bacterium, which causes genital ulcer disease (GUD) with inguinal lymphadenitis. Bacterial GUD is relatively common in geographic pockets of certain tropical, resource poor regions in Africa and Asia. The prevention of GUD would be an important strategy to control the spread of HIV infection in these countries [14] [15]. However, chancroid reinfection is common among patients, suggesting that a nonprotective immune response is most often generated in response to natural infection [16]. As early as 1889, Ducrey was able to serially propagate the infectious agent by repeated inoculations (up to 15 times) to the same individual, demonstrating that little if any immunity occurs in natural infection.

6.2.2 Virulence factors

H ducreyi secretes a cytotoxin with high cytotoxic activity specific to human cell lines, the cytolethal distending toxin (HdCDT) [17] [18], which causes apoptosis of dendritic cells (DC) in vitro. CDT inhibits the production of TNF-alpha, IL-6 and IL-12 by DCs or macrophages, hampering early stage immune responses [19]. Paradoxically, cytotoxin antibodies produced during the course of natural infection in humans did not appear to provide protection from disease [17] and deletion of the *cdtABC* gene cluster remained without effect on virulence [20].

The identification of *H ducreyi*'s virulence factors that lead to the development and persistence of disease was undertaken [21] [22] by different means, using either the rabbit model or the swine model of chancroid infection [23] [24], or inoculation to human volunteers, as well as a variety of *in vitro* assays such as for example the resistance of the bacterium to human serum (Rev in [22]).

The ability of the bacterium to evade complement-mediated serum lysis, an important feature in the pathogenesis of *H ducreyi* infection, was found to be determined mostly by the outer membrane protein (OMP) serum resistance protein A (DsrA) [25], a major fibronectin-binding determinant of *H ducreyi* [26] [27]. Another OMP, the 22kD lectin A (DltA) was also identified that binds to fibronectin, GalNAc and several N-glycosylated glycoproteins [28], and contributes to serum-resistance. Serum-susceptible *dsrA* mutants were found to be highly attenuated in human volunteers, whereas *dltA* mutants were partially attenuated [29] [30].

Other *H ducreyi* virulence factors have been identified that inhibit phagocytosis by macrophages and myelocytic cells *in vitro* and act as virulence factors both in the rabbit model and in human volunteers. These are the LspA1 and LspA2 proteins that compose with LspB a two-partner secretion system [21] [31] [32]. Still another *H ducreyi* virulence factor has been identified that is involved in the transport of iron: the hemoglobin receptor, HgbA [33]. A crucial role for HgbA in virulence was established by showing the inability of an *hgbA* mutant to initiate experimental infection in human volunteers even at a dose 10 times higher than the infective dose of the parent strain [34]. It was also observed that *H ducreyi* is resistant to human antimicrobial peptides (alpha-defensins, beta-defensins and cathelicidin). A resistance protein, SapA, was recently identified that provides resistance to the LL-37 antimicrobial peptide [35].

6.2.3 Attempts at vaccine development

Studies in animal models have shown partial immune protection against a challenge with *H ducreyi* strains after immunization with whole bacteria, crude OMP preparations or purified protein vaccines [36] [37] [38].

An experimental vaccine made of purified HgbA mixed with Freund adjuvant efficiently prevented subsequent *H ducreyi* infection in swine. Antibodies from the HgbA-immunized animals blocked hemoglobin binding to HgbA, suggesting that the vaccine worked by blocking bacterial growth through prevention of heme/iron acquisition by the bacteria [39].

At this time however, there is not much activity in the field of vaccine development.

6.3 Chlamydia trachomatis

Chlamydia trachomatis is an obligate intracellular bacterial pathogen that causes trachoma and genital tract infections. The majority of the genital infections are asymptomatic and without clinical evidence of complications and appear to spontaneously resolve [40], although there only is limited knowledge about the clinical factors that influence the duration of untreated, uncomplicated genital infections in humans [8]. These infections tend to be chronic and recurring and associated with scarring complications possibly related to hypersensitivity mechanisms [41]. They can infrequently progress to symptomatic upper genital tract complications in men (epididymitis) and women (eg, PID). Public health interventions have thus far been ineffective at controlling the disease [42] [43] [44].

6.3.1 Disease burden and epidemiology

Chlamydia trachomatis infections are the most common bacterial STIs worldwide, and represent an enormous public health problem. WHO estimated that there were 92 million new cases worldwide in 1999 and the incidence of infection has continued to increase each year in both industrialized and developing countries [2] [45]. Sub-Saharan Africa and South and South-East Asia have particularly high burdens of disease. A 4.2 % prevalence rate was documented in a random sample of 18-26 year-old adults in the USA, highlighting the fact that infection is mainly observed in adolescents and young adults [46]. In 2005, 976 445 cases of genital *C trachomatis* infections were reported to the Centers for Disease Control and Prevention (CDC) in the USA. The figure increased to more than 1.1 million cases in 2007 [47]. In view of the fact that approximately 20% of chlamydial infections in women and up to

50% of chlamydial infections in men remain asymptomatic, it is estimated that there are approximately 2.8 million new cases of chlamydia in the USA every year [48]. It should be stressed that repeated chlamydial genital infections account for a substantial proportion of incident infections [8] [49] [50] [51].

C trachomatis can also cause pharyngeal infection, more than 80% of which remain undiagnosed by classical urine and urethra testing [52]. Rectal chlamydial infection is often observed in men who have sex with men, but recent observations suggest that it is not infrequent in women [53].

C trachomatis is transmitted from one partner to another by sexual intercourse. In men, it is the commonest cause of non-gonococcal urethritis, epididymitis and orchitis. Men with asymptomatic infection serve as carriers of the disease, spreading the infection while only rarely suffering long-term health problems. Infection in women can lead to a variety of symptoms including vaginal mucopurulent discharge, endometritis and more severe complications such as pelvic inflammatory disease (PID), whose long-term consequences are chronic pain, ectopic pregnancy and tubal factor infertility [54] [55] [56]. Contamination of the hands with genital discharge may also lead to 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.

Furthermore, an existing chlamydial infection increases the risk of contracting HIV [57] and/or Herpes simplex infections [58]. This is especially true with the *Lymphogranuloma venereum* (LGV) disease, an invasive and frequently ulcerative chlamydial infection involving lymphatic tissue. LGV diseases are endemic in certain areas of Africa, Asia, South America and the Caribbean and represent a major risk factor for HIV acquisition [59].

Worldwide, the most important disease caused by *C. trachomatis* is trachoma, which is one of the commonest infectious causes of blindness. The disease starts as an inflammatory infection of the eyelid and evolves to blindness due to corneal opacity. Despite long-standing control efforts, it is estimated that more than 500 million people 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. Trachoma is a communicable disease of families, with repeated reinfection occurring among family members. Transmission is driven by sharing of ocular secretions among young children in family or community groups, facilitated by the ubiquitous presence of flies. 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 facial hygiene is poor. Visual loss from trachoma is 2-3-times more common in women than men and is a major cause of disability in affected communities, attacking the economically important middle-aged female population. Global elimination of trachoma as a disease of public health importance has been targeted by WHO for 2020.

6.3.2 Bacteriology

The genus *Chlamydia* includes four major species: *C trachomatis*, an exclusively human pathogen; *C psittaci*, which infects a variety of animals and can cause pneumonia or psittacosis in humans; *C pneumoniae*, a relatively common cause of lower respiratory infection (LRI) in humans; and *C pecorum*, an exclusively animal pathogen. Other *Chlamydia* species that infect animal species are also known, such as *C abortus* (sheep and goats), *C suis* (pigs) and *C felis* (cats) (Review in [60]). Based on the type of disease produced, *C. trachomatis* has been divided into biovars, including the

lymphogranuloma (LGV) biovar, associated with *Lymphogranuloma venereum*; and the trachoma biovar, associated with human conjunctival or urogenital columnar epithelium infections.

C. trachomatis is a small obligate intracellular bacterium found in two forms: the extracellular elementary body (EB) and the intracellular reticulate body (RB). The infectious form is the metabolically inactive elementary body (EB). After attachment, EBs penetrate into the host cells where they reorganize into metabolically active and replicative RBs that accumulate by binary fission in a large chlamydial cytoplasmic inclusion [61] [62]. The process lasts for 48-72 h, after which RBs reorganize into infective EBs, which are released by host cell lysis or extrusion and propagate infection to neighboring cells. At the site of mucosal infection, intense inflammation can occur, resulting in mucopurulent cervicitis in women and urethritis in men.

C. trachomatis is further divided into 15 serotypes (or serovars), designated A-K and L1-L3, which differ by the antigenicity of their major outer membrane protein (MOMP; OmpA). Serovars A, B, Ba and C produce almost exclusively ocular trachoma, whereas serovars D, Da, E, F, G, H, I, Ia, and K and the *Lymphogranuloma venereum* (LGV) strains L1-L3 are associated with genital tract infections. The most prevalent serovars worldwide are serovars E, F, D and Ia, but prevalence of individual serovars has been reported to differ by age, sex, geographic region and racial groups (For a review, see [63]). The whole nucleotide sequence of the genome of *C. trachomatis* (serovar D) has been determined [64]. There is little knowledge of possible chlamydial virulence factors, their expression and how they affect disease severity [65].

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. Persistent chlamydia infection is characterized by a non-culturable but viable state in which RBs do not mature into EBs. Persistent infections seem to be resistant to antibiotics [66] and may be at the origin of antibiotic treatment failures [67].

6.3.3 Immune markers of protection

Immunity to Chlamydial reinfection could be demonstrated in all animal models of genital infection [68]. However, complete immunity to reinfection lasted only for a short period after resolution (1-3 months), whereas partial immunity to reinfection was maintained for exceedingly long periods [69]. Thus, the majority of guinea pigs challenged at 30 days after primary infection were completely immune to reinfection, whereas the animals challenged at 75 or 150 days after initial infection became reinfected, albeit with an abbreviated course of disease. This could be explained by showing that antigen-specific CD4⁺ Th1 T cells were present in the genital tract at the time when infection was resolved, then decreased rapidly by day 30, and were at baseline levels by day 75. At that time, chlamydiae could infect target cells and initiate infection before activated T cells finally entered the site and resolved the infection anew.

Evidence that immunity is also capable of resolving chlamydial infection in humans has been found in limited studies of natural history, which consistently show that humans clear chlamydial infections in the absence of antibiotics treatment [8] [70] [71]. Most elements of resolving and protective immunity defined in animal models, such as Chlamydia-specific CD4⁺ T cells, Th1-related cytokines including IFN- γ and IL-12, and the presence of neutralizing IgGs and mucosal IgAs at the endocervix have been documented to exist in humans [7] [72] [73]. These elements also play a role in either promoting or protecting against development of upper genital tract disease in women (review in [74]). However, IFN- γ production by PBMCs in response to chlamydial heat shock protein cHSP60 was significantly associated with protection against chlamydial infection in sex workers, contrary to endocervical IgGs and IgAs which were not [75], which suggests that the CD4⁺ Th1 response is the principal mechanism of host defense [74] [75] [76].

6.3.4 Attempts at vaccine development

There is no vaccine available at this time to prevent *Chlamydia* infections and the most advanced vaccine candidates are still at an early preclinical stage. Early chlamydial vaccine studies focused on the use of inactivated whole organisms, which produced short-term protection in some individuals but also led to disease exacerbation in others, due to enhanced delayed-type hypersensitivity reactions. Elementary bodies (EBs) were nevertheless successfully used to protect mice against genital challenge, either alone [77] [78] or after *in vitro* adsorption onto dendritic cells [79].

Attention eventually turned to the use of the *C trachomatis* 40kD major outer membrane protein (MOMP), also known as Omp-1 antigen, as a subunit vaccine. Antibodies against MOMP can neutralize infectivity of *C trachomatis* of the same serovar and contribute to a protective response against genital tract reinfection [80]. Neutralizing IgAs and IgGs in cervicovaginal lavage fluid were induced in animal models following intranasal or systemic immunization with MOMP subunit vaccines [81] [82] [83] [84] [85], resulting in partial protective immunity (For a review see [63]). This suggests that better adjuvants and delivery systems are needed that elicit a stronger Th1 response and/or a more efficient mucosal IgA response, so as to enhance the efficacy of the MOMP vaccines; or that MOMP alone is inadequate as a vaccine [86] [87].

The limited protective efficacy of MOMP vaccines was not significantly improved by using a DNA - ISCOMs prime-boost strategy to deliver the MOMP antigen [88], or by adding CpG oligonucleotides [89] or the cytokine GM-CSF [90] as adjuvants. Limited protection was observed when using MF59 or E coli LT detoxified mutant LT-R72 as adjuvants [91]. Antex Biologics nevertheless developed a subunit vaccine candidate (TRACVAX) which was based on conserved MOMP epitopes and was tested in a randomized Phase I trial.

A significant protection against *C trachomatis* genital challenge in mice was provided by a MOMP vaccine that was added with the outer surface protein A (OspA) of *Borrelia burgdorferi* [92]. Similarly, mice vaccinated with MOMP adjuvanted with Th1-promoting cationic adjuvant formulation 1 (CAF01) displayed high titers of IgG2b, IFN-gamma, and TNF-alpha and a profoundly reduced vaginal chlamydial load as compared with mice vaccinated with MOMP in alum [93]. A similar protection level was obtained by immunization with MOMP mixed with Montanide ISA 720 and CpG oligonucleotides [94]. Protection against genital challenge was also obtained by immunizing mice by the oral route with a MOMP vaccine added with CpG oligonucleotides and cholera toxin and emulsified into a lipid-based oral delivery system, lipid C [95]. Lipid C consists of 50% oleic acid, 25% palmitic acid, 15% stearic acid, 6% linoleic acid and 1% myristic acid [96] and was also successfully used as an adjuvant for transcutaneous immunization [97].

The search for other candidate vaccine antigens, which was greatly helped by the complete sequencing of the genome [98], led to the identification and testing of several candidate subunit vaccine proteins such as the polymorphic outer membrane proteins (POMPs or pmp), the conserved PorB family of membrane proteins [99], a plasmid protein, ppg3 [100], the chlamydial proteasome/protease-like activity factor, CPAF [101], and constituents of the type III secretory machinery [102]. Mice immunized with DCs loaded with the PmpG-1 protein were partially resistant to Chlamydial challenge both in the lung and in the genital tract [103].

Intranasal immunization with the protease-like activity factor CPAF mixed with IL-12 elicited protection against genital chlamydial challenge in mice in an antigen-specific CD4⁺ T cells- and HLA-DR4-dependent manner [104] [105] [106]. Adoptive transfer of CPAF-specific CD4⁺ T cells induced comparable protective immunity against genital challenge to that induced by transfer of CD4⁺ T cells primed by *Chlamydia* infection. IFN- γ production from adoptively transferred CPAF-specific CD4⁺ T

cells was sufficient in IFN- γ -deficient mice to induce early resolution of *Chlamydia* infection and reduction of subsequent pathology [107]. The use of CPAF as an alternative to, or in combination with MOMP, in order to induce robust anti-chlamydial immunity is currently the object of additional studies [108].

Using expression library immunization, other novel vaccine candidates were recently identified such as a DNA gyrase subunit, TC0462, and the ATP-dependent Clp protease, TC0559 [63]. Another antigen that induces strong CD4⁺ Th1 cell response in chlamydia-infected mice and in human patients with diagnosed infection is CT043. A DNA prime-subunit boost with CT043 was able to provide significant cross-serotype protection in mice against *Chlamydia* challenge [109].

Random genomic library screening for T-cell proliferation from in vitro-stimulated PBMCs was also used to identify 51 unique *C trachomatis* antigens, out of which eight were recognized by the majority of *Chlamydia*-infected individuals with no evident disease, eliciting strong T cell proliferative responses and serological responses. Mixtures of some of these antigens with the MPL- and QS21-containing AS01B adjuvant induced protection against genital *C trachomatis* serovar K infection using murine intravaginal and intrauterine challenge models, as measured by reduced bacterial shedding and prevention of spreading of the infection to the upper genital tract [78].

These vaccine candidates are currently been further evaluated for protective efficacy. They foster the hope that it will be possible to develop a recombinant subunit vaccine for genital *C trachomatis* infection, perhaps through mixing together some of the most promising candidates, although, at this time, there still is no report of human clinical trials of Chlamydia vaccines

6.4 Gonorrhoea

6.4.1 Disease burden

Gonorrhoea, caused by *Neisseria gonorrhoeae*, is a most commonly reported sexually transmitted infectious disease, with an estimated 62.4 million cases yearly global prevalence [2]. More than 358 000 cases were reported in 2006 in the USA alone [110], not counting the fact that gonococcal infections can often be asymptomatic, especially in women, and therefore are not reported [42] [111]. Similarly, anorectal and pharyngeal infections, which are not uncommon in women and frequent in men who have sex with men, remain often asymptomatic and constitute an important potential source of transmission [52] [112]. As for Chlamydia, there is a strong inverse relationship between age and prevalence of gonococcal infections, the highest rates of genital infections being among the less than 25-year age groups in both men and women [113].

Gonococcal infection in males is usually asymptomatic but eventually manifests itself by a purulent urethral discharge and dysuria. If untreated, the infection can result in epididymitis. In females, the most common symptom is purulent cervical discharge, which often goes unnoticed. As a result, the infection frequently spreads to the higher genital tract and generates pelvic inflammatory disease (PID), salpingitis and ectopic pregnancy. Ultimately, untreated infections in both males and females can lead to infertility. In about 1% of cases, infection becomes invasive and a bacteremia develops, leading to disseminated gonococcal infection characterized by fever, skin rash and asymmetrical septic arthritis. Gonococcal infection does not elicit protective immunity, a major challenge for the development of a vaccine.

Newborns from gonococcus-infected mothers are also at risk of contracting the disease. 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 or ophthalmic antibiotics to newborns.

However, the progressive development of antibiotic resistance in circulating *N gonorrhoeae* strains has been a major source of concern, forcing to abandon penicillin treatment in the 1970's, tetracycline treatment approximately 10 years later, and the use of fluoroquinolones in 2007 [114] [115].

Another concern regards the role played by gonorrhoea in the transmission of HIV, whose loads are higher in seminal plasma and cervicovaginal secretions of men and women with concurrent gonococcal infection, and decrease upon their treatment with antibiotics [116] [117]. These issues have hastened the search for a vaccine to protect against gonococcal infections, but, as reviewed below, the gonococcal surface antigens are extremely variable, undergoing rapid and frequent phase and antigenic variation (Review in [118] [119]) and there is at this time no vaccine to prevent the disease.

6.4.2 Bacteriology

N. gonorrhoeae is a Gram-negative diplococcus, whose complete nucleotide sequence has been determined. The life cycle of the bacterium was studied using a variety of cell culture systems. These studies have shown that gonococci not only adhere to epithelial cells but also penetrate and transit across the epithelial layer and exit into the subepithelial space where the symptoms of the disease are actually elicited.

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 (pil + to pil -) occurs when the rearrangement involves a defective pilin gene. Variant pilin proteins arise at a frequency of approximately 1/1 000 per cell per generation, explaining the high degree of pilin heterogeneity found within gonococcal populations [120]. This considerable diversity precludes the use of pilin as a base for vaccine development.

Another membrane protein, the opacity protein (Opa) is encoded by up to 11 different Opa loci, each of which consists of a promoter and a set of repeats, so that in vivo up to 5 Opa proteins are expressed simultaneously [121]. Moreover, recent evidence indicates that Opa protein binding to host cell receptors results in suppression of T cell activation and proliferation, thus impeding the immune response [122]. The receptor to Opa proteins is the carcino-embryonic antigen-related cellular adhesion molecule, CEACAM1 (Cd66a) [123] [124]. The interaction of the opacity protein and CEACAM1 at the surface of human peripheral B cells results in inhibition of antibody production and the induction of cell death [125].

Complement is a key arm of innate immune defenses against gonococcal infections. The bacterium evades killing by human complement by either sialylation of its lipooligosaccharides (LOS) or through expression of the porine (Por) protein, which allows the bacterium to bind to the alternative pathway complement inhibitor, factor H [126] [127] and to the complement inhibitor C4b-binding protein (C4bp) [128] [129]. The specificity of *N gonorrhoeae* for the human species is readily explained by the fact that it is killed by the complement from rodents, lagomorphs or nonhuman primates, due to the fact that none of the gonococcal porins can bind to C4bp or fH from these species. Chimpanzees are

susceptible to Por 1B but not to Por 1A *N gonorrhoeae*, again due to the fact that chimp C4bp binds to Por 1B-bearing gonococci but not to Por 1A-bearing bacteria.

6.4.3 Vaccines

Development of a gonorrhoea vaccine has been deeply hampered by the lack of a suitable animal model and the considerable variability of *N gonorrhoeae* antigens. Also, there is no evidence that natural infection leads to immunity, as seen by the incidence of repeat gonococcal infections with the homologous strain or serotype [130]. In addition, whole-cell vaccines were found to elicit a nonprotective immune response [131].

Attachment of gonococci to mucosal cells is mediated in part by their pili, and rabbit antibody to pili can be shown to reduce attachment of the bacteria to mammalian cells. In spite of its variability, pilin was therefore chosen as a vaccine candidate and tested for efficacy in military recruits and in volunteers challenged urethrally [132]. This approach was met with some success, but protection was strain-specific only.

A major protein in the gonococcal outer membrane is Porin (PorB 1A and PorB 1B), which forms anion-selective transmembrane channels through the outer membrane. Antibodies to porin are bactericidal [133], but strain-specific. It has been possible to distinguish at least 24 PorB 1A serovars and 32 Por 1B serovars. Also, a human vaccine trial with Por 1B did not protect against intraurethral challenge with the homologous strain (cited in [118]).

The gonococcal transferring-binding proteins TbpA and TbpB have generated particular interest because they exhibit low inter-strain variability and are not subject to antigenic or phase variation [134] while being ubiquitous and mandatory for gonococcal virulence in humans [135]. The TbpAB protein system is employed by gonococci to bind transferrin and relieve it of iron, which is then transported through the outer bacterial membrane to be taken up by the FbpA and FbpBC transporter chain. Recombinant gonococcal transferrin-binding proteins TbpA and TbpB were conjugated to the cholera toxin B subunit and administered as a vaccine by the intranasal route to mice. Resulting antibodies were bactericidal in the presence of human complement [136]. Importantly, bactericidal activity was detected against both homologous and heterologous gonococcal strains.

In another study, the aminoterminal half of TbpB was fused to the sequence of TbpA loop 2, then to the A2 subunit of the cholera toxin. When administered by the intranasal route, the resulting fusion protein elicited cross-reactive, bactericidal antibodies in both vaginal secretions and plasma that interfered with transferrin-dependent growth of homologous and heterologous gonococcal strains [137].

While the TbpAB transferring receptor signal is required to initiate infection in human males, it is not however required for gonococcal survival in the female mouse model, probably because other iron sources are available in the female genital tract besides transferrin, lactoferrin and hemoglobin [138]. Other putative gonococcal iron transporters were identified (TdfF, TdfG, TdfH and TdfJ [139] [140]), out of which one, TdfF, was found to be expressed and required for growth within human cervical epithelial cells, making it a candidate of interest for future vaccine study [119].

Other surface proteins are either too variable to serve as vaccine candidates, or are known to elicit bactericidal-blocking antibodies [141]. Of note, however, is the recent attempt at using an Opa conserved internal loop as a vaccine, which succeeded in eliciting broad spectrum agglutinating antibodies [142].

It should be noted that other modes of vaccine delivery could also be developed such as recently illustrated by Thomas and co-workers using nonreplicating Venezuelan Equine Encephalitis viral replicons to deliver the *N gonorrhoeae* PorB or TpbB protein to mice by the intranasal route [143] [144]. Inclusion of a eukaryotic secretion signal (tPA) to the TpbB construct resulted in a gonococcal-specific IgG and IgA response in mucosal secretions and a Th1-biased immune response.

The “reverse vaccinology” approach [145] [146] was also applied to the study of the gonococcal genomic organization, leading among other to the identification of a peptidyl-prolyl isomerase that is involved in persistence of the bacteria in macrophages and their survival in the mouse female genital tract [147] [148]. Other, as yet uncharacterized, potential vaccine antigens have also been identified.

A successful gonorrhoea vaccine will probably necessitate the incorporation of antigenically and functionally diverse gonococcal antigens into a cocktail subunit-based vaccine.

6.5 Syphilis

6.5.1 Overview

Syphilis, a chronic sexually transmitted infection caused by the spirochaetal bacterium *Treponema pallidum* subsp. *pallidum*, remains a global public health problem despite effective drug treatment regimens. It is for example estimated that about 4% to 15% of women in Africa test positive for syphilis, but actual figures of disease burden remain scarce and most are outdated.

Understanding host-pathogen interactions and disease pathogenesis has been hampered by the paucity and imperfection of animal models and the fact that the organism cannot be grown *in vitro*. Many questions remain about how *T pallidum* escapes immune responses, allowing infection to persist in the presence of a robust host response, and its propensity for neuro-invasion and congenital transmission (for a review, see [149]). Fetal infection may lead to fetal death or pre-term delivery and severe growth retardation or other manifestations of congenital syphilis

6.5.2 The quest for a vaccine

Protective immunity against *T pallidum* infection can be demonstrated in the rabbit model where animals systemically infected for 3-6 months are resistant to superinfection with the homologous treponeme strain, and where immunization with gamma-irradiated *T pallidum* elicited protection against further challenge with viable treponeme [150]. However, the unusual biology of *T pallidum* has hindered progress towards the development of a vaccine [151].

T pallidum is believed to be an extracellular pathogen and, as such, the identification of its outer membrane proteins that could serve as targets for opsonizing or bactericidal antibodies has been a priority research. Potential outer membrane proteins TP0155, TP0326, TP0483 and TP0956 were used separately or in combination to vaccinate New Zealand rabbits but this failed to protect the animals against intradermal virulent challenge, in spite of achieving high levels of serum antibodies [152]. Another antigen, TP0136, a lipoprotein that is expressed on the surface of the bacteria and binds to fibronectin and laminin, was used as a candidate vaccine in rabbits. The vaccine delayed the formation of ulcers at the site of challenge but did not prevent infection nor the formation of lesions [153].

The 1041 open reading frames (ORFs) of the genome of *T pallidum* have been sequenced. A family of repeat genes, the *Tpr* genes, was found to encode proteins resembling the major sheath proteins of

another spirochaetal bacterium, *T denticola*. Antibodies to the TprK protein were found to be opsonic. Immunization studies in the rabbit model showed that TprK could induce significant protection against challenge with a homologous strain [154] [155] [156]. However, there was no protection against challenge with heterologous *T pallidum* strains, due to the absence of cross-reactivity of the antibodies to diverse TprK variable regions. Seven variable regions have been identified in the TprK protein [157] [158]. The T cell epitopes are located in the conserved regions of the protein, while B cell epitopes are in the variable regions [159]. Another Tpr protein, the TprI antigen, was similarly used to vaccinate rabbits but failed to elicit protection against intradermal *T pallidum* challenge [160].

The quest for *T pallidum* antigens that might induce protective immunity in rabbits should not be daunted. It is obvious that a better understanding of syphilis immunology is warranted at this time. It is even quite plausible that no protection will be obtained by *T pallidum* candidate vaccines short of eliciting both humoral and cell-mediated immune responses [152].