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Chlamydia Vaccines
Strategies and Status

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Abstract

The ultimate goal of current chlamydial vaccine efforts is to utilise either conventional or modern vaccinology approaches to produce a suitable immunisation regimen capable of inducing a sterilising, long-lived heterotypic protective immunity at mucosal sites of infection to curb the severe morbidity and worldwide prevalence of chlamydial infections. This lofty goal poses tremendous challenges that include the need to clearly define the relevant effectors mediating immunity, the antigens responsible for inducing these effectors, the anti-chlamydial action(s) of effectors, and establishment of the most effective method of vaccine delivery. Tackling these challenges is further compounded by the biological complexity of chlamydia, the existence of multiple serovariants, the capacity to induce both protective and deleterious immune effectors, and the occurrence of asymptomatic and persistent infections. Thus, novel molecular, immunological and genetic approaches are urgently needed to extend the frontiers of current knowledge, and develop new paradigms to guide the production of an effective vaccine regimen. Progress made in the last 15 years has culminated in various paradigm shifts in the approaches to designing chlamydial vaccines. The dawn of the current immunological paradigm for antichlamydial vaccine design has its antecedence in the recognition that chlamydial immunity is mediated primarily by a T helper type1 (Th1) response, requiring the induction and recruitment of specific T cells into the mucosal microenvironment. Additionally, the ancillary role of humoral immune response in complementing the Th1-driven protective immunity, through ensuring adequate memory and optimal Th1 response during a reinfection, has been recognised. With continued progress in chlamydial genomics and proteomics, select chlamydial proteins, including structural, membrane and secretory proteins, are being targeted as potential subunit vaccine candidates. However, the development of an effective adjuvant, delivery vehicle or system for a potential subunit vaccine is still an elusive objective in these efforts. Promising delivery vehicles include DNA and virus vectors, bacterial ghosts and dendritic cells. Finally, a vaccine still represents the best approach to protect the greatest number of people against the ocular, pulmonary and genital diseases caused by chlamydial infections. Therefore, considering the urgency and the enormity of these challenges, a partially protective vaccine preventing certain severe sequelae

would constitute an acceptable short-term goal to control *Chlamydia*. However, more research efforts and support are needed to achieve the worthy goal of protecting a significant number of the world's population from the devastating consequences of chlamydial invasion of the human mucosal epithelia.

1. Introduction

Chlamydia are a group of obligate intracellular Gram-negative-like bacteria that have become a major concern in both developed and developing nations as a result of the spectrum of severe diseases associated with different species in humans. Genital and ocular infections caused by *Chlamydia trachomatis* account for significant morbidity and socioeconomic burden to several nations.[1] Acute urethritis and cervicitis are prominent sexually transmitted diseases (STDs) caused by chlamydial genital infection in humans, and complications include endometritis, salpingitis, pelvic inflammatory disease (PID) [pelvic pain], ectopic pregnancy and infertility.[2] Reports suggesting that genital chlamydial infection may predispose to HIV-related AIDS and human papillomavirus (HPV)-associated cervical dysplasia have heightened these concerns. Ocular infection by *C. trachomatis* causes a spectrum of diseases ranging from conjunctivitis to trachoma, the world's most common preventable disease causing blindness. *Chlamydia pneumoniae,* previously known to cause mild respiratory infections, has emerged as an important pathogen in recent years because of its association with atherosclerosis, adult-onset asthma and certain other chronic diseases.[3,4] The zoonotic *Chlamydia psittaci* constitutes an occupational hazard for workers in the poultry and farming industry, and people exposed to infected avian species.[5]

Diagnosed chlamydial infections are treatable by antibacterial therapy; however, the staggering widespread incidence of the infections worldwide, and frequent asymptomatic and possibly persistent infections require simple and accurate diagnostic procedures to ensure timely application of anti-infectives.[6,7] Alternatively, a reliable preventive agent, such as a vaccine, is needed for a successful intervention, control of infections and prevention of disease complications in the population.[8] This review focuses on the strategies aimed at developing an efficacious vaccine against human chlamydial infections. Special attention is given to challenges facing vaccine development efforts, which include the immunological bases or operational paradigms for designing vaccine regimens, the need for more effective delivery systems, and the prospects of specific vaccine strategies. The information is discussed in the context of a status report that reflects the prospects for the development of a protective chlamydial vaccine.

2. Problem of Chlamydial Infections and Need for a Vaccine

The Gram-negative bacteria, *Chlamydia*, are obligate intracellular microbes that are dependent on the infected host cell for energy and growth nutrients. Four species recognised in the current taxonomy are *C. trachomatis, C. psittaci, C. pneumoniae*, and *Chlamydia pecorum*. A newly proposed taxonomic re-classification of *Chlamydia* species is still being debated,[9-11] and so the familiar classification based on four species will be adopted in this review. *C. trachomatis* is the aetiological agent of major ocular and STDs worldwide. Of the 15 major serologically defined serotypes or serovars of *C. trachomatis* (i.e., serovars A through K and L1 to L3), serovars A, B, Ba and C are the causative agents of trachoma. Trachoma is common in several developing nations, including Africa, South East Asia and the Middle East. Serovars D through K and the lymphogranuloma venereum (LGV) strains L1, L2, and L3 are major agents of oculogenital infections and STDs worldwide. In fact, *C. trachomatis* is the most common bacterial STD in industrialised nations, including the US, UK, Germany, Australia and France, where it poses a serious concern in the adolescent population.[1] A recent World Health Organization report revealed that 90 of 500 million annual new cases of STDs are caused by *C. trachomatis*, and, in the US alone, four million reported annual cases involve an expenditure in excess of $US2 billion.[1,12] Acute urethritis in women and men, and cervicitis in women are the immediate clinical presentations of genital chlamydial infections. The pathological consequences of genital infection by *C. trachomatis* in women are severe sequelae associated with ascending infection, including PID, fallopian tube scarring, ectopic pregnancy and infertility.[2] Inclusion conjunctivitis due to *C. trachomatis* can lead to severe pneumonitis in infants, and reactive arthritis is a secondary manifestation of chlamydial genital infection. The diseases caused by *C. pneumoniae* include acute respiratory infections such as sinusitis, pharyngitis, bronchitis and pneumonia. The single strain of *C. pneumoniae*, designated as TWAR, is a strictly human pathogen with no known animal reservoir. Infections by *C. pneumoniae* are common, with up to 60% of the population in North America, Europe and Japan showing serological evidence of exposure.[3,4] The recent association of *C. pneumoniae* with cardiovascular diseases such as atherosclerosis and coronary artery disease,[13-16] and chronic diseases such as asthma,[17,18] Alzheimer's disease[19] and multiple sclerosis,[20] continues to generate both challenges and controversies. *C. psittaci,* principally a bird pathogen, is a zoonotic chlamydial species that occasionally causes psittacosis or parrot fever-like illness in humans, and systemic or CNS spread could be fatal.[5] The fourth species of

Chlamydia, C. pecorum, has not been associated with any human disease.

Chlamydial infections are treatable with antibacterials, such as tetracycline derivatives, especially doxycycline, and the macrolides or azalides such as erythromycin and azithromycin; however, infections are often asymptomatic, with severe complications usually presenting as the first symptoms of an infection. The dilemma posed by chlamydial infections in both the developed and developing nations has intensified efforts to design preventive and control measures, of which frequent screening for early detection and treatment, and the administration of an efficacious vaccine, have become a priority.[8] However, there are obvious difficulties in establishing acceptable and cost-effective, community-wide screening programmes or rational antibacterial prophylaxis. Therefore, timely diagnosis using contemporary methodologies,[6,7] and application of chemotherapy to arrest silent or persistent infections have not been established to control chlamydial infections. This situation has resulted in the deep concern that chlamydial infections may pose a serious threat to human reproduction, longevity and general health quality, as well as constituting a considerable burden on national healthcare budgets and management. Thus, the development of preventive or control strategies is a high priority in chlamydial research efforts. Of these efforts, the design of an efficacious vaccine has become a *sine qua non* in controlling *Chlamydia* in the human population.[8] A vaccine is the best approach to delivering long-lasting protection to the largest number of people worldwide. In fact, computer modelling has indicated that even a partially successful vaccination programme would have a remarkable global impact in reducing chlamydial infections, disease prevalence and related expenditure.[21] Therefore, while the ultimate goal of a chlamydial vaccine is ideally to achieve sterilising immunity, a vaccine effective against disease sequelae, such as PID, blinding trachoma and tubal scarring would be acceptable as a first-generation product. Moreover, since it appears that only a subset of infected individuals are at risk of serious sequelae,[22] these high-risk individuals might be the primary target of a vaccine that provides protection against complications.

3. Challenges Facing Chlamydial Vaccine Efforts

A major goal of current chlamydial vaccine efforts is to produce a conventionally based immunisation regimen capable of inducing long-lived heterotypic protective immunity at mucosal sites of infection. However, *Chlamydia* present considerable obstacles to achieving this goal because they exist as multiple serovariants,[23] the infection and disease are restricted mostly to mucosal surfaces,[24,25] they have a complex biology and antigenic composition,[26] and infection is thought to generate both protective and pathological immune responses.[8,27-29] These characteristics, coupled with the lack of tools needed to genetically manipulate the pathogen, present a formidable challenge to vaccine development. Despite these obstacles, an efficacious vaccine is probably the best approach to controlling chlamydial infection or its complications. Moreover, there is evidence, although controversial, that a partial short-lived immunity develops after natural chlamydial infection.[30,31] For instance, clinical findings have shown that persons recently genitally infected with *C. trachomatis* were less likely to experience a reinfection.[30-34] In addition, resistance to trachoma appears to increase with age and hence exposure, [34,35] and vaccination with inactivated organisms does produce a short-lived protection against ocular re-challenge.[33] The successful development of an efficacious vaccine requires a better understanding of the immune parameters that mediate antichlamydial immunity, the identification of protective antigens, and the development of novel delivery vehicles such as vectors or adjuvants capable of targeting long-term protective immunity at mucosal surfaces. These goals have in fact been the focus of intense research efforts over the past 15 years, and the results have generated findings that support the working hypothesis that developing an efficacious antichlamydial vaccine is achievable. Experimental animal models including non-human primates have provided valuable information in our knowledge of protective immunity to infection and for testing promising vaccine candidates. We will attempt to synthesise the status of this work and provide what we believe are rational strategies towards the development of a conventional vaccine against human chlamydial infection of oculogenital epithelia.

3.1 Definition of the Immunological Requirements for Designing an Efficacious Antichlamydial Vaccine

While there is strong evidence for the existence of acquired immunity to chlamydial infection, and the generation of both humoral and cell-mediated immune responses in infected individuals (see recent reviews by Byrne,[32] and Grayston and Wang[33]), the immune parameters mediating antichlamydial protective immunity have only begun to be defined. Findings in several laboratories using animal models of experimental ocular, respiratory and genital infections, analysis of specific lymphocyte clones, genetically engineered gene knockout mice, adoptive transfer studies with specific antibodies or T-cell subsets, and murine and human strains of *C. trachomatis* have established that antichlamydial protective immunity is mediated primarily by T helper type 1 (Th1) response, involving the induction and recruitment of Th1 cells into the local mucosae.[36-45] The induction of spe-

cific humoral immune responses, including secretory and systemic antibodies, appears to play an ancillary role in protective immunity,[32,39,40,46] or at best is complementary to the Th1-driven T-cell-mediated immunity (CMI), possibly moderating the course or severity of a reinfection.[47-51] In addition, certain accessory cells, such as dendritic cells (DCs), contribute to antichlamydial immunity,[43,52,53] possibly because of their dynamic presence in the mucosal tissues, their motility and ability to transport antigen from the mucosal epithelium to the draining mucosal inductive sites,[54] and their efficient processing and presentation of antigens.[52,53,55,56] Moreover, dendritic cells have a proclivity for preferential activation of Th1 response,[57-59] which is due in part to their potent co-stimulatory ability associated with an elevated density expression of co-stimulators such as interleukin (IL)-1, IL-12, intercellular adhesion molecule (ICAM-1), lymphocyte function-associated antigen 3 (LFA-3), CD40 and B7 molecules.[59-61]

As shown in figures 1 and 2, and table I, the ability of an experimental vaccine regimen to confer short- and long-term protection against genital chlamydial infection in mice correlates with its capacity to induce a high frequency of chlamydial-specific Th1 cells at the genital mucosa. In addition, protection against primary genital chlamydial infection conferred by immune T cells from mice immunised with major outer membrane protein-immune stimulating complexes (MOMP-ISCOMS) [an experimental subunit vaccine regimen] or *ex vivo* chlamydial-pulsed dendritic cells (a candidate cellular vaccine) correlates more with the presence of secretory Th1-associated immunoglobulin (Ig) G2a than IgA (table II). The detection of a systemic Th1 response in successfully vaccinated mice should provide a more convenient method of assessing the induction of a Th1 response by these vaccine regimens.[62] In addition, more recent reports have essentially confirmed that a T-cell response is required for protective chlamydial immunity in humans.[32,40,45,63,64] Furthermore, there is usually a robust CMI-associated secretory IgG-2a and IgA accompanying the Th1-mediated protective immunity induced in experimental wild-type immunocompetent animal study groups.[38,65-67] Our studies in the murine genital chlamydial infection model using Fc receptor-deficient mice indicated that antibodies may contribute to antichlamydial immunity partly by facilitating chlamydial antigen uptake, processing and presentation by FcR+ antigen-presenting cells (APCs) for enhanced Th1 induction in pre-exposed animals (unpublished observation). Other supporting reports have indicated that B cells could contribute to memory response during chlamydial reinfection.[49-51] The CD8+ T-cell subsets contribute to the Th1-dependent protective immunity [68,69] through cytokine production that complements the CD4+ Th1-driven response rather than cytotoxicity *per se*.[51,70,71] Thus, current findings indicate that a potentially efficacious antichlamydial vaccine should elicit high levels of both mucosal and systemic Th1 response as well as a humoral response that might contribute to enhanced Th1 induction following reinfection.

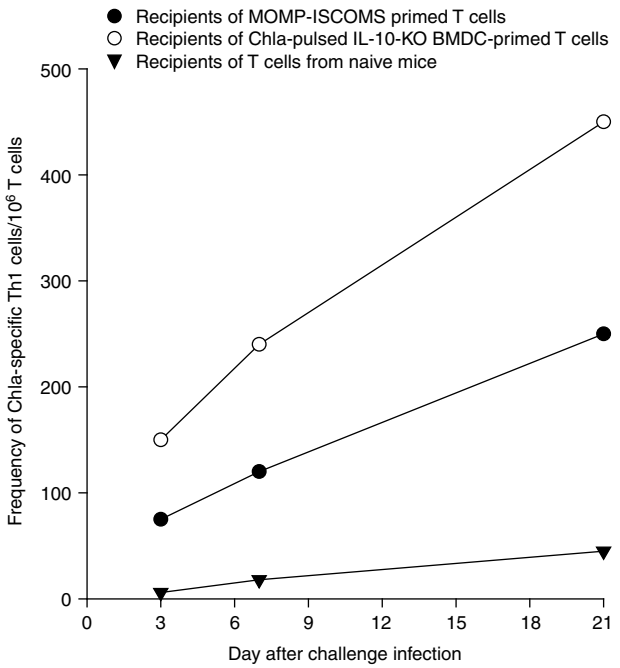

Fig. 1. Frequency of T helper-1 (Th1) cells in the genital mucosa of vaccinated mice. Protection against a primary genital infection conferred by immune T cells from mice immunised with major outer membrane protein-immune stimulating complexes (MOMP-ISCOMS) or ex vivo chlamydial (Chla)-pulsed interleukin-10-knockout (IL-10-KO) bone marrow-derived dendritic cells (BMDC) correlates with the recruitment of Chla-specific Th1 cells into the genital mucosa (see Igietseme et al.[72] and figure 2).

A nebulous aspect of the definition of the immune parameters that mediate anti-chlamydial immunity is how a predominantly Th1 response would conceivably confer protection without immunopathology. In this respect, the IL-10 knockout (IL-10-KO) dendritic cell-based vaccine findings (figures 1 and 2, and table I) would suggest that a rapid and vigorous Th1 response could quickly arrest chlamydial replication, clear the infection, eliminate residual antigens, and prevent the establishment of a latent infection. On the other hand, an inadequate Th1 response does not rapidly clear the pathogen, leading to the establishment of a latent infection, which fuels a low-grade chronic immune response that causes tissue damage. This proposition is supported by recent findings in experimental pulmonary chlamydial infection in mice in which IL-10-KO mice resolved the infection, and exhibited potent Th1 and strong delayed hypersensitivity (DTH) responses without tissue damage;[73] however, interferon (IFN)-KO mice exhibited a poor DTH and Th1 response profile, which was associated with lack of clearance of infection.[74] In addition, ICAM-

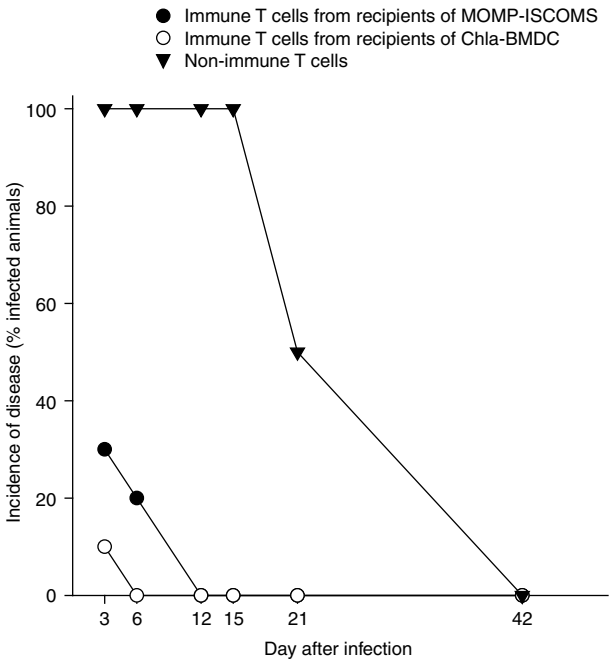

Fig. 2. Degree of protection conferred by immune T cells from immunised mice. T cells from mice immunised with major outer membrane protein-immune stimulating complexes (MOMP-ISCOMS) or *ex vivo* chlamydial (Chla)-pulsed bone marrow-derived dendritic cells (BMDC) conferred protection against a primary genital infection. There was an association between the level of protection achieved and the intensity of the local T helper-1 response induced by the vaccine regimens (see figure 1).

1-KO mice displayed a delayed Th1 response to chlamydial genital infection, severe acute cervical and ascending infection, and a high rate of hydrosalpinx, which was associated with the slow activation of specific Th1 cells by the ICAM-1-KO APCs.[75] Finally, immunopathology could be engendered through certain immune evasive mechanisms of chlamydia [e.g., inhibition of apoptosis,[76] and down-regulation of major histocompatibility complex (MHC) class I and II antigen expression [77,78]], with potential to modulate Th1 response and function, leading to inadequate effector function, chlamydial latency or persistence, chronic host reaction, and tissue damage. Thus, an efficacious antichlamydial vaccine should rapidly induce a strong Th1 response to arrest an infection in order to avert immunopathology.

3.2 Targeting Vaccines to Mucosal Inductive Sites

In designing vaccines against *Chlamydia*, it is crucial to select a route of administration that targets a vaccine to the appropriate draining lymphoid tissue(s) containing the primary APCs such as DCs and other efficient accessory cells capable of optimising mucosal Th1 response. In general, systemic immunisation routes are not effective for inducing significant protective immunity in mucosal tissues.[79-81] However, it is now appreciated that optimal induction of mucosal immunity generally requires targeting antigens to the specialised APCs of the mucosa-associated lymphoid tissues (MALT) in specific mucosal inductive sites.[82] MALT includes the nasal-associated lymphoid tissue (NALT), gut-associated lymphoid tissue (GALT), and bronchus-associated lymphoid tissue (BALT).[82,83] In this respect, orally administered antigens are targeted to GALT to induce protective immunity against pathogenic micro-organisms normally acquired via the gastrointestinal tract. Since the inductive and effector sites of the common mucosal immune system appear to be compartmentalised, specific inductive sites interact optimally with certain effector sites to produce effective immunity.[82,83] Therefore, it is important to choose a route of immunisation that would favour an effective cooperation between the mucosal inductive site(s) and a target effector site of infection.

The route of immunisation and the mucosal inductive sites that optimise mucosal Th1 response against *Chlamydia* are currently being studied. We have reported that intranasal immunisation-

Table I. Induction of long-term protection against chlamydial (Chla) infection in mice[a]

Day after immunisation[b]	Incidence of disease (% infected animals)		
	MOMP-ISCOMS- primed T-cell recipients	Chla-pulsed IL-10-KO BMDC-primed T-cell recipients	Recipients of T cells from naive mice
99	50 (3/6)	10 (1/6)	100 (6/6)
102	33 (2/6)	0 (0/6)	100 (6/6)
105	16.5 (1/6)	0 (0/6)	67 (4/6)
108	0 (0/6)	0 (0/6)	33 (2/6)
111	0 (0/6)	0 (0/6)	16.5 (1/6)
114	0 (0/6)	0 (0/6)	0 (0/6)

a Result: Immune T cells from mice immunised with MOMP-ISCOMS or *ex vivo* chlamydial-pulsed dendritic cells conferred long-term protective immunity against a secondary genital chlamydial infection. The level of protection was a function of the intensity of the T helper-1 cells in the genital mucosa induced by the vaccine regimen (see figure 1).

b Mice were reinfected 96 days after the primary challenge.

IL-10-KO BMDC = interleukin-10-knockout bone marrow-derived dendritic cells; **MOMP-ISCOMS** = major outer membrane protein-immune stimulating complexes.

Table II. Production of secretory immunoglobulin (Ig) A and IgG-2a after vaccination against Chlamydia (Chla) in mice[a]

Day after infection[b]	Mean antibody concentrations (μg/L) ± SEM		
	Recipients of MOMP-ISCOMS-primed T cells	Recipients of Chla-pulsed IL-10-KO BMDC-primed T cells	Recipients of T cells from naive mice
IgA antibody			
7	18.3 ± 2.3	18.8 ± 4.2	4.5 ± 0.5
14	26.5 ± 3.1	24.5 ± 2.2	14.8 ± 1.2
21	45.6 ± 5.0	35.2 ± 3.4	15.5 ± 0.6
24	57.7 ± 4.6	42.0 ± 6.8	20.2 ± 2.4
99	35.0 ± 3.7	28.6 ± 5.4	8.6 ± 3.2
108	50.8 ± 5.6	46.20 ± 4.3	16.4 ± 2.2
IgG-2a antibody			
7	28.8 ± 3.2	21.2 ± 3.2	5.5 ± 0.6
14	32.4 ± 4.3	42.6 ± 4.2	7.4 ± 2.4
21	41.6 ± 7.0	56.5 ± 5.4	8.6 ± 0.5
24	54.4 ± 6.4	72.8 ± 4.8	12.8 ± 3.2
99[b]	33.3 ± 2.6	58.4 ± 5.4	6.8 ± 2.4
108	45.0 ± 8.5	66.6 ± 5.1	14.4 ± 1.5

a Result: Protection against primary genital chlamydial infection conferred by immune T cells from mice immunised with MOMP-ISCOMS or *ex vivo* chlamydial-pulsed dendritic cells correlated better with the presence of secretory IgG-2a than IgA.[38]

b Mice were reinfected 96 days after the primary challenge.

IL-10-KO BMDC = interleukin-10-knockout bone marrow-derived dendritic cells; **MOMP-ISCOMS** = major outer membrane protein-immune stimulating complexes; **SEM** = standard error of the mean

induced protective antichlamydial immunity correlated with rapid elicitation of a genital mucosal Th1 response and the production of CMI-associated secretory IgG-2a and IgA.[38] Other corroborative reports have shown that the nasal route of immunisation caused rapid generation of effector lymphocytes detectable within days of the exposure,[84,85] and was superior to vaginal, gastric, peritoneal, or rectal immunisation for the induction of mucosal anti-HIV or anti-herpes simplex virus immune responses.[86,87] Moreover, nasal immunisation with live chlamydiae or an acellular outer membrane complex preparation protected mice against certain complications of genital chlamydial infection.[66,88,89] A recent report revealed that an experimental antichlamydial DNA-based vaccine delivered intranasally protected against *C. pneumoniae* in a lung infection model.[90] Thus, in terms of compartmentalisation of a common mucosal immune system, there is a strong link between NALT, BALT and the genital mucosal effector site(s).[83] However, the APCs and other accessory cells at the mucosal inductive sites of NALT have not been well defined, and the pathway for trafficking and recruitment of Th1 cells from NALT to the mucosal effector sites in the genital tract, as well as the critical molecular elements involved in regulating cellular recruitment and maintenance in the genital mucosa, remain unestablished. Among others, adhesion molecules, cytokines and chemokines will play a major role in this process.[91,92] In this respect, our studies and those of others have revealed that the temporary protective immunity associated with intravaginal infection with live chlamydiae was associated with the induction of the α4β1-vascular cell adhesion molecule (VCAM) and the α4β7-mucosal addressin cell adhesion molecule-1 (MAdCAM) pathways for T-cell recruitment into the genital mucosa.[93-95] Besides, direct epithelial T-cell interaction via the ICAM1/LFA-1 adhesion pathway is required for the efficient killing of intracellular chlamydiae in infected cells.[96] Future studies should define and molecularly characterise these adhesion, homing, trafficking and interacting molecules in the mucosal inductive and effector sites involved in genital, respiratory and ocular mucosal immunity, so that they can be better targeted to optimise immunity against *Chlamydia* and other agents of STDs. Moreover, the recruitment and retention of immune effectors in the genital, respiratory or ocular mucosa are important for maintaining long-term immunity against chlamydial infections,[97] and the molecular pathways and regulatory elements should be established.

The intramuscular route has been used in experimental DNA immunisations against chlamydial respiratory infections,[67,98-101] although the success of this route and regimen has not been demonstrated in genital tract infection models (Pal et al.[102] and unpublished observations). These findings may suggest that different vaccine regimens will require different immunisation routes, since intramuscular delivery of MOMP-ISCOMS induced genital mucosal Th1 response and protection against genital challenge in

mice.[62] The relevant APCs and molecular pathways for trafficking of effectors elicited under different immunisation protocols would need to be established in order to better understand the dynamics of immune elicitation and function under those conditions. While intranasal and intramuscular routes have been established for specific experimental vaccines, intrarectal, intravaginal and subcutaneous routes may deserve consideration in the future as more vaccine regimens are developed and if acceptability is gained in those areas. Ultimately, different regimens would probably require different routes of immunisation to target the inductive site(s) that induces optimal mucosal Th1 response against *Chlamydia*.

3.3 Antichlamydial Mechanisms of Immune Effectors

A detailed knowledge of the molecular and biochemical mechanisms underlying the antichlamydial action of immune effectors could furnish potential targets for therapeutic intervention. *In vitro* experimental systems involving chlamydial colonisation, intracellular multiplication and inclusion formation in permissive cells have aided studies designed to elucidate the molecular and biochemical mechanisms of chlamydial inhibition by host immune effectors. Such mechanistic analyses, including recent *in vivo* corroborative studies, have revealed that T-cell-derived cytokines, especially IFNγ and tumour necrosis factor-α, are crucial for antichlamydial immunity in humans and in experimental animals.[63,70,103-110] The biochemical basis of the antimicrobial action of these cytokines includes the following:

- the activation of phagocytes (e.g. macrophages) to rapidly take up and degrade chlamydiae or infected cells[111,112]
- the induction of indoleamine 2, 3-dioxygenase, an enzyme that catalyses the decyclisation of L-tryptophan into N-formylkynurenine,[113-115] thereby limiting the availability of the essential amino acid, and consequently inhibiting chlamydial growth
- the activation of the inducible nitric oxide synthase, which catalyses the production of various antimicrobial reactive nitrogen intermediates, most notably nitric oxide from L-arginine[110,116-120]
- the induction of intracellular iron deficiency by a process that at least partly involves the down-regulation of transferrin receptors, and restriction of microbial growth.[121]

We have reported that the last three mechanisms (as shown in figure 3), are involved in the antichlamydial action of T-cell-derived cytokines.[118] Iron deprivation has recently been confirmed to influence the growth of different species of *Chlamydia*.[122,123] In the case of antibodies, it is now established that their role is complementary to the predominant Th1 mechanism controlling *Chlamydia* in infected hosts. Thus, besides the potential role in

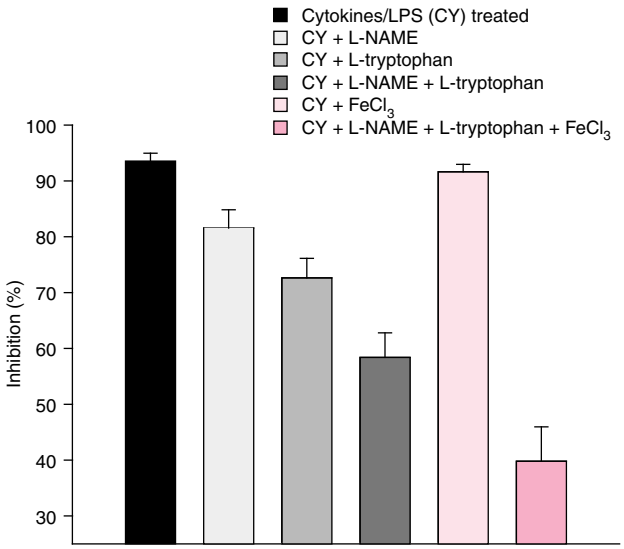

Fig. 3. Role of inducible nitric oxide synthase (iNOS), indoleamine 2,3-dioxygenase (IDO) and Fe systems in cytokine-induced inhibition of chlamydial growth in RT4 cells. Cultures of cytokine-treated RT4 cells were infected with *Chlamydia trachomatis* serovar E, in the presence or absence of N(omega)-nitro-L-arginine methyl ester (L-NAME), L-tryptophan, $FeCl_3$, or a combination of the reagents. The combination of the three antimicrobial systems was the most effective in chlamydial inhibition. Reproduced from Igietseme et al,[118] with permission.

neutralisation of extracellular infectious elementary bodies,[124-126] antibodies appear to function in antibody-dependent cellular cytotoxicity (ADCC) mediated by macrophages and natural killer (NK) cells, and are important for establishing an adequate memory response against chlamydial reinfection[49-51,71] and in enhancing the magnitude of Th1 response during reinfection.[127] Although *Chlamydia* is an obligate intracellular pathogen, CD8+ cytotoxic T cells contribute to antichlamydial immunity via cytokine-mediated antimicrobial effects rather than their cytolytic action.[70,71] In fact, apoptosis via Fas/Fas ligand, or both perforin and Fas ligand, was irrelevant to the antichlamydial action of T cells in mice.[128] It was, however, recently reported that *Chlamydia* could inhibit apoptosis of infected cells,[76] and that chlamydial protease down-regulated class I and II MHC expression by degrading critical transcription factors.[77,78] The significance of these findings in the context of persistent chlamydial infection, evasion of immune effectors and targets for intervention, and boosting host immune effectors against *Chlamydia* is yet to be analysed.

3.4 Antigenic Requirements for a Vaccine

3.4.1 Whole Versus Subunit Vaccines

Early human trials of candidate vaccines composed of whole bacterial cells [28,33,129-131] revealed that vaccinated individuals

experienced exacerbated disease during subsequent infection episodes.[28] Thus, the use of whole chlamydial agents appears to be unattractive because of the potential existence of immunopathogenic components.[27] However, considering the complexity of certain intracellular pathogens, such as *Chlamydia, Mycobacteria, Listeria* and *Legionella*, and the need for multiple epitopes to induce protection against different isolates, the significance of using live attenuated whole organisms as vaccines has been emphasised.[132,133] In the case of *Chlamydia*, no stable genetic systems for successful transformation and production of attenuated strains have been developed, except for some preliminary reports that are yet to be extended.[134,135] Besides, only one report has demonstrated the isolation of *C. trachomatis* mutants.[136] However, live attenuated *C. psittaci* strains have been developed and can successfully protect ewes from chlamydia-induced abortion,[77,137,138] suggesting that there is hope for producing live attenuated vaccines if the immunopathogenic concerns are addressed. Nevertheless, progress made in molecular and cellular immunology and genetic bioengineering in the last 2 decades has led to a gradual shift in the philosophy of vaccine development, from the classical whole vaccines consisting of inactivated (e.g. rabies, pertussis, cholera and Salk poliovirus), and live-attenuated (e.g. measles, mumps, rubella, tuberculosis and Sabin poliovirus) intact pathogens or their inactivated toxins (e.g. toxoids of tetanus and diphtheria), to a new focus on epitope, peptide, oligosaccharide, oligoglycopeptide or subunit vaccines. This change is due partly to the availability of technology enabling the identification, isolation and purification of relevant antigenic determinants of a complex antigen, as well as mass production for human use. In a growing list of accomplishments in these new approaches to vaccinology, subunit human vaccines are currently available against pneumococci, meningococci, *Haemophilus influenzae*, hepatitis B and influenza viruses. A subunit vaccine is therefore attainable for preventing chlamydial infections and its complications.

3.4.2 Identification and Selection of Vaccine Candidates

The goal of human chlamydial vaccine research is to identify an immunogenic protein containing adequate Th1 epitopes to elicit a vigorous Th1 response, and sufficient Th2 and antibody epitopes to induce a humoral immune response required for optimal protection from reinfection. The identification of an immunogenic and protective antigen(s) that can serve as a subunit or peptide vaccine has been a major focus of chlamydial research for almost 3 decades.[32]

Although recent strides in chlamydial genomics have predicted additional immunogenic proteins,[25,139-141] there are eight major serologically defined chlamydial antigens recognised during human infection by immunoblotting analysis of sera from women with *C. trachomatis* cervical infections.[27,142-144] These antigens range in size from 10 to 75kD and most of the encoding genes have been cloned.[27] *DnaK* and *GroEL* encode the 75 and 60kD heat-shock proteins, respectively, while *Omp-1*, *Omp-2* and *Omp-3* encode the 40, 60 and 15kD outer membrane proteins (OMPs), respectively. Considering a potential vaccine candidate, the 40kD Omp-1 antigen, also called the major outer membrane protein (MOMP), is regarded as the most promising. First, MOMP is both highly immunogenic, immunoaccessible, and elicits T-cell responses and neutralising antibodies. Second, MOMP is the dominant surface protein (60% of the total protein mass in the outer membrane), and is expressed in all phases of the life-cycle of *Chlamydia*.[27,144,145] The multifunctional attributes of MOMP include its contribution to the structural integrity of the chlamydial elementary body through disulphide bonding with other membrane components, and possible function as a porin,[146-148] as well as its role in attachment to the eukaryotic cell surface.[149] Typically, the *Omp-1* gene has a 1,182 base-pair open-reading frame, encoding a 394 amino acid polypeptide with 8 cysteine residues and a 22 amino acid signal peptide, and harbours 2 or more tandem promoters.[150,151] Comparative sequence analysis revealed that MOMP is 84 to 97% identical in nucleotides and amino acids among several *C. trachomatis* serovars, but variation in amino acid sequence is clustered into 4 variable surface-exposed sequence domains (VD1, VD2, VD3 and VD4) that are interspersed by invariable sequences.[152-155]

Immunological analysis has shown that MOMP harbours extensive species- and genus-specific immunogenic epitopes,[27,152] suggesting that a MOMP-based vaccine with either a narrow- or broad-spectrum effect is feasible. However, vaccine effectiveness based upon MOMP or other chlamydial proteins has been limited in part because of poor immunogenicity. Thus, in previous MOMP-based vaccine studies, intact MOMP, oligopeptide, cloned DNA or recombinant protein fragments corresponding to MOMP have been used for immunisation with different adjuvant systems, and various degrees of immunogenicity or levels of protective immunity were observed.[27,44,65,67,90,98,101,102,156-166] The lack of satisfactory protective immunity with these MOMP-based vaccine regimens would suggest that either MOMP alone is inadequate as a vaccine candidate or better delivery systems are needed to optimise the effect of MOMP. Current efforts in chlamydial genomics and proteomics will probably lead to the identification of additional immunogenic antigens [25,139-141,167-169] that may be compared with or added to MOMP in future vaccine design.

In addition to immunogenicity and ability to induce a high frequency of Th1 cells, a potential subunit vaccine candidate aimed at wide acceptability should possess broad specificity, inducing protection across species and across serovars, and should lack

toxicity. For instance, a recent report revealed that OMP2 of several *C. trachomatis* serovars, *C. pneumoniae* and *C. psittaci* harbours a sequence homologous to the pathogenic epitope of the human α-myosin heavy chain capable of inducing autoimmune myocarditis in mice.[170] The pathogenic sequence was not found in OMP1 or OMP3. Although the potential role of this epitope in microbial pathogenesis in man is currently unknown, it will be important to screen for such epitopes in selected vaccine candidates and to observe these candidates over time to determine whether an adverse reaction occurs in recipients when compared with non-recipients. Thus, specific strategies for analysing vaccine toxicity are additional requirements for evaluating any candidate subunit vaccine.

3.4.3 Significance of Delivery Vehicles and Adjuvants in Modern Vaccinology

In general, the efficacy of a vaccine is influenced by the immunogenicity of the antigen and the immune status of the host. In particular, the mucosal immune response to a vaccine directed at mucosally laden pathogens can be affected by additional factors that include vector, adjuvant, delivery vehicle or route of administration, and hormones associated with the estrous cycle (for genital mucosal response).[171] These combined requirements are important because even the most promising vaccine formulations may fail to establish the desired protective immunity because of an inadequate delivery vehicle that does not optimise, or that adversely affects, mucosal immune elicitation and maintenance. While the era of epitope or subunit vaccines has obviated the concerns inherent in inactivated or live-attenuated whole pathogens,[172-174] including infectious, noxious or integrating nucleic acid contents, induction of nonprotective blocking antibodies,[175] epitope destruction during inactivation [176] and presence of pathogenic antigenic determinants,[28,174] modern vaccinology has also encountered a major set-back associated with the relatively poor immunogenicity of candidate vaccines. Thus, the preference for epitopic or subunit vaccines has necessitated the search for more efficient delivery vehicles, such as adjuvants or vectors, to boost immune responses against the antigens.

3.4.4 Current and Potential Strategies

In the light of current knowledge of the immunobiology of chlamydial infection and antigenic requirements for a vaccine, the challenge at this point is to design a vaccine regimen equipped with an effective delivery vehicle(s) and targeted to the appropriate APCs, such as dendritic cells, to induce high levels of Th1 response with the accompanying humoral immune response in the ocular or genital mucosa. This challenge can be crystallised into two crucial objectives at this time: first, the judicious selection of an immunogenic antigen(s) or immunogen; and, second, the development of appropriate and effective delivery vehicles, such as adjuvants and vectors, or biological manipulations capable of boosting Th1 response and targeting it to the genital or ocular mucosa.[25] Various strategies have been used to deliver chlamydial antigens to enhance immunogenicity and protective immunity. Earlier strategies using non-ionic detergents, such as octyl-β-D-glucopyranoside, to deliver nondenatured chlamydial proteins produced mixed results in various animal models of experimental chlamydial infections.[156,157] Vector-mediated immunisation with naked DNA has received the most attention in recent times,[65,90,98,99,177-179] and although this approach has been mostly successful in the murine lung model of *Chlamydia* infection,[102] it has also been useful for rapid screening of vaccine candidates identified through advances in chlamydial genomics and proteomics.[169] The use of DNA vectors alone or in a prime-boost strategy [180] that includes delivery of chlamydial MOMP with lipophilic immune-stimulating complexes (ISCOMS) was also protective in the mouse lung infection model.[65] ISCOMS used as an adjuvant in the mouse genital infection model was immunogenic, inducing both genital mucosal and systemic Th1 responses that were protective in limited challenge studies that evaluated microbial shedding alone (Igietseme and Murdin[62] and see figures 1 and 2). These findings would suggest that some success has been achieved with the use of vehicles and adjuvants to deliver chlamydial proteins as vaccine regimens, and more attention should be paid to this aspect of chlamydial vaccine design. At the present phase of the technology, the DNA vaccine strategy should be highly useful when screening for potential vaccine candidates in experimental models, pending the alleviation of DNA integration and toxicity concerns. As *Chlamydia* infection is a major health threat, all contemporary vaccine strategies favouring immune induction against intracellular pathogens and STDs[181-183] should be explored to design an efficacious vaccine against this disease.

Promising Adjuvants and Vectors

DNA or vector delivery strategies are amenable to fusion of immunostimulatory CpG motifs[184,185] or specific APC-targeting domains, such as the ligands for the co-stimulatory B7,[186] CD40[187] or genes expressing specific chemokines, to gene sequences encoding a candidate chlamydial vaccine to induce protective immunity. A promising delivery system deserving serious consideration is the use of recombinant viral vectors. Apart from earlier reports of recombinant poliovirus hybrid constructs harbouring chlamydial sequences as potential immunogenic vaccine regimens,[161,188] the use of viral vectors as delivery systems for designing experimental chlamydial vaccines has been limited. Noninfectious adenovirus,[189] canarypox virus,[190,191] vaccinia virus,[192,193] and alphavirus replicons [194-196] are some of the well characterised viral

delivery systems that are yet to be applied to chlamydial vaccine design. In addition, experimental mucosal adjuvants, including cholera toxin, heat-labile enterotoxin, mutant toxin (LTK63 and LTR7), polymerised liposomes, microparticles and ILs/immunomodulators, are potential strategies yet to be extended to chlamydial vaccine research.[197]

Bacterial Delivery Systems

The use of live attenuated or non-living bacteria as delivery systems, and designer APCs in immunotherapeutic cellular vaccines,[72,198-200] needs to be evaluated in chlamydial vaccine design. For instance, the *Lactobacillus*,[201,202] *Salmonella* and *Listeria* [203] delivery systems could be better analysed for use in delivering candidate chlamydial vaccines for inducing mucosal immunity. With respect to non-living bacterial systems, recombinant bacterial ghosts represent a novel delivery vehicle that can appropriately direct immune response against multiple antigens.[100,204] Bacterial ghosts are non-living bacterial cells devoid of cytoplasmic contents that maintain their native surface antigenic structures and cellular morphology. In the novel recombinant bacterial ghost vaccine strategy, an appropriate bacterium is transformed with the gene of interest to express high levels of the antigen in a targeted location on the cell, then the ghosts are produced by controlled lysis of the cells. Bacterial ghost preparations have intrinsic adjuvant properties that enhance immune responses against the antigen of interest, including enhanced T-cell activation and mucosal immunity, which are important for controlling *Chlamydia.* Since multiple genes can be expressed on these ghosts in a deliberately controlled manner, high levels of the antigen or different antigens from the same or different pathogens can be presented to the immune system simultaneously to produce effective combination vaccines against multiple agents.[204]

The recombinant ghost technology can be used to develop a multi-subunit vaccine against *Chlamydia.* Multiple chlamydial proteins can be expressed on the membrane of an appropriate bacterial vector that possesses intrinsic adjuvanticity and mucosal tropism. Recent efforts in designing multi-subunit experimental ghost vaccines by expressing MOMP and other select chlamydial proteins on the epitheliotropic *Vibrio cholerae* ghosts (VCGs) and testing the vaccine in an established mouse model of genital chlamydial disease appear to be promising.[205]

The use of VCGs in vaccine design as carriers of heterologous antigens, such as MOMP and other proteins of *C. trachomatis,* offers a number of immunological and strategic advantages in the development of multi-component vaccines against mucosal pathogens. First, there are several *V. cholerae*-based vaccines currently in use in different countries, so VCGs would not pose any special or unknown risks to humans. In addition, VCGs possess intrinsic adjuvant activity that boosts immune responses against the antigens carried, and are currently being used as a vehicle/adjuvant for designing specific vaccines, at different phases of clinical trials in Europe and Australia. Second, since *V. cholerae* is a mucosal pathogen, VCG would foster the elicitation of mucosal immunity. Third, VCGs are non-toxic, free of DNA or cholera toxin, relatively easy and cheap to produce, support the expression of heterologous genes and are efficient carriers of large amounts of foreign proteins that maintain their native conformations, thereby ensuring efficient antigen processing for immune activation. Fourth, since there is no size limitation of foreign protein moieties inserted into the cell envelope, multiple antigenic determinants from the same or different pathogens can be presented simultaneously as a combination vaccine. Finally, VCGs are capable of inducing both mucosal humoral and T-cell immune responses that protect experimental animals against *V. cholerae.*[204]

Cellular Vaccine Delivery

Cellular vaccines are adoptive immunotherapeutics in which *ex vivo* antigen-primed autologous or syngeneic immune cells are adoptively transferred into recipients to boost specific immunity against an antigen *in vivo*.[198-200] A variety of adoptive cellular immunotherapeutic strategies have been developed using various immune cells, especially in response to the necessity of developing novel alternative technology to fight cancers and certain infectious diseases where conventional therapeutic approaches, such as chemotherapy and antigen-based vaccine, have proven to be inadequate.[200] A promising immunotherapeutic tool is the use of dendritic cells as a vehicle, and APCs to present antigens for immune activation *in vivo*.[200,206] To further enhance immunogenicity and effectiveness, adoptively transferred cells have been genetically modified in various ways, including deletion of suppressive genes [72] or tagging with cytokine genes.[199] Regarding cellular vaccines as an alternative immunotherapeutic regimen against *Chlamydia*,[56,72] a reasonable argument is that an adoptive cellular immunotherapy is less likely to be suitable for controlling a widespread infectious disease like *Chlamydia*, because of the need for an autologous or syngeneic system for adoptive cell transfers. However, chlamydial-pulsed dendritic cells appear to possess the necessary antigenic, co-stimulatory and immunomodulatory features for inducing high levels of Th1 response and the accessory IgA and IgG effectors required for optimal protective immunity against *Chlamydia*[56,72] (figures 1 and 2, and tables I and II). This phenomenal efficacy of the dendritic cell-based cellular vaccine makes them dendritic cells 'natural adjuvants or preeminent delivery vehicles', useful as tools to guide the designing of effective delivery systems for immunising against chlamydial infections.

4. Current Chlamydial Vaccine Candidates and Prospects

A concerted effort has been made to crystallise available knowledge of the immunological and antigenic requirements for designing a chlamydial vaccine into an efficacious vaccine regimen. These efforts include the use of cellular and acellular immunogens such as *ex vivo* antigen-pulsed DCs, chlamydial outer membranes, synthetic oligopeptides, recombinant proteins, and naked DNA. Some promising vaccine regimens have emerged that may pave the way for the development of an acceptable efficacious vaccine against *Chlamydia* in the near future. We will highlight the findings using models of chlamydial genital, respiratory and ocular infections that are most relevant to the pathogens and human diseases that are the focus of this review.

4.1 Subunit Vaccines

A significant effort has been made regarding the testing of various types of subunit-based vaccines both with respect to immunogenicity and protective efficacy in models of *C. trachomatis* and more recently *C. pneumoniae* infection. Several MOMP-based candidate vaccines, including whole subunits, oligopeptides, cloned DNA or recombinant fragments have been delivered with or without adjuvants or vectors, and several degrees of immunogenicity and levels of protective immunity were observed.[27,44,65,67,98,101,156,158-161,164-166] In all these efforts, no sterile immunity was observed, and most reports showed only partial protective immunity, as measured by either reduction in infectious burden or pathology. Recent efforts in naked DNA immunisation using genes encoding MOMP and HSP60 proteins have shown the most promise,[21,64,98] but only using MOMP DNA and in models of respiratory chlamydial infection, not infection of the genital tract. Clearly, this approach deserves more investigation to determine whether the levels of protective immunity generated by MOMP DNA immunisation can be increased and whether protection can be expanded to include the genital mucosae – the primary target of a *C. trachomatis* vaccine.[65,101,102] Interestingly, the immunogenicity and protective immunity induced by MOMP DNA vaccine were enhanced by use of an adjuvant.[65] This would suggest that proper selection of adjuvants and a delivery system to target immunity in the genital tract could enhance the immunogenicity and protective immunity induced by MOMP-based formulations. In addition, it has become clear that MOMP alone may be inadequate as an antichlamydial vaccine, prompting a search for other protective antigens to form the basis of a multicomponent vaccine. A major advantage of the multiple component approach is that the combination of epitopes furnished by MOMP and other protein antigens should be more effective at inducing a high frequency of immune effectors than MOMP alone, and could therefore induce a more solid and long-lasting immunity. Several other potential candidate vaccines, including the polymorphic outer membrane proteins (POMP) [207] have been predicted by recent advances in chlamydial genomics and proteomics.[25,26] In fact, these genomics efforts have identified a chlamydial ADP/ATP translocase of *C. pneumoniae* as a protective antigen in a DNA delivery and mouse lung infection model.[169] Comparative genomic[167,168] analysis is likely to identify other candidate vaccine antigens, which should lead to the judicious selection of a combination of immunogens that can be tested for immunogenicity and protective efficacy in relevant animal models of chlamydial oculogenital infection. The potential for non-protein antigens, such as the common chlamydial exoglycolipid antigen,[208] to form the basis of a subunit chlamydial vaccine candidate capable of inducing protective T-cell immunity is yet to be determined.

4.2 Cellular Vaccines

Both normal and genetically engineered dendritic cell-based immunotherapeutic cellular vaccines have been proposed for controlling *Chlamydia*.[56,72] This is because of the difficulty of developing an efficacious vaccine to date, the known contribution of dendritic cells to antichlamydial immunity after a natural infection,[43,52,53] the efficiency of processing and presentation of chlamydial antigens for activating high levels of Th1 response,[52,53,55,56] and to serve as a reliable alternative vaccination strategy against *C. trachomatis*. Dendritic cell-based therapy induced protective immunity against chlamydial genital infection that was at least equivalent to a natural infection in terms of pathology, clearance of infection and induction of Th1 response and the associated secretory IgA and IgG-2a. In the IL-10 suppressed dendritic cell-based cellular vaccine,[72] long-term protective immunity was associated with an elevated frequency of Th1 cells, and has potential to induce cross-protection from other *C. trachomatis* serovars or species. In any case, it has been shown that inactivated chlamydial elementary bodies possess sufficient immunogenic epitopes that can be used, in conjunction with an effective delivery system, to mount a protective immune response against *Chlamydia*. Moreover, since dendritic cell vaccines appear to possess all the elements of an efficient delivery system for an antichlamydial immunity, they could be used as a tool to unravel the necessary vaccine machinery in terms of antigens, immunity and homing requirements. Besides, the remarkable ability of dendritic cells to process whole antigens or components of an antigen and select the appropriate immunodominant epitope(s) for presentation to and activation of specific Th1 cells is a property that may obviate the current search for protective antigens, and laborious mapping

of immunogenic epitopes. Moreover, the potential for clinical application of dendritic cells in immunotherapies has resulted in the establishment of the technologies and protocols for efficient *ex-vivo* propagation of dendritic cells from peripheral blood cells of humans.[59,199,206,209,210]

Since only a subset of individuals infected with *Chlamydia* experience major complications, an efficacious and reliable cellular vaccine targeted at the high-risk population is better than the current situation of having no available efficacious or acceptable subunit vaccine. Thus, in terms of a more practical application, the dendritic cell-based delivery system should provide a model for efforts to design effective delivery vehicles that mimic the action of dendritic cells. In this respect, the dendritic cell-based cellular vaccine model system could be utilised to identify protective antigens and epitopes, which could be incorporated into suitable delivery vehicles. Some of the techniques useful for such studies include peptide elution from MHC molecules isolated from antigen-pulsed APCs and a proteomics approach involving mass spectrometry, which was recently presented by Dr. Donald F. Hunt of the University of Virginia, Charlottesville, VA, USA, at the President's Forum of the 101 General Meeting of the American Society for Microbiology (May 23, 2001). Alternatively, the cellular vaccine could furnish a reliable alternative for high-risk persons should other vaccine options fail.

5. Future Perspectives

There is little doubt that an efficacious vaccine represents the best approach to protect against chlamydial infections and their complications, but it must also be appreciated that more progress in this area of research is needed to achieve this goal. Specifically, more emphasis should be placed on research efforts aimed at the identification of protective antigens that could lead to a multicomponent vaccine. The effectiveness of the immunotherapeutic dendritic cell-based cellular vaccine using killed chlamydiae demonstrates that an effective conventional vaccine is feasible; however, in designing a subunit vaccine, it will be crucial to identify and mimic those immunostimulatory properties of dendritic cells that mediate an enhanced level of protective immunity. These efforts should be aimed at the following: (i) the identification of vectors capable of stimulating and retaining a regional chlamydial-specific type 1 cellular immune response at the genital, respiratory or ocular mucosa; (ii) the identification of new antigens that are key targets of cellular immunity as well as B-cell immunity; and (iii) the testing of candidate vaccines in preclinical animal models that are relevant to the targets of a vaccine in human infections of the eye, lung or genital tract. Lastly, it is possible that it may not be feasible to produce a conventional vaccine against oculogenital chlamydial infections in humans because of the epithelial tropism of the parasite, the necessity for multiple effector mechanisms to establish an optimum protective immune response, and the complex antigenic repertoire and multivariant features of the pathogen. In this context, it does not seem unreasonable to consider the use of attenuated chlamydial mutants as potential vaccines if the immunopathogenic concerns can be alleviated. Given the caveat of safety, an effective attenuated vaccine might in fact be an important approach to satisfy the apparent complex requirements in the development of an efficacious chlamydial vaccine. This approach requires modification of key chlamydial virulence factors and awaits the development of a chlamydial transformation system capable of modifying or knocking out targeted chlamydial genes.

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References

1. Schachter J, Grayston JT. Epidemiology of human chlamydial infections. In: Stephens RS, Byrne GI, Christiansen G, et al., editors. Chlamydial Infections. ICS Berkeley, San Francisco (CA), 1998: 3-10
2. Paavonen J, Wolner-Hanssen P. *Chlamydia trachomatis*: a major threat to reproduction. Hum Reprod 1989; 4: 111-24
3. Grayston JT. *Chlamydia pneumoniae*, strain TWAR pneumonia. Annu Rev Med 1992; 43: 317-23
4. Kuo CC, Jackson LA, Campbell LA, et al. *Chlamydia pneumoniae* (TWAR). Clin Microbiol Rev 1995; 8: 451-61
5. Saikku P, Wang SP, Kleemola M, et al. An epidemic of mild pneumonia due to an unusual strain of Chlamydia psittaci. J Infect Dis 1985; 151: 832-9
6. Black C. Current methods of laboratory diagnosis of *Chlamydia trachomatis* infections. Clin Microbiol Rev 1997; 10: 160-84
7. Schachter J. Diagnosis of human chlamydial infections. In: Stephens RS, Byrne GI, Christiansen G, et al., editors. Chlamydial infections. ICS Berkeley, San Francisco (CA), 1998: 577-586
8. Cohen CR, Brunham RC. Pathogenesis of Chlamydia induced pelvic inflammatory disease. Sex Transm Infect 1999; 75: 21-4
9. Everett KD, Bush RM, Andersen AA. Emended description of the order Chlamydiales, proposal of Parachlamydiaceae fam. nov. and Simkaniaceae fam. nov., each containing one monotypic genus, revised taxonomy of family Chlamydiaceae, including new genus and five new species, and standards for the identification of organisms. Int J Syst Bacteriol 1999; 49: 415-40
10. Bush RM, Everett KD. Molecular evolution of Chlamydiaceae. Int J Syst Evol Microbiol 2001; 51: 203-20
11. Schachter J, Stephens RS, Timms P, et al. Radical changes to chlamydial taxonomy are not necessary just yet. Int J Syst Evol Microbiol 2001; 51: 251-3
12. World Health Organization (WHO). Global prevalence and incidence of selected curable sexually transmitted diseases: overview and estimates. Geneva: WHO, 1996
13. Grayston JT. Background and current knowledge of *Chlamydia pneumoniae* and atherosclerosis. J Infect Dis 2000; 181: S402-10

14. Grayston JT, Campbell LA. The role of *Chlamydia pneumoniae* in atherosclerosis. Clin Infect Dis 1999; 28: 993-4
15. Saikku P, Leinonen M, Mattila K, et al. Serological evidence of an association of a novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. Lancet 1988; 2: 983-6
16. Saikku P, Leinonen M, Tenkanen L, et al. Chronic *Chlamydia pneumoniae* infection as a risk factor for coronary heart disease in the Helsinki Heart Study. Ann Intern Med 1992; 116: 273-8
17. Grayston JT, Aldous MB, Easton A, et al. Evidence that *Chlamydia pneumoniae* causes pneumonia and bronchitis. J Infect Dis 1993; 168: 1231-5
18. Hahn DL, Dodge RW, Golubjatnikov R. Association of *Chlamydia pneumoniae* (strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset asthma. JAMA 1991; 266: 225-30
19. Balin BJ, Gerard HC, Arking EJ, et al. Identification and localization of *Chlamydia pneumoniae* in the Alzheimer's brain. Med Microbiol Immunol (Berl) 1998; 187: 23-42
20. Sriram S, Stratton CW, Yao S, et al. *Chlamydia pneumoniae* infection of the central nervous system in multiple sclerosis. Ann Neurol 1999; 46: 6-14
21. De la Maza MA, De la Maza LM. A new computer model for estimating the impact of vaccination protocols and its application to the study of *Chlamydia trachomatis* genital infections. Vaccine 1995; 13: 119-27
22. Richey CM, Macaluso M, Hook EW. Determinants of reinfection with *Chlamydia trachomatis*. Sex Transm Dis 1999; 26: 4-11
23. Stern JE, Gardner S, Quirk D, et al. Secretory immune system of the male reproductive tract: effects of dihydrotestosterone and estradiol on IgA and secretory component levels. J Reprod Immunol 1992; 22: 73-85
24. Grayston JT, Wang SP. New knowledge of chlamydiae and the diseases they cause. J Infect Dis 1975; 132: 87-105
25. Stephens RS. Chlamydial genomics and vaccine antigen discovery. J Infect Dis 2000; 181: S521-3
26. Rockey DD, Stephens RS. Genome sequencing and our understanding of chlamydiae. Infect Immun 2000; 68: 5473-9
27. Brunham RC, Peeling RW. *Chlamydia trachomatis* antigens: role in immunity and pathogenesis. Infect Agents Dis 1994; 3: 218-33
28. Grayston JT, Wang SP, Yeh LJ, et al. Importance of reinfection in the pathogenesis of trachoma. Rev Infect Dis 1985; 7: 717-25
29. Morrison RP. Immune responses to chlamydia are protective and pathogenic. In: Bowie WR, editors. Chlamydial Infections. New York: Cambridge University Press, 1990: 163-172
30. Katz BP, Batteiger BE, Jones RB. Effect of prior sexually transmitted disease on the isolation of *Chlamydia trachomatis*. Sex Transm Dis 1987; 14: 160-4
31. Parks KS, Dixon PB, Richey CM, et al. Spontaneous clearance of *Chlamydia trachomatis* infection in untreated patients. Sex Transm Dis 1997; 24: 229-35
32. Byrne GI. Immunity to Chlamydia. In: Stephens RI, Byrne GI, Christainsen G, et al., editors. Chlamydial infections. 1998 ed. ICS Berkley, San Francisco (CA); 1998: 365-374
33. Grayston JT, Wang SP. The potential for vaccine against infection of the genital tract with *Chlamydia trachomatis*. Sex Transm Dis 1978; 5: 73-7
34. Grayston JT, Wang SP, Yang YF, et al. The effect of trachoma virus vaccine on the course of experimental trachoma infection in blind human volunteers. J Exp Med 1962; 115: 1009-22
35. Bailey R, Duong T, Carpenter R, et al. The duration of human ocular *Chlamydia trachomatis* infection is age dependent. Epidemiol Infect 1999; 123: 479-86
36. Cotter TW, Ramsey KH, Miranpuri GS, et al. Dissemination of *Chlamydia trachomatis* chronic genital tract infection in gamma interferon gene knockout mice. Infect Immun 1997; 65: 2145-52
37. Igietseme JU, Ramsey KH, Magee DM, et al. Resolution of murine chlamydial genital infection by the adoptive transfer of a biovar-specific, Th1 lymphocyte clone. Reg Immunol 1993; 5: 317-24
38. Igietseme JU, Uriri IM, Kumar SN, et al. Route of infection that induces a high intensity of gamma interferon-secreting T cells in the genital tract produces optimal protection against *Chlamydia trachomatis* infection in Mice. Infect Immun 1998; 66: 4030-5
39. Johansson M, Schon K, Ward M, et al. Genital tract infection with *Chlamydia trachomatis* fails to induce protective immunity in gamma interferon receptor-deficient mice despite a strong local immunoglobulin A response. Infect Immun 1997; 65: 1032-44
40. Johansson M, Schon K, Ward M, et al. Studies in knockout mice reveal that anti-chlamydial protection requires TH1 cells producing IFN-gamma: is this true for human? Scand J Immunol 1997; 46: 546-52
41. Patton DL, Rank RG. Animal models for the study of pelvic inflammatory disease. In: Quinn TC, editor. Sexually transmitted diseases. New York: Raven Press Ltd., 1992: 85-111
42. Perry LL, Feilzer K, Caldwell HD. Immunity to *Chlamydia trachomatis* is mediated by T helper 1 Cells through IFN-gamma-dependent and -independent pathways. J Immunol 1997; 158: 3344-52
43. Stagg AJ, Tuffrey M, Woods C, et al. Protection against ascending infection of the genital tract by *Chlamydia trachomatis* is associated with recruitment of major histocompatibility complex class II antigen-presenting cells into uterine tissue. Infect Immun 1998; 66: 3535-44
44. Su H, Caldwell HD. CD4+ T cells play a significant role in adoptive immunity to *Chlamydia trachomatis* infection of the mouse genital tract. Infect Immun 1995; 63: 3302-8
45. Yang X, Brunham RC. T lymphocyte immunity in host defense against . Can J Infect Dis 1998; 9: 99-108
46. Bailey RL, Kajbaf M, Whittle HC, et al. The influence of local antichlamydial antibody on the acquisition and persistence of human ocular chlamydial infection: IgG antibodies are not protective. Epidemiol Infect 1993; 111: 315-24
47. Cotter TW, Meng Q, Shen Z-L, et al. Protective efficacy of outer membrane protein-specific immunoglobulin A (IgA) and IgG monoclonal antibodies in a murine model of *Chlamydia trachomatis* genital tract infection. Infect Immun 1995; 63: 4704-14
48. Morrison RP, Feilzer K, Tumas DB. Gene knockout mice establish a primary protective role for major histocompatibility complex class II-restricted responses in *Chlamydia trachomatis* genital tract infection. Infect Immun 1995; 63: 4661-8
49. Morrison SG, Morrison RP. Resolution of secondary *Chlamydia trachomatis* genital tract infection in immune mice with depletion of both CD4+ and CD8+ T cells. Infect Immun 1995; 69: 2643-9
50. Su H, Feilzer K, Caldwell HD, et al. *Chlamydia trachomatis* genital tract Infection of antibody-deficient gene knockout mice. Infect Immun 1997; 65: 1993-9
51. Yang X, Brunham RC. Gene knockout B cell-deficient mice demonstrate that B cells play an important role in the initiation of T cell responses to *Chlamydia trachomatis* (mouse pneumonitis) lung infection. J. Immunol 1998; 161: 1439-46
52. Ojcius DM, Bravo de Alba Y, Kanellopoulos JM, et al. Internalization of Chlamydia by dendritic cells and stimulation of Chlamydia-specific T cells. J Immunol 1998; 160: 12970-1303
53. Stagg AJ, Stackpoole A, Elsley WJ, et al. Acquisition of chlamydial antigen by dendritic cells and monocytes. Adv Exp Med Biol 1993; 329: 581-6
54. Neutra MR, Pringault E, Kraehenbuhl J-P. Antigen sampling across epithelial barriers and induction of mucosal immune responses. Annu Rev Immunol 1996; 14: 275-300
55. Su H, Caldwell HD. Kinetics of chlamydial antigen processing and presentation to T cells by paraformaldehyde-fixed murine bone marrow-derived macrophages. Infect Immun 1995; 3: 946-53
56. Su H, Messer R, Whitmire W, et al. Vaccination against chlamydial genital tract infection after immunization with dendritic cells pulsed ex vivo with nonviable chlamydiae. J Exp Med. 1998; 188: 809-18
57. Heufler C, Koch F, Stanzl U, et al. Interleukin-12 is produced by dendritic cells and mediates T helper 1 development as well as interferon-gamma production by T helper 1 cells. Eur J Immunol 1996; 26: 659-68

58. Macatonia SE, Hosken NA, Litton M, et al. Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4+ T cells. J Immunol 1995; 154: 5071-9
59. Steinman RM. The dendritic cell system and its role in immunogenicity. Annu Rev Immunol 1991; 9: 271-96
60. Jenkins MK, DeSilva DR, Johnson JG, et al. Costimulating factors and signals relevant for antigen presenting cell function. Adv Exp Med Biol 1993; 329: 87-92
61. King PD, Ibrahim MAA, Katz DR. Adhesion molecules: co-stimulators and co-mitogens in dentritic cell-T cell interaction. Adv Exp Med Biol 1993; 329: 53-8
62. Igietseme JU, Murdin A. Induction of protective immunity against *Chlamydia trachomatis* genital infection by a vaccine based on major outer membrane protein-lipophilic immune response-stimulating complexes. Infect Immun 2000; 68: 6798-806
63. Cohen CR, Nguti R, Bukusi EA, et al. Human immunodeficiency virus type 1-infected women exhibit reduced interferon-gamma after *Chlamydia trachomatis* stimulation of peripheral lymphocytes. J Infect Dis 2000; 182: 1672-7
64. Stagg AJ. Vaccines against Chlamydia: approaches and progress. Mol Med Today 1998; 4: 166-73
65. Dong-Ji Z, Yang X, Shen C, et al. Priming with *Chlamydia trachomatis* major outer membrane protein (MOMP) DNA followed by MOMP-ISCOM boosting enhances protection and is associated with increased immunoglobulin A and Th1 cellular immune responses. Infect Immun 2000; 68: 3074-8
66. Pal S, Peterson EM, de la Maza LM. Intranasal immunization induces long-term protection in mice against a *Chlamydia trachomatis* genital challenge. Infect Immun 1996; 64: 5341-8
67. Zhang DJ, Yang X, Shen C, et al. Characterization of immune responses following intramuscular DNA immunization with the MOMP gene of *Chlamydia trachomatis* mouse pneumonitis strain. Immunology 1999; 96: 314-21
68. Buzoni-Gatel D, Guilloteau L, Bernard F, et al. Protection against *Chlamydia psittaci*in mice conferred by Lyt2+ T cells. Immunology 1992; 77: 284-8
69. Starnbach MN, Bevan MJ, Lampe MF. Protective cytotoxic T lymphocytes are induced during murine infection with *Chlamydia trachomatis*. J Immunol 1994; 153: 5183-9
70. Lampe MF, Wilson CB, Bevan MJ, et al. Gamma interferon production by cytotoxic T lymphocytes is required for resolution of *Chlamydia trachomatis* infection. Infect Immun 1998; 66: 5457-61
71. Morrison SG, Su H, Caldwell HD, et al. Immunity to murine *Chlamydia trachomatis* genital tract reinfection involves B cells and CD4+ T cells but not CD8+ T cells. Infect Immun 2000; 68: 6979-87
72. Igietseme JU, Ananaba GA, Bolier J, et al. Suppression of endogenous IL-10 gene expression in dendritic cells enhances antigen presentation for enhanced specific Th1 induction: potential for cellular vaccine development. J Immunol 2000; 164: 4212-9
73. Yang X, Gartner J, Zhu L, et al. IL-10 gene knockout mice show enhanced Th1-like protective immunity and absent granuloma formation following *Chlamydia trachomatis* lung infection. J Immunol 1999; 162: 1010-7
74. Wang S, Fan Y, Brunham RC, et al. IFN-gamma knockout mice show Th2-associated delayed-type hypersensitivity and the inflammatory cells fail to localize and control chlamydial infection. Eur J Immunol 1999; 29: 3782-92
75. Igietseme JU, Ananaba GA, Bolier J, et al. The intercellular adhesion molecule type-1 is required for rapid activation of T helper type 1 (Th1) lymphocytes that control early acute phase of genital chlamydial infection in mice. Immunology 1999; 98: 510-9
76. Fan T, Lu H, Hu H, et al. Inhibition of apoptosis in chlamydia-infected cells: blockade of mitochondrial cytochrome c release and caspase activation. J Exp Med 1998; 187: 487-96
77. Rodolaki A, Salinas J, Papp J. Recent advances on ovine chlamydial abortion. Vet Rec 1998; 29: 275-88
78. Zhong G, Liu L, Fan T, et al. Degradation of transcription factor RFX5 during the inhibition of both constitutive and interferon gamma-inducible major histocompatibility complex class I expression in chlamydia-infected cells. J Exp Med 2000; 191: 1525-34
79. Holmgren J, Czerkinsky C, Lycke N, et al. Mucosal immunity: implications for vaccine development. Immunobiology 1992; 184: 157-79
80. McGhee JR, Mestecky J, Dertzbaugh MT, et al. The mucosal immune system: from fundamental concepts to vaccine development. Vaccine 1992; 10: 75-88
81. Mestecky J, Jackson S. Reassessment of the impact of mucosal immunity in infection with the human immunodeficiency virus (HIV) and design of relevant vaccines. J Clin Immunol 1994; 14: 259-72
82. Moldoveanu Z, Russell MW, Wu H-Y, et al. Compartmentalization within the common mucosal immune system. Adv Exp Med Biol 1995; 371: 97-101
83. Wu H-Y, Russell MW. Nasal lymphoid tissue, intranasal immunization, and compartmentalization of the common mucosal immune system. Immunol Res 1997; 16: 187-201
84. Kelly KA, Robinson EA, Rank RG. Initial route of antigen administration alters the T-cell cytokine profile produced in response to the mouse pneumonitis biovar of *Chlamydia trachomatis* following genital infection. Infect Immun 1996; 64: 4976-83
85. Wu H-Y, Nikolova EB, Beagley KW, et al. Induction of antibody-secreting cells and T-helper and memory cells in murine nasal lymphoid tissue. Immunology 1996; 88: 493-500
86. Gallichan WS, Rosenthal KL. Specific secretory immune responses in the female genital tract following intranasal immunization with a recombinant adenovirus expressing glycoprotein B of herpes simplex virus. Vaccine 1995; 13: 1589-95
87. Staats HF, Montgomery SP, Palker TJ. Intranasal immunization is superior to vaginal, gastric, or rectal immunization for induction of systemic and mucosal anti-HIV antibody responses. AIDS Res Hum Retroviruses 1997; 13: 945-52
88. Pal S, Fielder TJ, Peterson EM, et al. Protection against infertility in a BALB/c mouse salpingitis model by intranasal immunization with a mouse pneumonitis biovar of *Chlamydia trachomatis*. Infect Immun 1994; 62: 3354-62
89. Pal S, Theodor I, Peterson EM, et al. Immunization with an acellular vaccine consisting of the outer membrane complex of *Chlamydia trachomatis* induces protection against a genital challenge. Infect Immun 1997; 65: 3361-9
90. Svanholm C, Bandholtz L, Castanos-Velez E, et al. Protective DNA immunization against *Chlamydia pneumoniae*. Scand J Immunol 2000; 51: 345-53
91. Bonecchi R, Bianchi G, Bordignon PP, et al. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. J Exp Med 1998; 187: 129-34
92. Fresno M, Kopf M, Rivas L. Cytokines and infectious diseases. Immunol Today 1997; 18: 56
93. Igietseme JU, Portis JL, Perry LL. Inflammation and clearance of *Chlamydia trachomatis* in enteric and nonenteric mucosae. Infect Immun 2001; 69: 1832-40
94. Kelly KA, Rank RG. Identification of homing receptors that mediate the recruitment of CD4 T cells to the genital tract following intravaginal infection with *Chlamydia trachomatis*. Infect Immun 1997; 65: 5198-208
95. Perry LL, Feilzer K, Portis JL, et al. Distinct homing pathways direct T lymphocytes to the genital and intestinal mucosae in Chlamydia-infected mice. J Immunol 1998; 160: 2905-14
96. Igietseme JU, Uriri IM, Hawkins R, et al. Integrin-mediated epithelial-T cell interaction enhances nitric oxide production and increased intracellular inhibition of Chlamydia. J Leukoc Biol 1996; 59: 656-62
97. Igietseme JU, Rank RG. Susceptibility to reinfection after a primary chlamydial genital infection is associated with a decrease of antigen-specific T cells in the genital tract. Infect Immun 1991; 59: 1346-51
98. Brunham RC, Zhang DJ. Transgene as vaccine for Chlamydia. Am Heart J 1999; 138: S519-22
99. Brunham RC, Zhang DJ, Yang X, et al. The potential for vaccine development against chlamydial infection and disease. J Infect Dis 2000; 181: S538-43
100. Liu MA. Vaccine developments (vaccine supplement). Nat Med 1998; 4: 515-9

101. Zhang DJ, Yang X, Berry J, et al. DNA vaccination with the outer membrane protein gene induces acquired immunity to *Chlamydia trachomatis* (mouse pneumonitis) infection. J Infect Dis 1997; 176: 1035-40
102. Pal S, Barnhart KM, Wei Q, et al. Vaccination of mice with DNA plasmids coding for the *Chlamydia trachomatis* major outer membrane protein elicits an immune response but fails to protect against genital challenge. Vaccine 1999; 17: 459-65
103. Byrne GI, Krueger DA. Lymphokine-mediated inhibition of Chlamydia replication in mouse fibroblasts is neutralized by anti-gamma interferon immunoglobulin. Infect Immun 1983; 42: 1152-8
104. Holtmann H, Shemer-Avni Y, Wessel K, et al. Inhibition of growth of *Chlamydia trachomatis* by tumor necrosis factor is accompanied by increased prostaglandin synthesis. Infect Immun 1990; 58: 3168-72
105. Rank RG, Ramsey KH, Pack EA, et al. Effect of gamma interferon on resolution of murine chlamydial genital infection. Infect Immun 1992; 60: 4427-9
106. Shemer Y, Kol R, Sarov I. Tryptophan reversal of recombinant human gamma-interferon inhibition of *Chlamydia trachomatis* growth. Curr Microbiol 1987; 16: 9-13
107. Shemer Y, Sarov I. Inhibition of growth of *Chlamydia trachomatis* by human gamma interferon. Infect Immun 1985; 48: 592-6
108. Shemer-Avni Y, Wallach D, Sarov I. Inhibition of *Chlamydia trachomatis* growth by recombinant tumor necrosis factor. Infect Immun 1988; 56: 2503-6
109. Shemer-Avni Y, Wallach D, Sarov I. Reversion of the antichlamydial effect of tumor necrosis factor by tryptophan and antibodies to beta interferon. Infect Immun 1989; 57: 3484-90
110. Woods ML, Mayer J, Evans TG, et al. Antiparasitic effects of nitric oxide in an in vitro murine model of *Chlamydia trachomatis* infection and an *in vivo* model of *Leishmania* major infection. Immunol Ser 1994; 60: 179-95
111. Kordova N, Wilt JC. Phagocytic and chlamydiae inhibiting activities of stimulated and nonstimulated peritoneal mouse macrophages. Can J Microbiol 1976; 22: 1169-80
112. Zhong G, de la Maza LM. Activation of mouse peritoneal macrophages in vitro or in vivo by recombinant murine gamma interferon inhibits the growth of *Chlamydia trachomatis* serovar L1. Infect Immun 1988; 56: 3322-5
113. Byrne GI, Lehmann LK, Landry GJ. Induction of tryptophan catabolism is the mechanism for gamma interferon-mediated inhibition of intracellular *Chlamydia psittaci*replication in T24 cells. Infect Immun 1986; 53: 347-51
114. Gupta SL, Carlin JM, Pyati P, et al. Antiparasitic and antiproliferative effects of indoleamine 2,3-dioxygenase enzyme expression in human fibroblasts. Infect Immun 1994; 62: 2277-84
115. Murray HW, Szuro-Sudol A, Wellner D, et al. Role of tryptophan degradation in respiratory burst-independent antimicrobial activity of gamma interferon-stimulated human macrophages. Infect Immun 1989; 57: 845-9
116. Chen B, Stout R, Campbell WF. Nitric oxide production: a mechanism of *Chlamydia trachomatis* inhibition in interferon-gamma-treated RAW264.7 cells. FEMS Immunol Med Microbiol 1996; 14: 109-20
117. Igietseme JU. The molecular mechanism of T cell control of Chlamydia in mice: role of nitric oxide. Immunology 1996; 87: 1-8
118. Igietseme JU, Ananaba GA, Candal DH, et al. Immune control of chlamydial growth in the human epithelial cell line RT4 involves multiple mechanisms that include nitric oxide induction, tryptophan catabolism and iron deprivation. Microbiol Immunol 1998; 42: 617-25
119. Igietseme JU, Perry LL, Ananaba GA, et al. Chlamydial infection in inducible nitric oxide synthase knockout mice. Infect Immun 1998; 66: 1282-6
120. Igietseme JU, Uriri MI, Chow M, et al. Inhibition of intracellular multiplication of human strains of *Chlamydia trachomatis* by nitric oxide. Biochem Biophys Res Commun 1997; 232: 595-601
121. Byrd TF, Horwitz MA. Regulation of transferrin receptor expression and ferritin content in human mononuclear phagocytes. Coordinate upregulation by iron transferrin and downregulation by interferon gamma. J Clin Invest 1993; 91: 969-76
122. Freidank HM, Billing H, Wiedmann-Al-Ahmad M. Influence of iron restriction on *Chlamydia pneumoniae* and C. trachomatis. J Med Microbiol 2001; 50: 223-7
123. Raulston JW. Response of *Chlamydia trachomatis* serovar E to iron restriction in vitro and evidence for iron-regulated chlamydial proteins. Infect Immun 1997; 65: 4539-47
124. Peterson EM, Zhong G, Carlson E, et al. Protective role of magnesium in the neutralization by antibodies of *Chlamydia trachomatis* infectivity. Infect Immun 1988; 56: 885-91
125. Zhang YX, Stewart S, Joseph T, et al. Protective monoclonal antibodies recognize epitopes located on the major outer membrane protein of *Chlamydia trachomatis*. J Immunol 1987; 138: 575-81
126. Zhang Y-X, Stewart SJ, Caldwell HD. Protective monoclonal antibodies to *Chlamydia trachomatis* serovar- and serogroup-specific major outer membrane protein determinants. Infect Immun 1989; 57: 636-8
127. Moore T, Ananaba GA, Bolier J, et al. Fc receptor regulation of protective immunity against C. trachomatis. Immunology 2002; In press
128. Perry LL, Feilzer K, Hughes S, et al. Clearance of *Chlamydia trachomatis* from the murine genital mucosa does not require perforin-mediated cytolysis or Fas-mediated apoptosis. Infect Immun 1999; 67: 1379-85
129. Dhir SP, Agarwal LP, Detels R, et al. Field trial of two bivalent trachoma vaccines in children of Punjab Indian villages. Am J Ophthalmol 1967; 63: 1639-44
130. Grayston JT, Woolridge RL, Wang S, et al. Field studies of protection from infection by experimental trachoma virus vaccine in preschool-aged children on Taiwan. Proc Soc Exp Biol Med 1963; 112: 589-95
131. Woolridge RL, Grayston JT, Chang IH, et al. Long-term follow-up of the initial (1959-1960) trachoma vaccine field trial on Taiwan. Am J Ophthalmol 1967; 63: 1650-5
132. Lagrange PH, Hurtrel B, Stach JL. Vaccines against mycobacteria and other intracellular bacteria. Ann Inst Pasteur Immunol 1985; 136D: 151-62
133. Su H, Messer R, Whitmire W, et al. Subclinical chlamydial infection of the female mouse genital tract generates a potent protective immune response: implications for development of live attenuated chlamydial vaccine strains. Infect Immun 2000; 68: 192-6
134. O'Connell CMC, Maurelli AT, editors. Introduction of foreign DNA into Chlamydia and stable expression of chloramphenicol resistance. ICS Berkeley, San Francisco (CA), 1998
135. Tam JE, Davis CH, Wyrick PB. Expression of recombinant DNA introduced into *Chlamydia trachomatis* by electroporation. Can J Microbiol 1994; 40: 583-91
136. Wylie JL, Wang LL, Tipples G, et al. A single point mutation in CTP synthetase of *Chlamydia trachomatis* confers resistance to cyclopentenyl cytosine. J Biol Chem 1996; 271: 15393-400
137. Chalmers WS, Simpson J, Lee SJ, et al. Use of a live chlamydial vaccine to prevent ovine enzootic abortion. Vet Rec 1997; 141: 63-7
138. Rodolakis A, Bernard F. Vaccination with temperature-sensitive mutant of *Chlamydia psittaci*against enzootic abortion of ewes. Vet Rec 1984; 114: 193-4
139. Kalman S, Mitchell W, Marathe R, et al. Comparative genomes of *Chlamydia pneumoniae* and *C. trachomatis*. Nat Genet 1999; 21: 385-9
140. Read TD, Brunham RC, Shen C, et al. Genome sequence of *Chlamydia trachomatis* MoPn and *Chlamydia pneumoniae* AR39. Nucleic Acids Res 2000; 28: 1397-406
141. Stephens RS, Kalman S, Lammel C, et al. Genome sequence of an obligate intracellular pathogen of humans: *Chlamydia trachomatis*. Science 1998; 282: 754-9
142. Brunham RC, Peeling R, Maclean I, et al. Postabortal *Chlamydia trachomatis* salpingitis: correlating risk with antigen-specific serological responses and with neutralization. J Infect Dis 1987; 155: 749-55
143. Jones RB, Batteiger BE. Human immune response to *Chlamydia trachomatis* infections. In: Oriel J, Ridgway G, Schachter J, et al., editors. Chlamydial infections. London: Cambridge University Press, 1986: 423-432
144. Ward ME. Chlamydial vaccine - future trends. J Infect 1992; Suppl. 1: 11-26

145. Caldwell HD, Kromhout J, Schachter J. Purification and partial characterization of the major outer membrane protein of *Chlamydia trachomatis*. Infect Immun 1981; 31: 1161-76
146. Bavoil P, Ohlin A, Schachter J. Role of disulfide bonding in outer membrane structure and permeability in *Chlamydia trachomatis*. Infect Immun 1984; 44: 479-85
147. Wyllie S, Ashley RH, Longbottom D, et al. The major outer membrane protein of *Chlamydia psittaci*functions as a porin-like ion channel. Infect Immun 1998; 66: 5202-7
148. Wyllie S, Longbottom D, Herring AJ, et al. Single channel analysis of recombinant major outer membrane protein porins from *Chlamydia psittaci*and *Chlamydia pneumoniae*. FEBS Lett 1999; 445: 192-6
149. Su H, Watkins NG, Zhang Y-X, et al. *Chlamydia trachomatis*-host cell interactions: Role of the chlamydial major outer membrane protein as an adhesin. Infect Immun 1990; 58: 1017-25
150. Stephens RS, Mullenbach G, Sanchez-Pescador R, et al. Sequence analysis of the major outer membrane protein gene from *Chlamydia trachomatis* serovar L2. J Bacteriol 1986; 168: 1277-82
151. Stephens RS, Wagar EA, Edman U. Developmental regulation of tandem promoters for the major outer membrane protein gene of *Chlamydia trachomatis*. J Bacteriol 1988; 170: 744-50
152. Baehr W, Zhang Y-X, Joseph T, et al. Mapping antigenic domains expressed by *Chlamydia trachomatis* major outer membrane protein genes. Proc Natl Acad Sci USA 1988; 85: 4000-4
153. Fitch WM, Peterson EM, de la Maza LM. Phylogenetic analysis of the outer membrane protein gene of Chlamydiae, and its implication for vaccine development. Mol Biol Evol 1993; 10: 892-913
154. Kaltenboeck B, Kousoulas KG, Storz J. Structures of and allelic diversity and relationships among the major outer membrane protein (ompA) genes of the four chlamydial species. J Bacteriol 1993; 175: 487-502
155. Yuuki H, Yoshikai Y, Kishihara K, et al. Deletion of self-reactive T cells in nude mice grafted with neonatal allogeneic thymus. J Immunol 1990; 144: 474-9
156. Batteiger BE, Rank RG, Bavoil PM, et al. Partial protection against genital reinfection by immunization of guinea-pigs with isolated outer-membrane proteins of the chlamydial agent of guinea-pig inclusion conjunctivitis. J Gen Microbiol 1993; 139: 2965-72
157. Campos M, Pal S, O'Brian TP, et al. A chlamydial major outer membrane protein extract as a trachoma vaccine candidate. Invest Ophthalmol Vis Sci 1995; 36: 1477-91
158. Conlan JW, Ferris S, Clarke IN, et al. Isolation of recombinant fragments of the major outer-membrane protein of *Chlamydia trachomatis*: their potential as subunit vaccines. J Gen Microbiol 1990; 136: 2013-20
159. Hayes LJ, Conlan JW, Everson JS, et al. *Chlamydia trachomatis* major outer membrane protein epitopes expressed as fusions with LamB in an attenuated aroA strain of Salmonella typhimurium: their application as potential immunogens. J Gen Microbiol 1991; 137: 1557-64
160. Knight SC, Iqball S, Woods C, et al. A peptide of *Chlamydia trachomatis* shown to be a primary T-cell epitope in vitro induces cell-mediated immunity in vivo. Immunology 1995; 85: 8-15
161. Murdin AD, Su H, Manning DS, et al. A poliovirus hybrid expressing a neutralization epitope from the major outer membrane protein of *Chlamydia trachomatis* is highly immunogenic. Infect Immun 1993; 61: 4404-14
162. Su H, Parnell M, Caldwell H. Protective efficacy of a parenterally administered MOMP-derived synthetic oligopeptide vaccine in a murine model of *Chlamydia trachomatis* genital tract infection: serum neutralizing IgG antibodies do not protect against genital tract infection. Vaccine 1995; 13: 1023-32
163. Tan T-W, Herring AJ, Anderson IE, et al. Protection of sheep against *Chlamydia psittaci* infection with a subcellular vaccine containing the major outer membrane protein. Infect Immun 1990; 58: 3101-8
164. Taylor HR, Whittum-Hudson J, Schachter J, et al. Oral immunization with chlamydial major outer membrane protein (MOMP). Invest Ophthalmol Vis Sci 1988; 29: 1847-53
165. Tuffrey M, Alexander I, Conlan W, et al. Heterotypic protection of mice against chlamydial salpingitis and colonization of the lower genital tract with a human serovar F isolate of C. trachomatis by prior immunization with recombinant serovar L1 major outer membrane protein. Eur J Immunol 1992; 23: 1169-72
166. Zhong G, Smith GP, Berry J, et al. Conformational mimicry of a chlamydial neutralization epitope on filamentous phage. J Biol Chem 1994; 269: 24183-8
167. Jackson JW, Maisonneuve J, Taylor RB, et al. Immunization with a high molecular weight protein (pmpG) from *Chlamydia trachomatis* confers heterotypic protection against infertility [abstract]. Proceedings of the 101st General Meeting of the American Society for Microbiology (ASM); 2001 May 20-24; Orlando (FL)
168. Jen SS, Stromberg EJ, Probst P, et al. Discovery of new vaccine candidates for prevention and treatment of Chlamydia [abstract]. Proceedings of the 101st General Meeting of the American Society for Microbiology (ASM); 2001 May 20-24; Orlando (FL), 2001: 343
169. Murdin AD, Dunn P, Sodoyer R, et al. Use of a mouse lung challenge model to identify antigens protective against *Chlamydia pneumoniae* lung infection. J Infect Dis 2000; 181 Suppl. 3: S544-51
170. Bachmaier K, Neu N, de la Maza LM, et al. *Chlamydia* infections and heart disease linked through antigenic mimicry. Science 1999; 283: 1335-9
171. Prabhala RH, Wira CR. Sex hormone and IL-6 regulation of antigen presentation in the female reprodutive mucosal tissues. J Immunol 1995; 155: 5566-73
172. Macadam AJ, Pollard SR, Ferguson G, et al. Genetic basis of attenuation of the Sabin type 2 strain of poliovirus in primates. Virology 1993; 192: 18-26
173. Minor PD. Attenuation and reversion of the Sabin vaccine strains of poliovirus. Dev Biol Stand 1993; 78: 17-26
174. Minor PD. The molecular biology of poliovaccines. J Gen Virol 1992; 73: 3065-77
175. Pedersen NC, Johnson L, Birch D, et al. Possible immunoenhancement of persistent viremia by feline leukemia virus envelope glycoprotein vaccines in challenge-exposure situations where whole inactivated virus vaccines were protective. Vet Immunol Immunopathol 1986; 11: 123-48
176. Fulginiti VA, Eller JJ, Downie AW, et al. Altered reactivity to measles virus: atypical measles in children previously immunized with inactivated measles virus vaccines. JAMA 1967; 202: 1075-80
177. Gurunathan S, Klinman DM, Seder RA. DNA vaccines: immunology, application, and optimization. Annu Rev Immunol 2000; 18: 927-74
178. Gurunathan S, Wu CY, Freidag BL, et al. DNA vaccines: a key for inducing long-term cellular immunity. Curr Opin Immunol 2000; 12: 442-7
179. Seder RA, Gurunathan S. DNA vaccine - designer vaccines for the 21st century. N Engl J Med 1999; 341: 277-8
180. Ramshaw IA, Ramsay AJ. The prime-boost strategy: exciting prospects for improved vaccination. Immunol Today 2000; 21: 163-5
181. Fletcher MA. Vaccine candidate in STD. Int J STD AIDS 2001; 12: 419-22
182. Hess J, Schaible U, Raupach B, et al. Exploiting the immune system: toward new vaccines against intracellular bacteria. Adv Immunol 2000; 75: 1-88
183. Seder RA, Hill AV. Vaccines against intracellular infections requiring cellular immunity. Nature 2000; 406: 793-8
184. Chu RS, Targoni OS, Krieg AM, et al. CpG oligodeoxynucleotides acts as adjuvants that switch on T helper 1 (Th 1) immunity. J Exp Med 1997; 186: 1623-31
185. Klinman DM, Barnhart KM, Conover J. CpG motifs as immune adjuvants. Vaccine 1999; 17: 19-25
186. Iwasaki A, Stiernholm BJ, Chan AK, et al. Enhanced CTL responses mediated by plasmid DNA immunogens encoding costimulatory molecules and cytokines. J Immunol 1997; 158: 4591-601
187. Gurunathan S, Irvine KR, Wu CY, et al. CD40 ligand/trimer DNA enhances both humoral and cellular immune responses and induces protective immunity to infectious and tumor challenge. J Immunol 1998; 161: 4563-71
188. Murdin AD, Su H, Klein MH, et al. Poliovirus hybrids expressing neutralization epitopes from variable domains I and IV of the major outer membrane protein of *Chlamydia trachomatis* elicit broadly cross-reactive C. trachomatis-neutralizing antibodies. Infect Immun 1995; 63: 1116-21

189. Babiuk LA, Tikoo SK. Adenoviruses as vectors for delivering vaccines to mucosal surfaces. J Biotechnol 2000; 83: 105-13
190. Hewson R. RNA viruses: emerging vectors for vaccination and gene therapy. Mol Med Today 2000; 6: 28-35
191. Tartaglia J, Excler JL, El Habib R, et al. Canarypox-virus-based vaccines: prime-boost strategies to induce cell-mediated and humoral immunity against HIV. AIDS Res Hum Retroviruses 1998; 3: S291-8
192. Bennink JR, Yewdell JW. Recombinant vaccinia viruses as vectors for studying T lymphocyte specificity and function. Curr Top Microbiol Immunol 1990; 163: 153-84
193. Lawson CM, Bennink JR, Restifo NP, et al. Primary pulmonary cytotoxic T lymphocytes induced by immunization with a vaccinia virus recombinant expressing influenza A virus nucleoprotein peptide do not protect mice against challenge. J Virol 1994; 68: 3505-11
194. Davis NL, Caley IJ, Brown KW, et al. Vaccination of macaques against pathogenic simian immunodeficiency virus with Venezuelan encephalitis virus replicon particles. J Virol 2000; 74: 371-8
195. Schlesinger S, Dubensky TW. Alphavirus vectors for gene expression and vaccine. Curr Opin Biotechnol 1999; 10: 434-9
196. Schultz-Cherry S, Dybing JK, Davis NL, et al. Influenza virus (A/HK/156/97) hemagglutinin expressed by an alphavirus replicon system protects chickens against lethal infection with Hong Kong-origin H5N1 viruses. Virology 2000; 278: 55-9
197. Singh M, O'Hagan D. Advances in vaccine adjuvants. Nat Biotechnol 1999; 17: 1075-81
198. Bartholeyns J, Romet-Lemonne JL, Chokri M, et al. Cellular vaccines. Res Immunol 1998; 149: 647-9
199. Gilboa E, Nair SK, Lyerly HK. Immunotherapy of cancer with dendritic-cell-based vaccines. Cancer Immunol Immunother 1998; 46: 82-7
200. Hoffman DM, Gitlitz BJ, Belldegrun A, et al. Adoptive cellular therapy. Semin Oncol 2000; 27: 221-33
201. Gerritse K, Posno M, Schellekens MM, et al. Oral administration of TNP-Lactobacillus conjugates in mice: a model for evaluation of mucosal and systemic immune responses and memory formation elicited by transformed lactobacilli. Res Microbiol 1990; 141: 955-62
202. Turner MS, Giffard PM. Expression of Chlamydia psittaci- and human immunodeficiency virus-derived antigens on the cell surface of Lactobacillus fermentum BR11 as fusion to bspA. Infect Immun 1999; 67: 5486-9
203. Gentschev I, Dietrich G, Spreng S, et al. Delivery of protein antigens and DNA by virulence-attenuated strains of Salmonella typhimurium and Listeria monocytogenes. J Biotechnol 2000; 83: 19-26
204. Eko FO, Witte A, Huter V, et al. New strategies for combination vaccines based on the extended recombinant bacterial ghost system. Vaccine 1999; 17: 1643-9
205. Eko FO, Lubitz W, Igietseme JU. Immunogenicity of a novel recombinant subunit candidate vaccine against *Chlamydia trachomatis* [abstract]. Proceedings of the 101st General Meeting of the American Society for Microbiology (ASM); 2001 May 20-24; Orlando (FL); 2001: 341
206. Hajek R, Butch AW. Dendritic cell biology and the application of dendritic cells to immunotherapy of multiple myeloma. Med Oncol 2000; 17: 2-15
207. Rank RG. Models of immunity. In: Stephens RS, editor. Chlamydia: intracellular biology, pathogenesis and immunity. Washington (DC): ASM Press, 1999: 239-295
208. Whittum-Hudson JA, Ann LL, Saltzman WM, et al. Oral immunization with an anti-idiotypic antibody to the exoglycolipid antigen protects against experimental *Chlamydia trachomatis* infection. Nat Med 1996; 2: 1116-21
209. Colaco CA. Why are dendritic cells central to cancer immunotherapy? Mol Med Today 1999; 5: 14-7
210. Reid CDL. The biology and clinical applications of dendritic cells. Transfus Med 1998; 8: 77-86

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