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REVIEW

Recent advances in molecular medicine techniques for the diagnosis, prevention, and control of infectious diseases

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Abstract In recent years we have observed great advances in our ability to combat infectious diseases. Through the development of novel genetic methodologies, including a better understanding of pathogen biology, pathogenic mechanisms, advances in vaccine development, designing new therapeutic drugs, and optimization of diagnostic tools, significant infectious diseases are now better controlled. Here, we briefly describe recent reports in the literature concentrating on infectious disease control. The focus of this review is to describe the molecular methods widely used in the diagnosis, prevention, and control of infectious diseases with regard to the innovation of molecular techniques. Since the list of pathogenic microorganisms is extensive, we emphasize some of the major human infectious diseases (AIDS, tuberculosis, malaria, rotavirus, herpes virus, viral hepatitis, and dengue fever). As a consequence of these developments, infectious diseases will be more accurately and effectively treated; safe and effective vaccines are being developed and rapid detection of infectious agents now permits countermeasures to avoid potential outbreaks and epidemics. But, despite considerable progress, infectious diseases remain a strong challenge to human survival.

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Introduction

Despite the great advances in medicine, particularly in new therapeutic drugs, diagnostic tools, and even ways to prevent diseases, the human species still faces serious health problems. Among these problems, those that draw the most attention are infectious diseases, especially in poor regions. An important feature of infectious disease is its potential to arise globally, as exemplified by known devastating past and present pandemics such as the bubonic–pneumonic plague, Spanish flu (1918 influenza pandemic), and the present pandemic of human immunodeficiency virus (HIV), in which an estimated 33.3 million persons were living with the HIV infection worldwide at the end of 2009 [1–3]. In addition, other non-viral diseases are significant public health problems, as exemplified by tuberculosis (TB). This infectious disease accounts for one third of the world's bacterial infections (TB infected), and in 2010 a total of 8.8 million people worldwide became sick with TB [1, 4].

In recent years, new forms of infectious diseases have become significantly important to medical and scientific communities; these forms are now widely known as emergent and re-emergent infectious diseases. With the appearance of new transmissible diseases, such as SARS, West Nile and H5N1/H1N1 Influenza viruses, in addition to reemerging diseases like dengue fever, the concerns about a global epidemic are not unfounded [5]. Moreover, in the tropical and subtropical regions of the world, parasitic infections are a common cause of death. Since one of the major characteristics of infectious diseases is its inter-individual transmission, advances in personal protection, effective public policy, and immunological procedures are efficient means of controlling the spread of these diseases. Thus, improvement of pre-existing technologies commonly used to monitor, prevent, and treat infectious diseases is of crucial importance not only to the medical community, but also to humankind.

Here, we describe recent strategies used to fight infectious diseases, with a special focus on recent advances in the fields of molecular biology, examining the development of different methods for their control. We emphasize that regardless of the great advances in science, in new drugs, and in methods used to diagnose infectious diseases, the number of available vaccines is still limited.

Prevention

Dengue fever is a significant disease that has spread around the tropical and subtropical regions of the globe. The virus is transmitted by infected mosquitoes, mainly *Aedes aegypti*, and it is estimated that annually the number of infections exceeds more than 50 million cases in more than 100 countries [6]. Initial attempts at vector control were based on the eradication of mosquitoes, by programs with extensive use of insecticides; thus, as a consequence many *Aedes aegypti* field populations are presenting increased resistance to such compounds (pyrethroids and organophosphates). A recent intervention, that aims to reduce the survival rate or life span of adult mosquitoes, limiting the potential of dengue infection by affecting the period of vector pathogen transmission, is now being tested in areas where dengue is endemic. This methodology employs a genetically modified strain of *Wolbachia pipientis* (an obligate intracellular bacterium). This bacterium was adapted to infect *Aedes aegypti* mosquitoes by continuous serial passage in mosquito cell culture for approximately 3 years. More importantly, the mosquitoes remained infected for a period of 30 generations, allowing its use in field populations [7]. Initial field tests showed that *Wolbachia* (strain wMelPop-CLA) successfully invaded two natural *A. aegypti* populations in Australia with almost 100 % fixation rate 5 months after release [8]. Thus, with low implementation costs, *Wolbachia*-based strategies can represent a practical approach to dengue fever suppression in endemic areas.

In women, HIV infection is a prevalent risk, mainly in sub-Saharan Africa. In this region women are unable or reluctant to negotiate condom use. An alternative strategy is the use of microbicide vaginal gels. Over the last few years, several formulations of microbicide gels were tested with variable efficacy, leading to disappointing results. As exemplified by a recent phase III clinical trial of PRO2000, a synthetic naphthalene sulfonate polymer of around 5 kDa in molecular weight with antiviral activity against HIV-1. In this trial a randomized, double-blind, parallel group showed that 0.5 % PRO2000 and 2 % PRO2000 formulations are not efficacious against vaginal HIV-1 transmission [9]. However, positive results were also reported; in 2010, a clinical trial demonstrated the effectiveness of tenofovir 1 % (a nucleotide reverse transcriptase inhibitor) intravaginal gel for the prevention of HIV acquisition in women. In a high gel adherence group, HIV incidence was statistically lower (54 % decrease), with 38 % and 28 % HIV reduction in intermediate gel adherence and low gel adherence groups respectively [10]. Also, other formulations are in the development phase. The SPL7013 Gel (VivaGel) is a microbicide that inhibits HIV, herpes simplex virus-2, and human papillomavirus in vitro and in animal models. A phase 1 clinical trial demonstrated that VivaGel is well tolerated with no serious adverse events when used by sexually active women aged 18–24 years [11]. Besides technological advances, simple strategies are also effective in HIV prevention. Education programs providing information for individual protection against infection, such as information about routes of transmission, condom use, lubrication, blood risks, and safe and risky sexual practices, are of fundamental importance [12].

Diagnosis tools

It is important to note that some infectious diseases are treatable. However, to achieve successful therapy rapid detection of the causative agent is a determinant factor. Since the discovery of microbes as causative agents of several transmissible diseases in the late 1800s, the advent of recent genetic methodologies has transformed the field of medical microbiology. Developed in 1983 by Kary Mullis, the polymerase chain reaction (PCR) is now an indispensable technique widely applied in medical and biological research [13]. Initially, PCR was a qualitative test that could be used to detect the presence of a specific gene segment, interpreting that if the gene sequence was present the pathogen would be present also. Nowadays, PCR has diversified into various subtechniques with important applications. In fact, there are several commercial kits available for the diagnosis of important pathogens. The principal advantage of PCR is that nucleic acid amplification allows the detection of lower levels of pathogen genetic material, emphasizing the high sensitivity of this technique [14]. Post-amplification analysis, such the traditional Sanger sequencing, allows the identification of microorganism species or genetic variants that could be related to drug resistance [14]. Genetic variant studies are now identifying new types and subtypes of important pathogens; these variations could represent a major determinant of microorganism pathogenesis [15].

Multiplex PCR assays are a useful approach that enables simultaneous amplification of several target genes in the same reaction. This technique has already been applied with success to the identification of influenza subtypes, the subtyping of dengue viruses, and the genotyping of rotaviruses, among others [16–19]. Multiplex PCR often requires posterior analysis methods; usually, post-amplification analysis includes methods such as sequencing, pyrosequencing,

reverse hybridization, and Luminex analysis [14]. Pyrosequencing is a high throughput DNA sequencing method based on the detection of pyrophosphate release after nucleotide incorporation [20]. This technology has been used for the study of highly variable pathogens, allowing rapid characterization of evolving quasispecies [21–24]. Moreover, pyrosequencing is a useful approach to identifying drug-resistant strains [25]. Widely applicable, real-time PCR is an important methodology for the molecular diagnosis of viral, bacterial, and even parasitic infections. The main advantage of this technique is that real-time PCR allows the quantification of the target sequence, which is directly related to the quantity of infecting microorganisms. Also, real-time PCR can be multiplexed for the detection of up to 3–5 targets simultaneously. Real-time PCR is being applied in a large spectrum of infectious diseases, such as dengue fever, HIV, malaria, and several other major infectious diseases [26–28]. PCR-related techniques revolutionized the field of disease diagnosis, and at the moment the sequencing of the genomes of pathogens is permitting the identification of individual genetic variants. This rapid and reliable methodology is simplifying the identification of the genetic basis of drug resistance in several pathogens.

For latent tuberculosis infection, the gold standard method is the tuberculin skin test; as an alternative, interferon gamma (IFN-γ) release assays have been introduced. Currently, two tests have been approved by the US Food and Drug Administration (FDA). These are: QuantiFERON®-TB Gold In-Tube test (QFT-GIT), which detects IFN-γ concentration released by patient T cells after incubation with a mixture of synthetic stimulatory peptides, and T-SPOT®. TB test (T-Spot), which works on the same principle, but detects the number of IFN-γ-producing cells instead of the concentration [29].

Successful diagnostic tools often require high sensitivity, reliability, and short periods to process samples. In accordance with these requirements, biosensors are becoming a valuable, large, applicable technology. Utilizing a classical antigen-antibody reaction, Mujika et al. [30] designed a magneto-resistive immunosensor, integrated into a microfluidic network, for the detection of *Escherichia coli* O157:H7 in food and clinical samples. This device detects magnetic field oscillations caused by monitoring the presence of super paramagnetic beads bound to *E. coli*-immobilized antigens. A significant advantage of this method is that the beads are strongly stable, permitting repetition of the assays several times [30]. An electrochemical biosensor, which employs DNA probes for the 16S rRNA of the most common uropathogens, was tested in a prospective clinical study in patients with neurogenic bladder. The authors report that the biosensor assay specificity and positive predictive values were of 100 % with a pathogen detection sensitivity of approximately 89 %, yielding a 76 % negative predictive value. A positive aspect of this device is that it allows the samples to be tested within 1–2 h of collection; however, the test failed to detect pathogens at a concentration of 4–3 10^4 CFU/mL or less [31].

Although the important advances in molecular diagnosis of infectious diseases are incontestable, serological techniques are still the principal application in infectious diseases. In this way, monoclonal antibody techniques gained an important role in the fields of microbiology and immunology. Monoclonal antibodies are now widely applied in serological techniques, such as enzyme-linked immunosorbent assay (ELISA), producing more sensitivity than traditional techniques in detecting and measuring antibodies to pathogens [1].

Vaccines

Additionally, after the implementation of control measures like sanitation, epidemiological vigilance, and therapy, vaccines are probably responsible for some of the world's greatest public health achievements. Over the last few years there has been an explosion of potential new strategies for vaccine development. Since the development of new techniques, especially those based on genetic engineering, the field of vaccinology has been exploring safer vaccines by the creation of recombinant immunogenic proteins, naked DNA vaccines, and viral recombinants/mutants [32–34]. Several techniques for safe immunization have presented positive results; an interesting example is the vaccine against human papillomaviruses, which uses a system to generate virus-like particles by inserting the genes encoding the L1 proteins of oncogenic serotypes into baculovirus, producing highly immunogenic proteins via infected insect cells. This vaccine is now licensed for use in humans [35].

An important obstacle that comes with recombinant protein immunization is that subunit vaccines are weak immunogens. To overcome this obstacle, viral vector vaccines have been used against several pathogens. Viral vector vaccines are potentially safe, able to induce strong immune responses (by simulating a natural infection), and easy to handle. Recombinant adenoviruses have emerged as promising viral vectors, with the potential to deliver genes of interest, inducing a protective immune response [36]. In fact, substantial protection in a pre-clinical study was provided against the pre-erythrocytic stages of malaria by vaccination first with an adenoviral and then with a modified vaccinia virus Ankara (MVA) poxviral vector encoding the same ME-TRAP transgene, heterologous prime and boost vaccination [37]. This alternative, the use of viral vectors to augment the immunogenic potential recombinant proteins, has been employed successfully in numerous infectious animal models [38–41]. Most importantly, viral vector

vaccines are now being tested in phase III clinical trials, as illustrated by the RTS,S/AS malaria vaccine. RTS,S/AS is a genetically engineered viral vector vaccine based on the hepatitis B surface antigen virus-like particle (VLP) expressing the circumsporozoite (CS) antigen (carboxy terminus amino acids 207–395) of the *P. falciparum* [42, 43]. In a phase 2a trial the RTS,S/AS malaria vaccine induced protection against challenge with infectious sporozoites in 40 % of the 80 subjects enrolled in the study. Protective immunity was associated with $CD4^+$ T cells frequency significantly higher in protected versus non-protected subjects [44].

Another phase III clinical trial vaccine is showing promising results. Among a cohort of healthy adults aged 16–65 years in China, the vaccine efficacy after three doses was 100.0 % with no vaccination-related serious adverse events [45]. This recombinant hepatitis E virus (HEV) vaccine, denominated HEV 239, has a 26-amino acid extension from the N terminal of another peptide, E2, of the HEV capsid protein expressed in bacteria. This vaccine was previously demonstrated to protect monkeys against HEV infection by inducing high levels of IgG anti-HEV serum antibody [46]. Moreover, a dengue tetravalent, live, attenuated vaccine (CYD-1-4), based on the backbone of the yellow fever vaccine 17D (YFV 17D), is presenting good safety and immunogenicity profiles. After a complete round of three immunizations, this formulation induces a strong immune response against all four dengue serotypes in a high proportion of the 6,000 vaccinated children [47]. A recent randomized, double-blind efficacy field trial with 8,323 women demonstrated that a herpes simplex virus type 2 (HSV-2) subunit vaccine, containing glycoprotein D, was associated with an increased risk of local reactions and more importantly, the vaccine efficacy against genital herpes disease was only 20 % [48].

Of the viral infections, HIV is actually the main cause of death in the world, accounting for 1.8 million deaths in 2011. Several groups have focused on developing an effective HIV vaccine, but without success so far. At present, four distinct vaccine regimens have been tested in phase III or IIb efficacy trials in human volunteers; two of these involve the Vaxgen's gp120 alone, which is a bivalent vaccine consisting of a preparation of recombinant gp120 from two types of HIV. The others consist of Merck's rAd5, a replication-defective recombinant adenovirus serotype 5, and ALVAC + gp120 (RV144), a recombinant canarypox vector carrying three synthetic HIV genes [49–51]. However, only ALVAC showed a modest degree of efficacy in preventing HIV-1 infection (31 % protective efficacy against HIV-1 acquisition) [52]. Additional important vaccines are available for the human population (Table 1). Although the list of diseases preventable by immunization is still limited, in recent years new vaccine candidates have been tested and it is expected that in the future they will become licensed.

Table 1 Human preventable infectious diseases for which human vaccines are available, according to the Centers for Disease Control and Prevention (CDC)

Infectious disease	Etiological agent
Bacterial disease	
Measles	Measles virus
Pneumococcal	*Streptococcus pneumoniae*
Typhoid fever	*Salmonella enterica* typhi
Pertussis (whooping cough)	*Bordetella pertussis*
Meningococcal	*Neisseria meningitidis*
Anthrax	*Bacillus anthracis*
Diphtheria	*Corynebacterium diphtheriae*
Haemophilus influenzae type b (Hib)—meningitis	*Haemophilus influenzae*
Tuberculosis	*Mycobacterium tuberculosis*
Tetanus	*Clostridium tetani*
Lyme disease[a]	*Borrelia burgdorferi*
Cervical cancer	Human papillomavirus (HPV)
Viral disease	
Rotavirus	Rotavirus
Swine flu	Influenza virus (H1N1)
Rabies	Rabies virus
Mumps	Mumps virus
Yellow fever	Yellow fever virus
Herpes zoster (shingles)	Varicella zoster virus (VZV)
Poliomyelitis (polio)	Poliovirus
Monkeypox	Monkeypox virus
Hepatitis A	Hepatitis A virus (HAV)
Influenza (seasonal flu)	Influenza virus
Human papillomavirus (HPV)	Human papillomavirus
Japanese encephalitis	Japanese encephalitis (JE) virus
Hepatitis B	Hepatitis B virus (HBV)
Rubella (German measles)	Rubella virus
Smallpox[b]	Variola virus
Varicella (chickenpox)	Varicella zoster virus (VZV)

[a] Discontinued by the manufacturer in 2002

[b] Smallpox is the only human infectious disease considered to be eradicated, in 1979 the WHO recommended that vaccination against smallpox could be stopped in all countries after a worldwide vaccination program

Conclusion

A variety of molecular-based strategies are now improving our knowledge of microorganism biology, and of preventive and therapeutic tools, providing humankind with better control of infectious agents. In addition, the advancements in the fields of immunology, bioinformatics, and nanotechnology are expected to have an impact on our interpretation of interaction mechanisms between host and pathogenic microorganisms. These advancements may lead to the discovery of new pathogens, new potential therapeutic targets, and

more effective diagnosis, contributing to the eradication of human infectious diseases. However, despite these great advances infectious agents still cause millions of deaths annually.

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Conflict of interest The authors declare that they have no conflict of interest.

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