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Development of Therapeutic Interventions for Emerging Diseases

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1. Introduction

New infectious diseases emerge with a high regularity; it has recently been estimated that a novel infectious disease agent either emerges or re-emerges approximately every 8 months. This latter statistic is supported by the observation that there have been over 335 emerging infectious disease (EID) events between 1940 and 2004 (Jones *et al.*, 2008). Of course, not all of these EID events represent a threat to human health, indeed many of these are infections of animals, although approximately 60% are also zoonotic infections (which by definition can be transmitted between animals and humans); another additional proportion also have the potential to cross the species barrier. Since 1970 there have been approximately 30 new species of pathogen emerge which cause human infection. Table 1 lists these pathogens (taken from World Health Organisation, 1999).

Year	Pathogen	Year	Pathogen
1972	Small Round Structured Viruses	**1989**	Hepatitis C virus
1973	Rotavirus	**1990**	Human herpesvirus-7
1975	Astrovirus	**1990**	Hepatitis E virus
1975	Parvovirus B-19	**1991**	Hepatitis F virus
1976	*Crytosporidium parvum*	**1992**	*Vibrio cholerae* 0139:H7
1977	Ebola virus	**1992**	*Bartonella henselae*
1977	*Legionella pneumophila*	**1993**	Sin Nombre virus
1977	Hantaan virus	**1993**	Hepatitis G virus
1977	*Cambylobacter jejuni*	**1994**	Sabia virus
1980	HTLV-1	**1994**	Human herpesvirus-8
1981	Toxigenic *Staphylococcus aureus*	**1995**	Hendra virus
1982	HTLV-II	**1996**	Prion (BSE/vCJD)
1982	*Borrelia burgdorferii*	**1997**	Influenza A virus (H5N1)
1983	*E.coli* 0157:H7	**1997**	Transfusion-transmitted virus
1983	HIV	**1997**	Enterovirus 71
1983	*Helicobacter pylori*	**1998**	Nipah virus
1988	Human herpesvirus-6	**1999**	Influenza A virus (Hong Kong 'flu)
1989	*Ehrlichia* spp.	**1999**	West Nile virus

Table 1. Emerging Infectious Disease Pathogens, 1972-1999.

An emerging infectious disease may be defined as one that has appeared in a population for the first time, or that may have existed previously but is rapidly increasing in incidence or geographic range (World Health Organisation [WHO]). This definition is quite generic and many consider EID's as those which are either genuinely novel infectious disease pathogens (examples include the SARS coronavirus which emerged in 2003) or those where there has been a paradigm shift in their genotype or phenotype such that it poses a new threat to health (examples include the appearance of multi-drug resistant *Mycobacterium tuberculosis* and other bacterial pathogens). These latter pathogens are frequently referred to as re-emerging infectious diseases, to discriminate them from completely novel disease agents. Because of the apparent rise in the incidence of EIDs during the 1980's and 90's (HIV/AIDS, vCJD *etc.*), factors involved in the process of emergence were analysed by a number of workers. One such study (Taylor *et al.*, 2001), concluded that although over half of EIDs were zoonotic in origin, the route of transmission had no effect on the likelihood of emergence, rather it was the taxonomy of the organism that was the root cause. They concluded that viruses and bacteria were of much higher likelihood of emergence, whereas parasites such as Helminths, were very unlikely to ever emerge.

Despite the advances in medical science, infectious diseases still constitute a threat to human survival, health and well-being and have done since human life began. Immediately following the discovery of penicillin, there was a mood of optimism that felt that the conquest of infectious diseases was a war that had been won; rather it seems it was merely the first skirmish in a very long-lasting battle. In the first decade of the 21st century, we know that infectious diseases represent a major global threat, accounting for some 41% of the global disease burden and in the UK alone, infectious diseases now account for approximately 70,000 deaths per annum and 40% of the population in the country consult a medical practitioner each year because of infection (Donaldson, 2001). It is clear, therefore, that infectious diseases remain a major global threat and that the burden of disease with an infectious aetiology is very high. There are a large number of interventions that can be applied to the control of infectious disease and for health protection. Interventions include simple public health control measures (hand-washing, quarantine, supply of clean water *etc.*), diagnostic tests, therapeutic treatments and vaccines. These different aspects will be discussed in greater depth in the following sections.

2. Vaccination

Vaccination is the process by which the adaptive immune system is stimulated to produce a deliberate response. Typically, vaccines comprise an antigenic component (or components) which are administered by a variety of routes and mimic the infection against which protection is sought. Modern vaccination was probably first described by Edward Jenner (there are reports that a similar approach had been used some years earlier) in 1796; indeed it was Jenner who coined the phrase vaccination. The term is derived from the Latin word *vacca* meaning "cow", so derived since the first "vaccination" used material from cowpox viral lesions on a milk-maids hands as protection against Smallpox infection (reviewed in Lombard *et al.*, 2007). Strictly, vaccination may be considered to be the process of introducing a foreign antigen into the body for the purpose of protection against infectious disease, whereas, immunisation is the process by which a vaccine induces an immune response against a foreign antigen – a subtle difference in meaning, although in practice the

two terms are often used interchangeably. From these early beginnings, there are 26 currently licensed vaccines widely available and administered as components of vaccination programmes. These 26 vaccines are shown in Table 2 below.

Vaccine	Vaccine
Anthrax	Pertussis
Cervical cancer (papilloma virus)	Pneumococcal infection
Chicken pox virus	Poliomyelitis
Cholera	Rabies
Diphtheria	Rotavirus
Group A & C Meningococcal infections	Rubella
Hib infection	Shingles
Hepatitis A	Smallpox
Hepatitis B	Tetanus
Japanese encephalitis	Tuberculosis
Influenza	Typhoid Fever
Measles	Varicella
Mumps	Yellow Fever

Table 2. Licensed Vaccines Currently Available for Use.

The table above illustrates that there are available vaccines for many of the "common" infectious diseases, yet despite this availability, there still exists a considerable disease burden for many of the diseases shown in Table 2. One might reasonably question why this is so, and the answer is multi-fold. Firstly the aspect of vaccine efficacy needs to be considered; the availability of a licensed vaccine does not, of course, indicate that use will result in 100% protection of the recipient. Due to the very different immune profiles observed within the human population, there will be a response curve which at one end results in next to no (or at least very low) levels of protection, whereas at the other end of the scale, recipients will show good protection. Taken on a population level, this means that there will always be a proportion of the population which are unprotected and thus the disease is still able to circulate. The above reasoning also assumes that take-up (and indeed availability) of any vaccine is 100% within a population; it is not due both to the cost involved in vaccinating entire populations and the choice which some make not to receive vaccination when offered. This latter point is very well illustrated by the recent issues surrounding the MMR (Measles, Mumps & Rubella) tri-valent vaccine within the UK. Adverse scientific publications (for an update on the current scientific evidence and lack of any link between these events see DeStefano & Williams, 2004), indicating a link between receipt of the vaccine and autism in children has meant that many parents decided not to allow their children to receive the vaccine and the UK now has a considerable measles outbreak due to much higher levels of susceptibility within the population (see ECDC measles Surveillance Report, 2011). It is of note, though, that the risk of real adverse events with the MMR vaccine are very low; the risk of brain damage due to receipt of the vaccine is calculated to be approximately 1/100,000 (this is due to the measles component of the vaccine), whereas the risk of brain damage if a child catches measles is approximately 1/1000, a figure which is considerably higher.

2.1 Types of vaccine

There are basically three ways of making vaccines against infectious diseases, all rely on a level of knowledge about the pathogenesis of the disease and ideally about the virulence factors which the pathogen employs. That vaccination is an effective method to protect the health of a population is well known and documented; the figure below illustrates the reduction in numbers of ill and in numbers of deaths for a fictitious respiratory disease, spreading with a population of approximately 60 million people (Fig. 1). A large number of assumptions have been made in running this very simple Susceptible, Infected, Recovered (SIR) model, one of which was that a new vaccine had to be developed and that the time taken to both produce a crude, whole-cell vaccine, plus manufacture enough to be used widely, resulted in an approximately 50% reduction in the overall numbers of fatalities (C. Norris & N J Silman, Unpublished). Of course, modelling the same disease, but making the assumption that a vaccine already exists can reduce the impact of the disease even more than that shown in Fig.1.

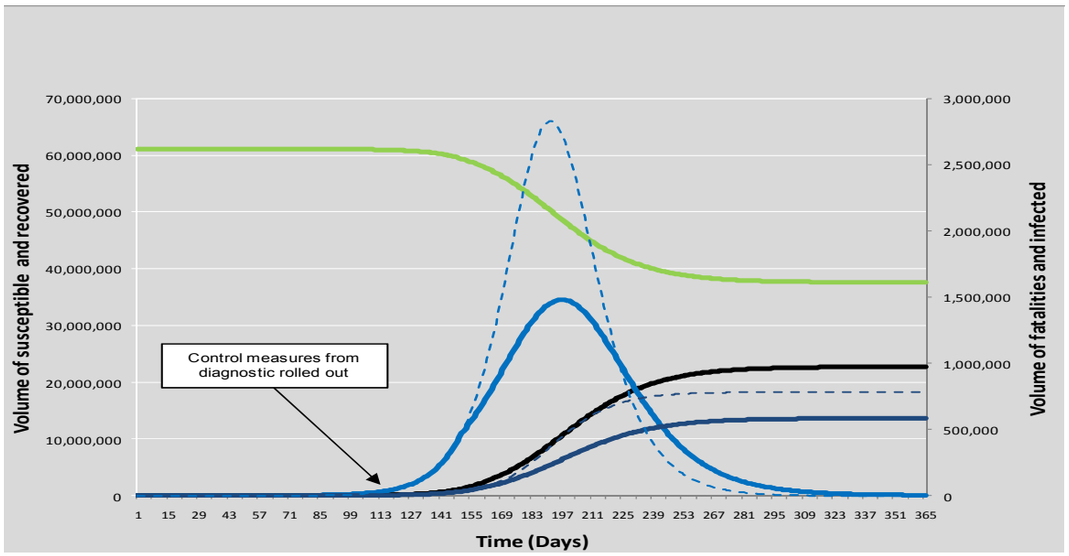

Fig. 1. SIR Model of the Impact of Vaccine Introduction on the Course of Infectious Disease. Key: ____ Susceptible population; _ _ _ Infected population; ___ Infected population after vaccine introduced; _ _ _ Fatalities if vaccine not used; _____ Fatalities when vaccine used; ___ Numbers Recovered

This figure is shown merely to illustrate the point that development of a new vaccine in the event of an emerging disease can have a very profound effect on the outcome of the disease (this point was well made during the recent H1N1 influenza pandemic). The illustration also includes an element of diagnostic roll-out, where we made the assumption that in the early stages of a newly emerged pathogen, simple public health control measures would be invoked to control person to person transmission and that these measures would be supported by a diagnostic test.

The three different approaches to vaccine development will be discussed in greater detail in the sections which follow, and their potential for use in producing vaccines against rapidly emerging pathogens will be discussed further.

2.1.1 Whole-cell vaccines

The simplest vaccines comprise growing up the pathogen and inactivating a crude, unfractionated preparation. This is an approach which has been used for a considerable length of time and vaccines made using this relatively simple rationale are still widely used. Examples taken from table 2 include Anthrax, Japanese encephalitis, influenza, yellow fever and typhoid fever; the reader will note that both bacterial and viral vaccines are made using the same approach and both types can show good efficacy in use. There are, of course, a number of examples where such an approach is not successful; examples here include vaccines that were produced against Cholera, Plague, meningitis and Smallpox. These vaccines failed because of a paucity of understanding of the mechanisms of virulence (*e.g.* the major virulence factor for Cholera is a toxin and thus a crude whole-cell preparation would not contain this secreted component). However, it is still considered that this method of rapidly producing a vaccine could be used in the event of a newly emerging infectious disease that had a high mortality rate and was highly transmissible within the population. Although there are examples of vaccines for both bacteria and viruses produced by this method, it is generally considered that the approach is probably more suited to production of viral vaccines due to their lower complexity. Probably the most commonly used vaccine of this type which is still produced and updated year on year is the split virion influenza vaccine that is produced seasonally. This vaccine comprises an unpurified preparation of virus typically grown in egg-culture (although there are notable examples of cell-culture grown influenza virus vaccines which are licensed for use or in clinical trial) and inactivated. The rationale is that the major antigenic components of the influenza virus are the haemagglutinin and neuraminidase, both of which are surface proteins against which considerable immune responses are mounted. Interestingly, despite the longevity of use of this vaccine and the considerable number of clinical trials and research work that has been conducted, the correlates of protection for influenza are still unknown (Montomoli *et al.*, 2011).

Looking at the list of recently emerged pathogens shown in Table 1, it is interesting to note that this approach has been used to develop vaccine candidates for a number of the pathogens. The most notable example is HIV/AIDS, for which there is still no effective vaccine and that an inactivated viral preparation did not exhibit high protective efficacy. Thus, for this method of vaccine development to be of value for an emergent pathogen a number of criteria need to be fulfilled, some of which will not be obvious when the disease first emerges. Firstly, the disease needs to have a high mortality rate, such that use of a vaccine is absolutely required. Secondly, it must be highly transmissible, since an infection with a low rate of transmission (R_0) can be controlled by other public health measures, a good example of such a pathogen was the SARS coronavirus, which was transmissible but had a relatively low R_0 and thus was adequately controlled by quarantine. Thirdly, natural infection should induce a protective immunity, otherwise an inactivated preparation is unlikely to induce immunity if the natural infection is incapable of so doing. This last consideration is very unlikely to be understood at the point when the pathogen emerges and will only be fully understood after a considerable length of time, most probably once an epidemic or pandemic situation has passed. The speed that a vaccine must be deployed is illustrated in Fig.2, which shows the effect on the numbers of infectious people within a population as the reproduction rate of the infectious disease pathogen is varied.

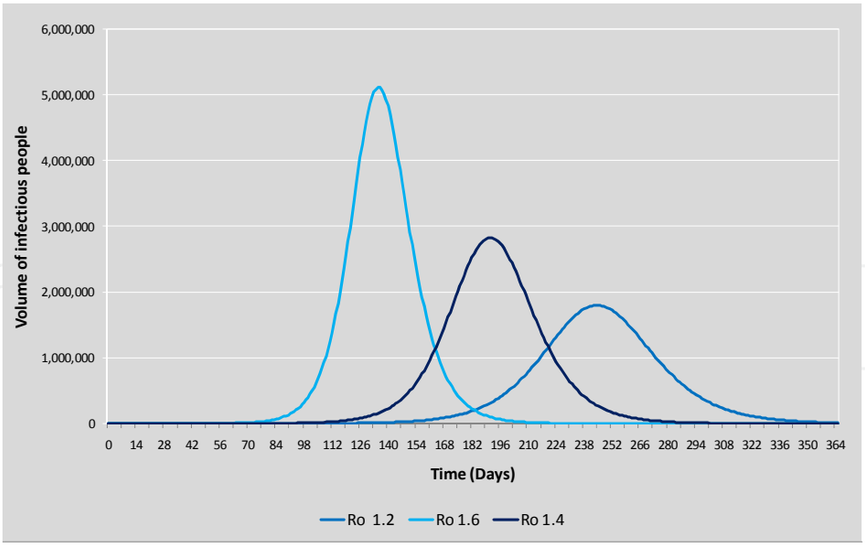

Fig. 2. Effect of Varying R_0 on the Number of Infectious People within a Population.

This figure illustrates a modest range of R_0 values between 1.2 and 1.6, the point to note is that even with small variations in the R_0 value, large differences in the numbers of infected people are observed and the time that maximum numbers of people are infected varies between approximately 130 and 240 days. This should be compared with the reproduction rate of typical influenza viruses which may be up to $R_0 = 3$ (that is, 3 uninfected people are infected by each infectious member of the population). The lower values used in this illustration were seen with the SARS coronavirus and perhaps illustrate why a vaccine was not developed against this pathogen during the epidemic phase.

2.1.2 Attenuated vaccines

Another approach to the rational design of vaccines for existing or emerging pathogens is the attenuation of virulent strains. Here, a strain is used which is not able to produce fulminant infection as a result of its' attenuation. In contrast to the inactivated vaccines described in section 2.1.1, these vaccines comprise live micro-organisms. Historically, attenuated strains were first obtained by serendipity often following prolonged sub-culture or passage (for example see Barrett *et al.*, 1990). Good examples of this occurrence are the Yellow fever 17D strain which was naturally attenuated by repeated passage and has been used as a vaccine against this disease since the 1950's. Interestingly, the mechanism of attenuation is still not known, although the vaccine has a long history of safe use, except in those members of the population with an egg allergy (the virus is propagated in egg culture). Another well-known example of an attenuated vaccine strain is the Polio virus vaccine, of which there are two vaccines in use. The first, the Salk vaccine is an inactivated viral preparation whilst the second, called the Sabin strain is an attenuated Polio virus (see Pearce, 2004). The Sabin vaccine was trialled between 1957 and 1962, when it was licensed for widespread use. The vaccine is taken orally and was attenuated by repeated passage in the brains of mice. By the seventh passage the virus was found to be no longer capable of infecting mice via the neurological route. A further 2 to 3 passages through rats confirmed the attenuation and the strain was considered safe for human inoculation. The development

of polio vaccines in the 1950's was a response to the large burden of disease caused by this virus and delivery of the Salk and Sabin vaccines constitutes the first mass-immunisation programmes. At the current date, Polio is still yet to be eradicated, despite efforts which began in 1988 between the WHO, UNICEF and the Rotary Foundation, however the hope is that this virus will soon be committed to history.

As the above example illustrates, the process of attenuation is not one which can be undertaken rapidly. There is no rational way of determining whether a strain may be attenuated by simple repeated passage or not and therefore the utility of such an approach is of limited value in responding to emergence of new infectious diseases. There are, of course, alternative ways of attenuating pathogenic strains of microorganism. For example, in the time between isolation of the Sabin strain and the present day, the mutations which are responsible for this strains lack of neuro-infectivity have been mapped to the internal ribosome entry site (IRES) of the virus in the currently used strain, a derivative of the original Sabin isolate. Extensive characterisation of the series of viruses that have been used as live-attenuated Polio vaccines has indicated that there are fifty-seven nucleotide substitutions which distinguish the attenuated Sabin 1 strain from its virulent parent (the Mahoney serotype), with a two further nucleotide substitutions between the Sabin 2 and parent strain, and ten more substitutions are involved in further attenuating the Sabin 3 strain (Kew *et al.*, 2005).

An alternative to repeated passage is the rational deletion of one or more genes from the genome of the organism. This approach requires in-depth knowledge of the virulence or pathogenicity factors which the infectious micro-organism uses to achieve infection in the human host. There are a number of examples where this approach has been successfully used. In view of the subject within this chapter to look at prospects for emerging infectious diseases, the live attenuated influenza vaccine (LAIV) will be used as a discussion example to illustrate this approach. There is currently only one licensed influenza vaccine based upon this LAIV technology, although, like other influenza vaccines, the virus backbone is used to construct vaccines against currently circulating strains by recombination with the genomic segments encoding the haemagglutinin and neuraminidase genes from the currently circulating strains. The LAIV is licensed in the USA by FDA and sold under the trade name of FluMist™. The backbone strain used for this vaccine is a cold-adapted strain which is attenuated since it is not able to complete the cycle of replication at normal human body temperatures. The relatively small genome of the influenza virus has been completely sequenced from this strain and many nucleotide substitutions have been made to ensure that the strain cannot revert and also to improve its growth properties in cell, rather than egg culture as this is a more scalable technology for rapid vaccine manufacture than is egg culture.

A similar approach may be envisioned for viral groups such as the Flaviviruses. This is an important group of viruses, whose members have been responsible for several infectious disease outbreaks during the last two decades. Their members include Yellow fever virus, West Nile virus and Dengue virus and they are viruses with segmented RNA genomes. One of the key factors in the emergence of new pathogens is the presence of an RNA genome as this allows rapid recombination, mutation and hence evolution and adaptation. This group is therefore of great importance when horizon scanning for the next emergent viral disease and only one flavivirus already has a licensed vaccine (Yellow fever). There are vaccines

against Dengue virus in clinical trial, since this is a disease with a worldwide distribution and serious complications can be observed if re-infection with a different serotype occurs (Dengue haemorrhagic fever). West Nile virus is a classic example of an emergent viral infection; it arose in the West Nile delta in North Africa and was transported by cervid birds into North America where, as it is a mosquito vector-transmitted disease, it has caused seasonal outbreaks in every subsequent year (see Campbell *et al.*, 2002). Chikungunya virus has similarly spread across the Indian Ocean and has also been imported into Europe where it caused a limited outbreak in Italy during 2009 (Beltrame *et al.*, 2007). It is likely that infection with one flavivirus induces an immunity against some other viruses within the group, based on the observation that antibodies against many of these viruses cross-react with flaviviral antigens in ELISA assays and thus make clinical infection with a particular virus impossible to diagnose by serology alone. By comparing different flaviviruses with the Yellow fever 17D vaccine, it should be possible to produce vaccines against this range of viruses by using the same approach to attenuate different viruses. What is unknown, once again, are the correlates of immunity for not only Yellow fever infection but for any of the other flavivirus infections. Care will also be needed to avoid any complications by priming the immune system as are seen in Dengue virus infection. As a method of rapidly producing a vaccine strain, attenuation by repeated passage is not useful due to the extensive length of time that it may take to produce an attenuated virus and the additional time required to demonstrate irreversible attenuation.

2.1.3 Sub-unit vaccines

Probably the most rapid way to make a vaccine against a newly emerging pathogen is to use a recombinant technology approach and identify a single immunogenic antigen, clone out and express the gene encoding that particular antigen. A common theme that we have observed whilst discussing vaccinology, is that rational vaccine design has an absolute requirement for good understanding of both the factors which affect pathogen virulence as well as those which contribute to immunity in the host. Although this has been discussed previously, it bears repeating that in the event of a newly emerging pathogen, these data will not be available and that the second best approach is to fall back to an inactivated whole pathogen preparation. Assuming, however, that we are aiming to develop a vaccine against a pathogen which is similar to one about which we have considerable knowledge, then a rational sub-unit approach is likely to be used. Once again the caveat is that a single sub-unit(s) may not induce complete protective immunity, as there may well be multiple components involved in protection following natural infection. There are numerous examples where single sub-unit vaccine candidates fail to provide complete or even any protection, a good illustration of this observation is the lack of complete protection afforded by single sub-unit vaccines in HIV infection (inactivated virus also fails to induce protection though).

Yersinia pestis, the bacterial causative agent of plague, is a re-emergent infection as it has caused recent outbreaks in geographical areas that have previously not experienced infections caused by this organism. Fig. 3 below illustrates that although there are a large number of surface antigens, against which an immune response is induced (as determined by the presence of human antibody response), only two are associated with protective immunity, these are the F1 and V antigens and both are virulence factors encoded by

transferrable plasmids. A vaccine containing the F1 and V-antigens is being developed, although because of the low prevalence of disease, it is unlikely to be widely used. As an exemplar of the approach that can be taken with emerging and re-emerging diseases, it illustrates perfectly the requirement for a thorough understanding of the pathogenesis of the organisms as well as understanding the host immune response.

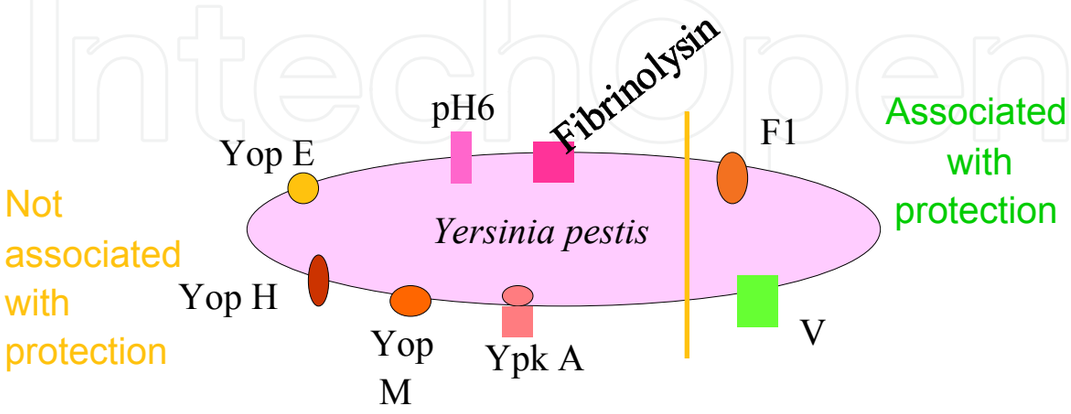

Fig. 3. Identification of Protective Antigens in *Yersinia pestis*.

3. Therapeutic interventions

There are a great number of therapeutic interventions that can be applied to the control of infectious diseases. The key type of interventions are discussed in greater detail below, and as a general trend, we have observed a move towards more targeted approaches to design of therapeutic compounds. During the last 30 years, the period under discussion when considering emerging infectious diseases, there has been a marked change in the scientific approaches to discovery of therapeutic molecules. There were a great many researchers using natural product libraries during the 1970's and 80's to discover compounds with activity against a wide range of biological activities; of relevance to infectious diseases are those which exhibited antimicrobial activity. A consequence of these high-throughput screening programmes was that several new classes of antibiotic were discovered during this time, but subsequently there have been very few new discoveries and there is now a real shortage of new antimicrobial compound groups to counteract the rise of antimicrobial resistance. Future research to meet clinical unmet needs, must incorporate the rational design and discovery of new ways of combating antimicrobial resistance, be it new antibiotics or other therapeutic approaches.

3.1 Antimicrobial compounds

The majority of currently licensed antibiotics were discovered between 40 and 60 years ago and modern drugs are mostly derivatives of existing classes of drug. Despite the increased need to treat expanding populations against micro-organisms exhibiting increasing levels of resistance, there has been a reduction in the effort by the major pharmaceutical companies to discover new antibiotics (Marinelli, 2009). There have been a number of high, but unrealised

expectations driven by high-throughput screening, combinatorial chemistry and microbial genome sequencing that have failed to deliver the new compounds required. It is interesting to note that during the "Golden Age" of antibiotic discovery (1940-1960) approximately 12,000 compounds were screened and resulted in 160 licensed products (0.01%). This statistic played a major part in the evolution of antibiotics where research effort was directed towards the improvement of existing chemotypes (increased potency, stability and pharmokinetics, reduced side-effects) rather than discovery of novel compound classes. Those that did continue to invest research funds into discovery rather than improvement were rewarded by the discovery of some new chemotypes such as thienamycin, daptomycin and echinocandins. One of the main confounders to the discovery of new compounds was the high rate of re-isolation of existing compounds, requiring a different approach to screening assays from the traditionally used inhibition of bacterial growth that has been used since Alexander Fleming discovered Penicillin in 1928. This need has led to the introduction of functional, cell-free assays, but this has not resulted in the desired increase in discovery of novel compounds. Moreover, the availability of whole genome sequence data for a wide range of pathogenic bacteria and viruses similarly has not resulted in discovery of novel chemotypes, despite high investment by the pharmaceutical companies.

There are, however, several prospects for the discovery of new classes of chemotype. The first of these is to harness the wealth of structural data that are now available, but this approach requires an in-depth knowledge of the biology of the target micro-organism. There are a number of documented examples of this approach being coupled with virtual high-throughput screening (VHTS) against virtual compound libraries. One example is that described by Reddy *et al.* (2006) for the rational discovery of small molecule inhibitors of prion protein, a key emergent disease. Here a virtual compound library was screened for molecules that would theoretically bind and inhibit prion protein from entering cells. A similar approach has been taken to the design of inhibitors of the Anthrax toxin cell-binding component (protective antigen; PA) which combines with two further toxin components to exert the toxic effects, which typically result in death of the animal or human host. The protein crystallography data were used for the PA molecule and the cell-receptor binding site was identified from published research (Bradley *et al.*, 2001). This crystal structure was screened using VHTS and small-molecule inhibitors of this binding reaction were identified and then synthesised and screened *in vitro* using functional assays for one of the two Anthrax toxins (lethal toxin, a combination of PA and lethal factor). Many of the molecules screened using this approach were subsequently found to have significant activity in the *in vitro* assays; approximately 3% of the compounds screened were found to possess activity, compared with the 0.01% that are typically obtained by high-throughput screening of compound libraries (B. Chen, personal communication). This approach clearly holds much promise for the rational design of novel chemotypes of antimicrobial compound and the approach works irrespective of whether the pathogen is a virus or bacteria. This is therefore, an attractive approach for the development of targeted inhibitors of a range of processes involved in the pathogenesis of disease. The main caveat, though, is that a thorough understanding of the virulence factors and pathogenesis of the disease are needed, along with suitable structural data to enable this approach to be used to combat emerging diseases. Clearly, when a pathogen first emerges, it is completely uncharacterised and such a directed approach is not possible.

Other approaches to the discovery of new chemotypes are the screening of compound libraries derived from different sources. This approach somewhat mimics the approach used during the "Golden Age", in that compound libraries are screened using high-throughput methods and inhibition of microbial growth at this stage is a precursor to further characterisation. The libraries from which most antibiotics were derived are soil organisms, but there are also untapped resources in the oceans, where there is a large number and range of micro-organisms and from plant extracts where a number of compounds have already been shown to possess antibacterial and/or antiviral activity. Examples are the extracts from the garlic and clove plants (Arora & Kaur, 1999). A range of spice plants were screened for activity and only these two extracts exhibited antimicrobial activity, however, they were active against a range of Gram positive and Gram negative bacteria and yeasts.

As an adjunct to antimicrobial compounds, we should also consider those compounds which modify activity of existing drugs. Such examples include ß-lactamase inhibitors such as clavulanic acid (used as a proprietary preparation in conjunction with amoxicillin and other ß-lactam antibiotics) and efflux inhibitors which maintain an elevated drug concentration within cells and hence improve antimicrobial efficacy.

3.2 Therapeutic antibodies

An alternative therapeutic approach to emerging infectious diseases is the use of antibodies to treat disease. This is a concept which has existed for a considerable time, however, only relatively recently have therapeutic antibodies really been used against infectious diseases. As of 2006, there were 20 therapeutic antibodies approved as therapeutics by the FDA (Das, 2006), but they offer considerable potential for the rapid treatment of emerging infectious diseases. One may question why this should be; the answer is straightforward, since protection from infection by pathogenic micro-organisms may be either active, and induced by immunization using prophylactic vaccines (see section 2.1), or passive. One of the issues discussed in section 2 was that prophylactic vaccines take a considerable time to make and formulate and these can be too slow for a pathogen with a high rate of reproduction (R_0) in a susceptible population. Passive immunity, where specific antibodies are administered, is a viable alternative here and also for the treatment of diseases where antimicrobial therapy may exacerbate the disease symptoms and also result in higher transmissibility (*e.g.* ulcerative colitis caused by *Clostridium difficile* infection and toxin). Many advances have been seen over the past decade which has allowed improved antibody engineering technologies along with improvements in safety and efficacy. These developments, along with a greater understanding of the immunomodulatory properties of antibodies, have paved the way for the next generation of new and improved antibody-based drugs for the treatment of human diseases. One major factor that makes this an attractive technology is that antibody "factories" can be rapidly turned around to produce a stock of antibodies for therapeutic use. This approach was recently described by Rogers *et al.* (2008) where they developed a panel of neutralising antibodies against the SARS coronavirus, a recently emerged pathogen, using a novel DNA display method. They describe their approach which involved panning the library using whole SARS virus rather than just the spike protein (which had been used by others, being a primary virulence factor for cell binding and entry). Other therapeutic antibody approaches in development include those to treat toxigenic effects following bacterial infection (*Clostridium difficile,* verotoxigenic *E.coli,* Anthrax toxin).

The use of therapeutic antibodies certainly holds much promise for the treatment of infectious diseases and is particularly attractive due to the rapidity that recombinant antibodies may be selected, produced and manufactured at scale.

3.3 Other therapeutics

There are a considerable number of other therapeutic approaches in development or at the research stage, far too many to comprehensively review here. Instead, we will concentrate on some of the key areas where there are noteworthy developments.

3.3.1 Therapeutic vaccines

Firstly, we will consider therapeutic vaccines. In section 2 previously, we have considered the use of vaccines for induction of prophylactic immunity. There is an alternative use of some vaccines, however, and this is for prophylaxis following exposure to an infectious disease micro-organism. A good example is the AIDS virus, HIV, which emerged in 1983 and for which, despite many attempts, there is no prophylactic vaccine. The Norwegian biotech company, Bionor Pharma recently released results from a study of its therapeutic HIV vaccine, Vacc-4x. There are recent data showing that the vaccine lowered patients' viral loads and negated the need for antiretroviral therapy (Fierce Vaccines, 2011).

3.3.2 Phage therapy

Phage therapy entails the use of bacteriophage viruses that infect bacteria for the treatment of bacterial infections. Phages are ubiquitously found in bacterial populations and control the growth of bacteria in many environments, including in the intestine, the oceans, and the soil. Phage therapy was in used in the 1920s and 1930s in the USA, Western & Eastern Europe, however, success rates of this therapy have never been firmly established, because only a limited number of clinical trials testing the efficacy of phage therapy have ever been conducted. These studies were performed mainly in the former Soviet Union. The development of antibacterial-resistant bacteria has once again sparked renewed interest in phage therapy with several companies, universities and foundations across the world now focusing on phage therapeutics. One of the main difficulties is that of delivery of the phage to the site of infection, making them potentially more suitable for treatment of respiratory or skin diseases than for deep-seated infections. There is also the safety concern about giving live viruses to human subjects.

3.3.3 Bacteriocins

Bacteriocins are peptides that can potentially be more readily engineered than small combinatorial chemistry generated molecules and are potential alternatives to conventional antibacterial compounds. Different classes of bacteriocins have different potential as therapeutic agents. Small-molecule bacteriocins (*e.g.* microcins and lantibiotics) are similar to the classic broad-spectrum antibiotics whereas colicin-like bacteriocins possess a much narrower activity spectrum, and require pathogen identification (and susceptibility testing) prior to therapy. Limitations of large-molecule antibacterials include reduced transport across membranes and within the human body. For this reason, they are usually used topically or gastrointestinally.

3.3.4 Chelation

A novel approach relies on the removal of essential nutrients for bacterial growth within the host by chelation. These compounds are not suitable for use alone, but may have utility in combination with conventional antibacterial compounds. A similar approach forms the basis of a treatment for lymphoblastic leukaemia, where bacterially-derived L-asparaginase is used as a therapeutic to remove L-asparagine, an essential amino acid for leukaemia cells to grow and divide, from the circulating blood stream and hence effectively "starving" the cancer cells.

3.3.5 Probiotics

Probiotics consist of live cultures of bacteria, which may become established as competing commensal organisms and thus inhibit or interfere with colonization by microbial pathogens. This approach has been used to reduce nasal carriage with Methicillin-resistant *Staphylococcus aureus* (MRSA) by replacement therapy using a skin commensal *Corynebacterium* sp. (Uehara *et al.* 2000).

4. Diagnostic tests

Diagnostic testing is not a therapeutic intervention, of course, but like other public health control measures, this chapter would be incomplete without mention of its use. In the early stages of an outbreak caused by any infectious disease pathogen, the public health control measures are inevitably supported by diagnostic testing. When a new disease emerges, the only factor that can be used for diagnosis is the clinical presentation. This is frequently compounded by the observation that many diseases present at the early stage with non-specific symptoms that are common with many less severe diseases. For example, the early symptoms of the SARS virus are very similar to many other respiratory infections caused by a range of viral pathogens, most of which do not require any intervention. Thus the power and value of a good diagnostic is in the differentiation of a pathogen causing severe infection from seasonally circulating infections of much lower consequence. Recently the Health Protection Agency undertook a study to model the value of rapid diagnostics development in the control of an outbreak caused by an emerging infectious disease (C. Noris & N. J. Silman, unpublished). The conclusion was that the diagnostic was of value only early in an outbreak when differential diagnosis was used to drive a policy of "containment" of the disease, that is, preventing onward transmission by preventing non-infected persons coming into contact with those who were infectious. Of course, quarantine cannot be enforced, but it was an effective tool used in controlling the spread of the H1N1 "swine" influenza pandemic during 2009. At the stage of the outbreak where containment is no longer possible, *i.e.* there are sufficiently high numbers of cases, then laboratory diagnosis ceases to have any real value in the CONTROL of the pandemic. That is not to say that it is of no value at all though.

5. Public health control measures

Although this chapter is focussing on the different therapeutic interventions that may be used to tackle the problems of emerging infectious diseases, it would be incomplete without mention of non-therapeutic means of controlling spread of infectious disease. In the initial

stages of responding to an emergent infectious disease, the only tools available to limit spread of the disease and prevent nosocomial infection of clinical staff are non-therapeutic public health interventions. Perhaps the earliest described successful approach was in 1854 when John Snow removed the handle from a water pump in Broad Street, Soho, London. Before the discovery of pathogenic micro-organisms, Snow, a physician, was sceptical about the "miasma" theories that surrounded what we know to be infectious disease outbreaks. Miasma theory suggested that the cause of disease was a form of pollution or bad air. Snow carefully pieced together the evidence surrounding the distribution of the Cholera cases in the Soho area of London and concluded that the water pump in Broad Street was the common denominator; he removed the pump handle to prevent people from drawing and hence consuming water contaminated by *Vibrio cholerae* and very effectively curtailed the outbreak.

The same general, non-therapeutic approach may be taken with newly emerging diseases. For example, many of the diseases that have emerged in the last 30 years have an insect vector involved in the dissemination of the infectious disease pathogen (Jones *et al.*, 2008). The most effective intervention for vector-borne diseases is the eradication or reduction in the numbers of the insect vector using pesticides, rather than prophylaxis (not generally available) or therapeutics (Rose, 2001). Another recent example is the reduction in the numbers of cases of hospital acquired infection (HCAI). A contribution to this reduction is the reminder that good hand hygiene is vital and re-education of medical and nursing staff in the UK on good hand-washing practice, as well as the introduction of hand sanitizer gels (Grayson *et al.*, 2009). Thus these important interventions, although mostly quite simple can have very pronounced outcomes in the control of infectious diseases.

6. Conclusion

There are a number of therapeutic interventions that can be used to combat emerging infectious diseases. When a new pathogen emerges and subsequently causes a widespread outbreak, the interventions that can be applied differ during the course of the outbreak. At the early stage, recognition of the disease is heavily reliant upon clinical case definition, as was used in differentiating the new variant H1N1 influenza virus in 2009, prior to the development of a molecular diagnostic assay. We have seen that the development of a diagnostic assay is used in support of clinical case definition. The most effective interventions are therapeutic or prophylactic ones, however, since they are able to either treat those infected or protect onward spread by inducing herd immunity within a population. The downside of a therapeutic approach against a newly emerging disease is the length of time that is required to develop an effective vaccine or therapeutic against any specific pathogen. Here we require more generic, broad-spectrum interventions such as antimicrobials. What is evident is that more investment in R&D to discover new therapeutic molecules that can be used against newly emerging pathogens. Currently we are relatively well-served by the availability of broad-spectrum antibiotics, but increasingly widespread multi-drug resistance is a major problem and new chemotypes are urgently required. Perhaps the greatest hope is available by using recombinant antibody technology, where using the high-throughput genome sequencing approaches now available, we can rapidly sequence newly emerged pathogens, clone out and express surface antigens for rapid development of therapeutic antibody preparations. Many countries have invested heavily in

infrastructure to build rapid vaccine facilities that can be turned around quickly in the event of the emergence of a new highly infectious pathogen and these sorts of adaptable facilities are of potentially great value in combating emerging infectious diseases.

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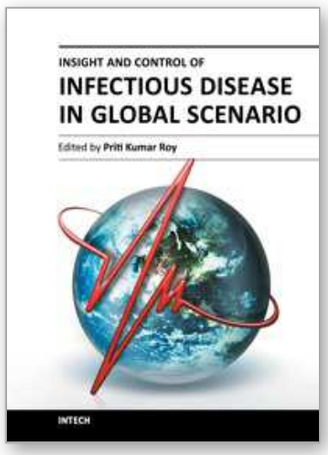

Insight and Control of Infectious Disease in Global Scenario
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This book is projected as a preliminary manuscript in Infectious Disease. It is undertaken to cover the foremost basic features of the articles. Infectious Disease and analogous phenomenon have been one of the main imperative postwar accomplishments in the world. The book expects to provide its reader, who does not make believe to be a proficient mathematician, an extensive preamble to the field of infectious disease. It may immeasurably assist the Scientists and Research Scholars for continuing their investigate workings on this discipline. Numerous productive and precise illustrated descriptions with a number of analyses have been included. The book offers a smooth and continuing evolution from the principally disease oriented lessons to a logical advance, providing the researchers with a compact groundwork for upcoming studies in this subject.

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