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Overview of measles and mumps vaccine: origin, present, and future of vaccine production

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Summary. – Measles and mumps are common viral childhood diseases that can cause serious complications. Vaccination remains the most efficient way to control the spread of these viruses. The manufacturing capability for viral vaccines produced in embryonated hen eggs and conventional/classical cell substrates, such as chicken embryo fibroblast or primary dog kidney cell substrates, is no longer sufficient. This limitation can be overcome by utilizing other recognized cell substrates such as Madin Darby Canine Kidney (MDCK), Chinese Hamster Ovary (CHO), Vero (monkey origin) cells, MRC-5 (human diploid) or as an alternative, introducing new cell substrates of human or avian origin. A very important factor in vaccine production is the safety and immunogenicity of the final vaccine, where the proper choice of cell substrate used for virus propagation is made. All substrates used in vaccine production must be fully characterized to avoid the contamination of hidden unknown pathogens which is difficult to achieve in primary cell substrates.

Keywords: measles virus; mumps virus; vaccines; cell substrates

Contents:

1. Introduction

Measles and mumps are highly contagious viral diseases caused by the enveloped viruses in the *Paramyxoviridae* family, members of the genus *Morbillivirus* and *Rubulavirus,* respectively. By the convention used for paramyxoviruses, the term „gene" refers to the genome sequence encoding a single mRNA, even if that mRNA contains more than one ORF and encodes more than one protein.

Measles virus contains a non-segmental negative-stranded RNA genome of 15,894 nucleotides in length. The genome contains six genes (N, P/V/C, M, F, HN, and L) that code six structural proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutin (H), and large protein (L) (Griffin and Bellini, 1996; Lamb and Kolakofsky, 1996). In addition, measles virus codes two nonstructural proteins: V protein and C protein.

Mumps virus carries a single-stranded RNA genome that is 15,384 nucleotides in length, with the viral genes arranged in linear sequence. The genome contains seven genes (N, V/P, M, F, SH, H, and L) that code nucleo- (N), phospho- (P), matrix (M), fusion (F), large (L), and V proteins as well as small hydrophobic (SH) protein, and hemagglutinin-neuraminidase (HN) (Elango *et al*., 1988, 1990).

The negative-sense RNA is encapsidated by N protein and associated with an RNA-dependent RNA polymerase complex composed of L and P protein subunits. This core

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Abbreviations: CCL = continuous cell line; CEF = chicken embryo fibroblast; CHO = Chinese Hamster Ovary; DCL = diploid cell line; F = fusion protein; H = hemagglutin; L = large protein; M = matrix protein; MDCK = Madin Darby Canine Kidney; MDG4 = United Nation´s Millennium Development Goal 4; MMR = measles, mumps, and rubella; N = nucleoprotein; P = phosphoprotein; PCC = primary cell culture; WHO = World Health Organization

is linked to the virion membrane by M protein. The outer surface of the virion is covered with glycoprotein spikes consisting of the H or HN protein, respectively. H or HN protein binds sialic acid to allow virion attachment to the cells. F protein induces viral and cellular membranes to fuse together during virus entry. The small hydrophobic (SH) protein prevents infected cells from undergoing apoptosis (Wilson *et al.*, 2006). The V protein is only found in virus-infected cells and prevents induction of interferon-induced antiviral responses (Kubota *et al.*, 2001, 2005; Ulane *et al.*, 2003).

2. Measles – epidemiology, pathogenicity, and reported cases

Measles is transmitted via droplets from the nose, mouth or throat of infected persons. Measles is one of the most readily transmitted communicable diseases and probably the best known and most deadly of all childhood rash/fever illnesses. Initial symptoms usually appear 10–12 days after infection; include high fever, runny nose, bloodshot eyes, and tiny white spots on the inside of the mouth. Several days later, a rash develops, starting on the face and upper neck and gradually spreading downwards. There is no specific treatment for measles and most people recover within 2–3 weeks. However, measles can cause serious complications, including blindness, encephalitis, severe diarrhea, ear infection and pneumonia, particularly in malnourished children and people with reduced immunity. Measles can be prevented by immunization.

Prior to the availability of measles vaccine, measles infected over 90% of children before they reached 15 years of age. These infections were estimated to cause more than two million deaths and between 15,000 and 60,000 cases of blindness annually worldwide (Semba and Bloem, 2004). Live attenuated viral measles vaccine was used from 1963, and immediately identified its use as highly cost-effective. In the year 2000, the World Health Organization (WHO) estimated that 535,000 children died of measles. The majority of these children were from developing countries, and this burden accounted for 5% of all under-five mortality (Levels & trends in child mortality report 2011, 2012). In some developing countries, case-fatality rates for measles among young children may still be at 5–6%. In industrialized countries, approximately 10–30% of measles cases require hospitalization, and one in a thousand of these cases among children result in death from measles complications.

In 2010, the World Health Assembly committed to reducing measles deaths by 95% of the 2000 levels to 2015. By 2010, estimated global measles mortality decreased by 74% from 535,300 deaths in 2000 to 139,300 in 2010 (Fig. 1) (WHO, 2012). Measles mortality was reduced by more than three-quarters in all WHO regions apart from the WHO South-East Asia Region. India accounted for 47% of estimated measles mortality in 2010, and the WHO African region accounted for 36%. Improving measles vaccination coverage and reducing measles-related deaths is a global imperative, particularly as it relates to the United Nation´s Millennium Development Goal 4 (MDG4) (The millennium development report 2009). Achieving MDG4 and global measles-mortality

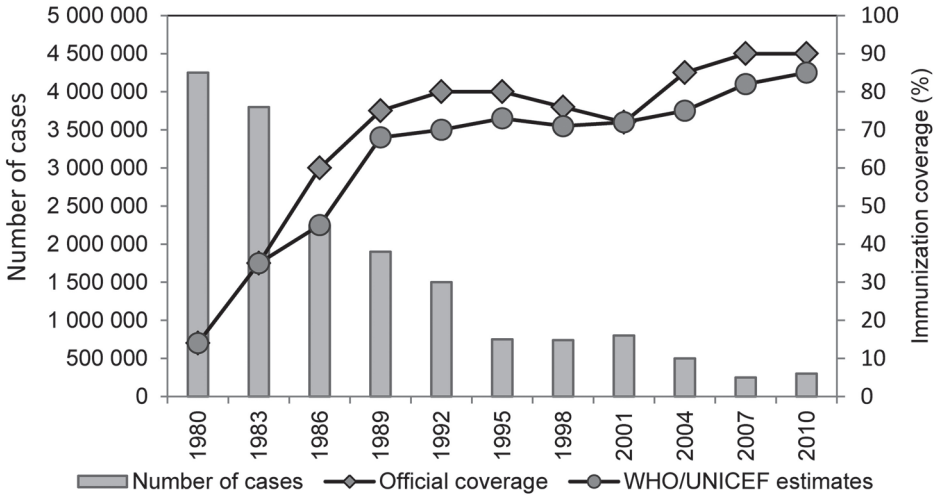

Fig. 1

Measles global annual reported cases and vaccine coverage (1980–2011)

http://www.who.int/immunization_monitoring/diseases/measles/en/index.html.

reduction goals will require a further increase in measles vaccine coverage. It is unacceptable that every day 380 children continue to die from measles and 300 children still enter the world with the disabilities of CRS despite the availability of effective, safe and inexpensive vaccines.

3. Mumps – epidemiology, pathogenicity, and reported cases

Mumps is a viral infection, primarily affecting the salivary glands. It is sometimes called infectious parotitis. The virus is transmitted by direct contact, or via airborne droplets from the upper respiratory tract of infected people. It most often effects children between five and nine years of age. However the mumps virus can also infect adults. Initial symptoms usually appear 2–3 weeks after infection, and include headache, muscle pain, low-grade fever and malaise. Soon after, swelling of one or both parotid glands appears. There is no specific treatment for mumps. The virus usually causes mild disease in children, but in adults can lead to complications such as meningitis and orchitis. Mumps can be prevented by immunization.

Before the 1960s, mumps was a common infectious disease in all parts of the world, with annual incidences ranging from approximately 0.1–1%, and up to 6% in certain populations. In hot climates the disease is endemic throughout the year, whereas in temperate climates incidence peaks in winter and spring. In countries where vaccines against mumps were introduced in the late 1960s, mumps incidence dropped dramatically. In most parts of the world, the annual incidence of mumps is in the range of 100–1,000 per 100,000 population. By the year 2000, approximately 120 countries or regions had included vaccination against mumps in their national immunization services, the vast majority combining mumps with measles, mums, and rubella (MMR) vaccines. However, in the African Region only Egypt has included vaccination against mumps, and in the South-East Asia Region, only Singapore, Thailand and Brunei have done so. In these two regions mumps incidence remains high, with epidemic peaks every two to five years, mostly affecting children five to nine years of age. The disease is not considered eradicable, and has a low priority in terms of public health efforts to control it. This is likely to be the reason that mumps global annual reported cases have a slightly raised tendency (Fig. 2) (WHO Vaccine-Preventable Diseases, 2012).

4. Vaccines

The first available measles vaccine was licensed in 1963 under the trade name Rubeovax™ (Merck & Co., Inc.). This

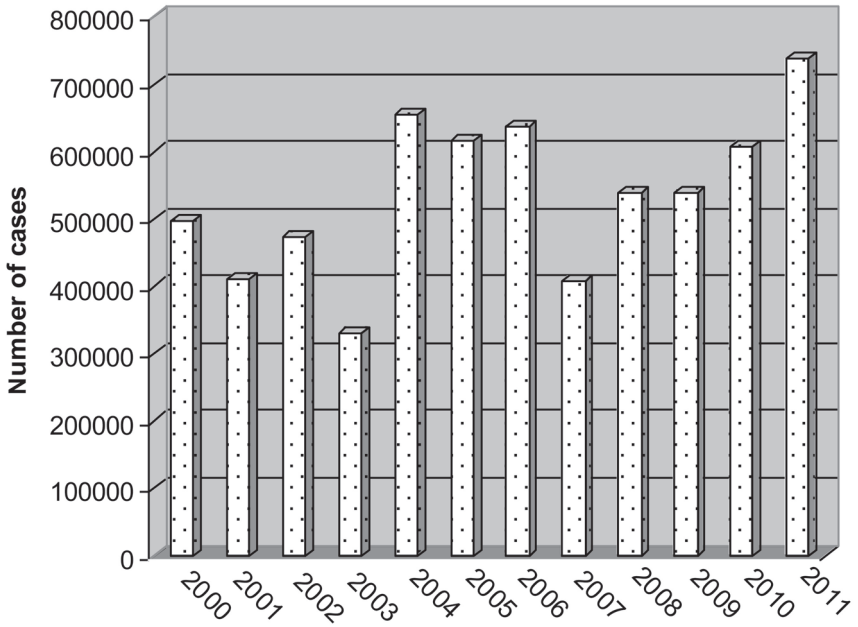

Fig. 2

Mumps global annual reported cases (2000–2011)

The data summarized from WHO (2012), Mumps reported cases. http://apps.who.int/immunization_monitoring/en/globalsummary/timeseries/tsincidencemum.html.

vaccine was widely used until 1975. The first available measles vaccine, Edmonston B was derived from Edmonston wild/type strain of measles virus, which was isolated from the blood of a boy with typical measles in 1954 (Enders and Peebles, 1954). The obtained virus was adapted to chicken embryo fibroblast (CEF) by serial passages to produce the Edmonston A and B seeds. The Edmonston A was attenuated in chick embryo culture at 32°C and Schwarz strain were derived after 156 passages in 1963. Attenuated Edmonston Enders strain was prepared by passages to produce the Edmonston B virus in CEF at 36°C and 32°C (Schwarz, 1962; Hilleman *et al.*, 1968). The Edmonston Enders strain is used in the vaccine Attenuvax™ produced by Merck & Co., Inc. GlaxoSmithKline Biologicals (GSK Biologicals) developed their own vaccine strain by additional passages of the Schwarz strain (Rimevax™, GSK Biologicals). Schwarz and Edmonston Enders strains have identical genomic sequences and are the most commonly used measles vaccine worldwide (Parks *et al.*, 2001a,b; WHO, 2004; Tillieux *et al.*, 2009). Additional attenuation of Schwarz strain on primary dog kidney cells at 34°C was Schwarz Sevapharma strain, previously used in Czechoslovakia. The Schwarz BA33 was adapted to MRC-5 cells from Schwarz Sevapharma by serial passages at 33°C (T. Betáková, unpublished data). The Edmonston wild-type strain was also used to prepare the attenuated viruses such as AIK-C and Zagreb, which are currently used for the preparation of measles vaccines (Rota *et al.*, 1994).

The most commonly used mumps vaccine strain is the Jeryl Lynn strain. This strain was isolated from women who developed mumps with unilateral parotitis in 1963 (Buynak and Hilleman, 1966). The strain was attenuated by passages in embryonated eggs and CEF cultures and sold worldwide by Merck, Sharp & Dohme/Merck & Co., Inc. Since the early 1970s it has been known under the trade name Mumpsvax™. The vaccine Mumpsax contains a 1:5 mixture of two substrains with substantially different sequences (Afzal *et al.,* 1992, 1994). GSK Biological developed a mumps virus vaccine based on a single cloned immunologically dominant strain JL1 derived from Jeryl Lynn. This strain was named RIT 4385. At least 11 strains are presently in use throughout the world: the Jeryl Lynn and Urabe Am9 strains have been the most commonly used followed by the Leningrad-Zagreb, Leningrad-3, and Rubini strains; the newer RIT 4385 strain has been derived from the Jeryl Lynn strain. The Jeryl Lynn BA33 was adapted to MRC-5 cells from Jeryl Lynn Sevapharma (adapted on primary dog kidney cells) by serial passages at 33°C (T. Betáková, unpublished data). The use of other available mumps strains has been limited in most cases to one country only. Mumps vaccines are available as monovalent vaccines or in combination with other vaccines (which are almost universal), such as the MMR combination (Hviid *et al.*, 2008).

5. Cell substrates

The manufacturing capability for viral vaccines produced in embryonated hen eggs and conventional/classical cell substrates, such as chicken embryo fibroblast, has now reached its capacity limit. This limitation can be overcome by utilizing other recognized cell substrates such as Madin Darby Canine Kidney (MDCK), Chinese Hamster Ovary (CHO) or Vero (monkey origin) cells or as an alternative, introducing new cell substrates of human or avian origin (Hess *et al.*, 2012). A new expression technology for vaccine development has been adopted using a cell substrate. A cell-based isolated virus may be more clinically relevant for use as a vaccine than an egg-based isolate (EMA/CHMP/BWP/68803/2010). It is well established that cell substrates themselves and events linked to cell growth can affect the characteristics and safety of the resultant biological products. Therefore, a thorough understanding of the characteristics of the cell substrate is essential in order to identify points of concern and to develop a quality control system that addresses those points (WHO, 2010).

Cell substrates are the cells used to manufacture a biological product. The cells may be i) primary cell culture (PCC), ii) diploid cell line (DCL), iii) continuous cell line (CCL), and iv) novel cell substrates. These cell lines may be grown in monolayer or suspension culture conditions.

Primary cells are established directly from trypsinized tissues of normal animals (CEF, kidney cells from dog, monkeys, rabbits and hamsters) (WHO, 1998).

The essential features of diploid cell lines of human (WI-38 and MRC-5) or monkey (FRhL-2) origin have a finite capacity for serial propagation, which ends in senescence. They are nontumorigenic and display diploid cytogenetic characteristics with a low frequency of chromosomal abnormalities of number and structure (Jacobs *et al.*, 1981). Human diploid cell substrates were used for the production of viral vaccines more than 35 years ago (Hayflick *et al.*, 1987). WHO has also overseen the establishment of seed stocks of MRC-5 for the production of vaccines. The WHO MRC-5 RCB was established in 2007 due to stability issues associated with the original vials of MRC-5 cells, which dated to 1966. This RCB was prepared in a qualified clean room environment and subjected to specified quality-control testing endorsed by the ECBS (WHO, 2010).

CCL have the potential for an infinite life span and can usually be cultivated as attached cells or in suspension in a bioreactor. They have been derived by i) serial subcultivation of a primary cell culture of a human or animal tumor cell, such as HeLa or Namalva cells; ii) transformation of a normal cell having a finite life span with an oncogenic virus, e.g. B lymphocyte transformed by the Epstein-Barr virus; iii)

serial subcultivation of a normal cell population generating a new cell population having an infinite life span; or iv) fusion between a myeloma cell and an antibody-producing B lymphocyte. CCL are now considered to be suitable for the production of many biological substances and possess distinct advantages over primary and diploid cell substrates (Grachev, 1990). CCL grow well using ordinary media and serum, and some do not require serum at all. However, many CCL express endogenous viruses and are tumorigenic.

Novel cell substrates originate predominantly from avian, human and other mammal sources. Two human cell substrates were developed by transforming or stably transfecting human embryonic kidney cells (HEK 293) or human embryonic retinal cells (PER.C6) with the early region 1 (E1) of adenovirus type 5 (Ad5). Today, additional novel cell substrates of avian origin are available, such as the duck embryonic stem cell line EB66 (Brown and Mehtali, 2010) and CR (Cairina Retina) cells obtained from muscovi duck retinal tissue. The CR cells were transfected with Ad5 pIX gene resulting in AGE.1CR.pIX cells (Jordan *et al.*, 2009; Lohr *et al.*, 2009).

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Conflict of interest. The authors declare that there is no conflict of interest with the ideas put forward in the final version of manuscript.

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