Vaccination against measles: a neverending story

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Measles, a highly contagious viral disease, is a major childhood killer in developing countries, accounting for almost 1 million deaths every year globally. Measles virus normally does not cause a persistent infection, no animal reservoir for measles virus exists, no vector is involved in its spread, only one serotype exists, the virus is antigenically stable and vaccination with the currently used live attenuated vaccines proved to be highly effective in preventing disease. Therefore, theoretically measles should be considered eradicable. This article provides a review of past and current measles vaccination efforts and development and need of new generation experimental measles vaccines.

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CONTENTS

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Inactivated vaccine

In theory, measles should be considered eradicable [1]. Large-scale vaccination against measles started in the 1960s. Children were vaccinated with formalin- or tween-ether inactivated, whole-virus vaccines adjuvated with alum. Although high seroconversion rates were observed (>95%), the virus-neutralizing antibody titers were short-lasting, which necessitated multiple immunizations [2]. Furthermore, upon natural infection with measles virus (MV), children vaccinated with the inactivated vaccine developed enhanced disease, referred to as atypical measles [3,4]. Atypical measles was characterized by a prolonged high fever, an atypical rash and severe pneumonitis, often requiring hospitalization [3,5,6]. Abdominal pain, hepatic dysfunction, headache, eosinophilia, pleural effusions, hilar adenopathy and edema were also described [7]. As a result of this apparent immunopathological predisposition, the use of inactivated vaccines was abolished. It took long before the underlying mechanism was elucidated and even today we do not have a full understanding of the postulated immunepathogenesis. One of the first hypotheses was that the disease resulted from a lack of functional antibody against the fusion protein [8]. However, reproduction in macaques suggested that atypical measles rather resulted from previous priming for a MV-specific, but nonprotective T-helper (Th)2 response, leading to a strong anamnestic response following challenge resulting in immune complex formation and a pulmonary hypersensitivity response associated with eosinophilia [9].

Live attenuated vaccine

Based on the safety and efficacy data obtained in early studies with live attenuated measles virus preparations, vaccination against measles was persued again in the 1970s [10,11]. The application of live attenuated measles vaccines (LAV) resulted in an impressive decline of measles cases, especially in developed countries [12]. Furthermore, recent data suggests that vaccination against measles also reduces mortality from many other causes [13,14]. Other merits but also demerits of LAV are listed in BOX 1.

Alternative strategies for vaccine administration

Currently, the first LAV dose is given at an age between 9 and 15 months. At this age maternal antibodies, which interfere with replication of the vaccine virus, have vanished in most children. However, in developing countries, measles frequently occurs at an early age (< 9 months) [15]. Ideally, a measles vaccine should be effective when administered to very young infants in the presence of

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maternally-derived antibody. In an attempt to overcome vaccine neutralization by pre-existing immunity against MV, LAV has been applied with a dose 100- to 1000-fold higher. However, this led to a poorly understood increased mortality in girls in subsequent years as compared with infants vaccinated with standard titer LAV [16–19]. The currently used LAV, when parenterally administered, has proven to be quite successful. However, vaccine failures may, at least in part, be attributed to an inadequate vaccine-induced mucosal immunity – the current vaccine protects against measles but not necessarily against MV infection [20]. Vaccination strategies that would allow the induction of adequate mucosal immunity may have advantages in this respect. If this could be combined with the easy, inexpensive and safe administration of a stable vaccine, the efforts to eradicate MV would be considerably facilitated. Besides the development of new generations of MV vaccines, the question has been raised whether it would be feasible to apply the existing LAV *via* mucosal routes instead of the currently used parenteral routes. This could lead to an improved immune response at the site of virus entry. An additional advantage of this strategy might be a more effective vaccination in the presence of pre-existing MV-neutralizing antibody [21,22]. For measles vaccines this phenomenon was reported years ago by Okune *et al.* and Ueda *et al.* [23,24]. They found that subcutaneously injected LAV was neutralized in the presence of low levels of neutralizing antibody, whereas LAV inhaled as aerosol was not. Since then, the concept of mucosal vaccination using the current LAV has been studied frequently. Different routes of administration have been explored [25]. Live measles vaccines for inhalation, already tested in thousands of children, usually show higher seroconversion rates than the LAV administered *via* a percutaneous injection [26–29]. However, the preparation of the aerosol vaccines requires advanced technologies to ensure their efficacy. Live measles vaccines for oral administration using enteric-coated tablets have been tested in laboratory animals with variable degrees of success putting this approach in arrears [30,31].

Despite the fact that ample experience has been obtained with the current LAV *via* the subcutaneous route, the same preparation but administrated *via* an alternative route will be considered a new vaccine according to current regulations [32]. Thus, as for new generation vaccine formulations, LAV administered *via* an alternative route would have to go through a complete process of registration and licensing.

Box 1. Advantages and disadvantages of live attenuated vaccines.

Advantages	Ref.
Protective	
Inexpensive	
Balance immune response	
Safe in immunocompetent individuals	[69]
More than 30 years of experience	[12]
Effective in measles control campaigns	[12,65]
Combination vaccine with rubella/mumps	
Disadvantages	
Less effective at young age	[70]
Interference with maternal antibody	[71]
Dependent on cold chain	[72]
Potential risk in immunocompromised individuals	[64]
Contraindication during pregnancy	[73]
Needles required	
Molecular basis of attenuation is not known	
Revertants not excluded	
Possible contaminations introduced during production	[74,75]
Three components (vaccine, diluent and syringe)	
Subclinical measles	[76,77]
Second dose required for effective control	[78]

Animal models used in evaluation of experimental vaccines

Over the past decades, several animal models have been used for studying the pathogenesis of measles as well as the evaluation of new vaccine candidates and vaccination strategies. Different rodents including mice [33], rats [34], ferrets [35] and hamsters [36] have been used to study aspects of experimental MV-induced encephalitis (EMVIE) as a model for neurologic disease and to study MV antigen-induced immune responses using EMVIE as a read-out for protection. These animal species are not susceptible to infection with wild type MV. However, several MV strains have been adapted for use in rodents, although virus replication is in general only detectable after intracerebral inoculation of very young animals. CBA/N mice, grafted with human PBL, were used to study MV vaccine-induced protection in transfer experiments [37]. It needs no explaining that these animals do not develop measles-like disease.

The most successful rodent model for measles research appeared to be the cotton rat (*Sigmodon hispidus*) model [38–42]. Cotton rats can be infected intranasally with LAV and nonculture adapted wild type MV isolates. The interference of pre-existing virus neutralizing (VN) antibody with vaccination in cotton rats was addressed by transferring MV-specific antibodies of human- or cotton rat-origin and by vaccination of the offspring from seropositive dams [41,43,44].

From the earliest days of measles vaccine research, primates have been used because of their high susceptibility to MV [45]. Nonhuman primates including marmosets (*Saguinus mystax*), cynomolgus- and rhesus macaques (*Macaca fascicularis* and *Macaca mulatta*, respectively) and baboons (*Papio hamadryas* and *Papio hybridus*), proved to be most relevant

for measles research [46,47]. Macaques have been shown to be highly susceptible to MV infection as illustrated by natural outbreaks and the fact that intratracheal inoculation with 1 $TCID_{50}$ is sufficient to cause MV viremia [48]. It has also been shown that the pathogenesis of MV infection and development of specific immunity in macaques is largely similar to that in humans [48–50]. Upon intratracheal infection with wild type MV, infectious MV can be quantitatively demonstrated in peripheral blood mononuclear cells (PBMC), lung lavage cells (LLC) and PEC, showing kinetics of viral loads that resemble MV viremia in humans. The macaque models have allowed research on vaccine efficacy, in the presence and absence of passively acquired VN antibody [51–53]; vaccine safety [54], including comparison of the virulence of different virus strains [48,55]. Today, techniques and reagents to study immunological mechanisms in nonhuman primates such as T-cell proliferation assays, methods to detect specific antibody and reagents to measure cytokine production and cytokine producing cells, are to a large extent available.

Conventional mouse- and rat-strains have been used for studying the antigenicity of MV-derived antigens, candidate vaccines and the type of immune response induced by these antigens. However, these studies are complicated by the fact that the type of immune reaction (Th1/Th2-like responses) varies among inbred laboratory animals. Transgenic and knockout mice have been used to study different aspects of MV-specific immune responses. Transgenic mice expressing human major histocompatibility complex (MHC) and CD8 molecules mounted cytotoxic T-lymphocyte (CTL) with similar specificities compared with humans with natural MV infection [56].

Although the pathogenesis of other morbillivirus infections in several animal species is often quite similar to that of MV infection in humans, models using other animal morbilliviruses, like canine distemper virus (CDV) in ferrets and dogs, are not selected for this review.

New generation vaccines

In the mid1980s the scientific community started working on the development of new generation vaccines. Vaccine effectiveness in children aged 4–5 months or younger and one-dose immunization were part of the new recommendations for MV vaccine development. The development of more efficacious vaccines and vaccination strategies for human and animal virus infections is subject of considerable effort [57]. Here, several approaches to develop a new generation of measles vaccines are addressed. A major theme was related to studies aiming at the induction of both VN antibodies and human lymphocyte antigen (HLA) class I-restricted CTL responses. The latter are considered to play a major role in the clearance of MV [58] – their role in the elimination of MV infected cells during infection is considered essential. To induce CTL or activate memory CTL, MV antigens have to enter the endogenous antigen processing and presentation pathway in an antigen-presenting cell (APC), which generally requires *de novo* protein synthesis [59]. Certain nonreplicating vaccine formulations however, may allow exogenous protein to enter this pathway. Developments in organic chemistry, biochemistry and molecular biology in the past decades have boosted efforts to formulate new generations of vaccines, which indeed allow the efficient induction of both VN antibodies and HLA class I-restricted CTL responses.

The new generation of candidate measles vaccines include: inactivated virus, live viral vectors, live bacterial vectors, subunit vaccines, synthetic vaccines and nucleic acid vaccines. TABLE 1 provides an enumeration of experimental measles vaccines showing the route of administration, the model and the parameters that were addressed. In addition, it is indicated when the vaccine candidate was tested in the presence of pre-existing MV-neutralizing antibodies. Given the diversity of the experimental set-up for instance in terms of immunization dose, number of immunizations, time interval between different immunizations, time interval between immunization and challenge and the kind of challenge infection, it is difficult to venture expressing which vaccine candidate would be the best to take part in future vaccination strategies against measles.

Synthetic vaccines have been shown to be efficient activators of CTL and to induce protective immune responses. However, such vaccines will most likely be unsuitable for vaccination of large populations because they would have to be 'tailor-made'. Epitope vaccines are designed for the individual on the basis of MHC restriction, which might be a stumbling block. Peptides are weak immunogens that will require further immunopotentiation if they are to be effective *in vivo*. The use of adjuvants may also reduce the amount of purified antigen required for successful immunization, thus making vaccine production more economical and practically feasible. At present the only adjuvants registered for human use are still aluminum hydroxide and aluminum phosphate. Synthetic vaccines would of course have several advantages, such as the option that their effectiveness would not necessarily be hampered by pre-existing immunity against measles, the option to orchestrate the type of immune responses (immunomodulation) and good possibilities to be produced under GMP conditions [60,61]. Furthermore, they are relatively stable and cheap and sequence variations can easily be implemented whenever required. Although VN antibodies induced by different MV strains are known to be cross-reactive, the reductionistic approach of a peptide-based vaccine may lead to mismatch between the vaccine and the wild type MV.

For measles, the only subunit and inactivated candidate vaccines that have been extensively studied are the Quil A-based preparations. MV-ISCOMS based on semipurified Quil A have been shown to induce both strong MV-specific VN antibody, which are long lasting and CMI responses both in the absence and presence of pre-existing VN antibody. These vaccine candidates have been tested in different preclinical models and it would now be interesting to test them for their ability to induce protective immune responses in early life. In addition, because of the history of atypical measles associated with inactivated vaccines it will be necessary to test these Quil A-based preparations in the macaque model for atypical measles.

Another novel vaccination approach which has been studied quite extensively for measles is nucleic acid vaccination (also

Table 1. Enumeration of experimental measles vaccines.

Category	Type	MV antigen	Admin.	Model	MV Ab transfer	Readout	Ref.
Synthetic	Lipopeptide	F CTL epitope	-	*In vitro*	-	CTL	[79]
	Peptide + CTB, IFA	N, F CTL epitope	in., ip.	Mouse	-	CTL, protection	[80]
	Peptide + CTB	F T- B-cell epitope	in.	Mouse	-	IgG, proliferation, protection	[81]
	Peptide + Freund	H T- B-cell epitope	ip.	Mouse	Yes	VN, Ig	[82]
Subunit	ISCOM	F, H	-	*In vitro*	-	CTL	[83]
		F, H	im.	Mouse	-	IgG subclasseses, IgM, VN	[83]
		F, H	im.	Macaque	-	VN	[66]
		F, H	im.	Cotton rat	-	VN, protection	[41]
		F, H	im.	Macaque	Yes	IgG, IgM, VN, CTL, proliferation, protection	[51]
		F, H	im.	Mac., mouse, rab.	-	DTH, VN, HI, IgG, T-cell clones	[84]
	Liposome	H	sc.	Mouse, *in vitro*	-	Proliferation	[85]
DNA	Plasmid	N, F, H	Gene gun	Macaque	-	IFN-γ, IgG, IgM, VN, protection	[86]
		N	Oral, PLGA	Mouse	-	IgG	[87]
		F, H	Gene gun, id.	Macaque	-	CTL, VN, Ig, protection, AMS	[88]
		N, F, H	?	Cotton rat	-	IgG, protection	[89]
		H	Gene gun, im.	Mouse	-	IgG subclasses	[90]
		N	im., in.	Mouse	-	IFN-γ, IL4, CTL, IgG subclasses, IgA	[91]
		H	?	Mouse	Yes	Ig, IFN-γ, IL5, CTL, proliferation	[92]
	(± DOTAP)	N (epitope)	id.	Mouse	-	CTL, protection	[93]
		H	ib., in., oral, ij.	Mouse	-	CTL	[94]
		F, H	Gene gun	Mouse	-	VN, IgG	[95]
		N	im.	Mouse	-	IgG, protection	[96]
		H	im.	Mouse	-	IgG subclasses, CTL, IL5, IFN-γ, Ig	[97]
		H	ip.	Mouse	-	IFN-γ, IL5, CTL, Ig	[98]
Inactivated	Quil A-adjuvated	BPL-inact. MV	-	*In vitro*	-	CTL	[83,88]
		BPL-inact. MV	im.	Mouse	-	IgG subclasses, IgM, VN	[83]
		BPL-inact. MV	im.	Macaque	-	VN	[66]
	Alum-adjuvated	BPL-inact. MV	im.	Cotton rat	-	CTL, IgG, IgM, VN, protection	[41]
		formalin-inact. MV	im.	Macaque	-	IgG, IgA, IgE, VN, CTL, protection, AMS	[9]
Live	Attenuated	MV Chicago-1	sc.	Macaque	-	IgG, IgA, IgE, VN, CTL, protection, AMS	[9]
		MV Schwarz	?	Mouse	Yes	IFN-γ, IL5, CTL, Ig, proliferation	[92]
		MV Schwarz	im.	Macaque	Yes	IgG, IgM, VN, CTL, proliferation, protection	[51]
		MV E-M, MV E-Z	im.	Cotton rat	Yes	VN, protection	[41]
		MV Schwarz	ip.	Mouse	-	IgG subclasses, CTL, IL5, IFN-γ, Ig	[97]
		MV L-16	im., in., oral	Macaque	-	VN, IgG, IgM, CD69, IL4, IFN-γ, protection	[31]
		MV-Moraten	im.	Macaque	-	VN	[99]
		MV-Moraten	sc.	Macaque	-	VN, CTL, Ig, protection	[88]
Recombinant	Vaccinia virus	N, F, H	id.	Mouse	-	VN, protection	[100]
		F, H	id.	Mouse	-	HI, VN, protection	[101]
		F, H	id.	Macaque	-	Ig, VN, CTL, protection	[52]
		F, H	id., im.	Macaque	Yes	IgG, IgM, VN, CTL, proliferation, protection	[51]
		N, P, M, F, H	ip.	Cotton rat	-	IgG, VN, protection	[44]
		N, P, M, F, H	ip.	Rat	-	Ig, DCD8, VN, proliferation, protection	[102]
		N, P, M, F, H	ip.	Rat	-	Ig, DCD8, proliferation, protection	[34]
	NYVAC	H	ip.	Mouse	-	IFN-γ, IL5, CTL, Ig	[98]
	ALVAC	H	?	Mouse	Yes	IFN-γ, IL5, CTL, Ig, proliferation	[92]
		F, H, N, M	im.	Cotton rat	Yes	VN, protection	[41]
		H	ip.	Mouse	-	IgG subclasses, CTL, IL5, IFN-γ, Ig	[97]
		H	ip.	Mouse	-	IFN-γ, IL5, CTL, Ig	[98]
	MVA	F, H	im., in.	Macaque	Yes	IgG, VN, CD69, protection	[53]
		F, H	im., in.	Macaque	Yes	Ig, VN, CTL, protection	[52]
		H	ip.	Mouse, cotton rat	Yes	IgG subclasses, DCD4, protection	[44]
	PIV3	H	in., it.	Macaque	-	VN, protection	[99]
		H	in.	Hamster	-	HI, VN	[103]
	VSV	H	in., ip.	Cotton rat	Yes	IgG, VN, protection	[43]
	Adenovirus	N	oral	Mouse	-	IgG	[87]
		F, H	oral, ip.	Mouse, cotton rat	-	IgG, VN, protection	[104]
		N	ip.	Mouse	-	CTL, IgG, protection	[105]
		N	oral	Mouse	-	CTL, IgG	[106]
	Protein	N	in., ip.	Mouse	-	IgG subclasses, proliferation, protection	[107]
	Streptococcus gordonii	F, H	sc.	Mouse	-	IgG, VN	[108]
	Shigella flexneri	N, F, H	in.	Mouse	-	IFN-γ, IL4, CTL, IgG subclasses, IgA	[91]
	Salmonella typhi	N	ip.	Mouse	-	IFN-γ, IL4, CTL, IgG subclasses, IgA	[91]
		F, N (minigene)	oral	Mouse	-	IgG, proliferation, protection	[109]
		F (minigene)	-	*In vitro*	-	Proliferation, CTL	[110]
	Escherichia coli	F (minigene)	-	*In vitro*	-	Proliferation, CTL	[110]
	BCG	N	in., id.	Macaque	-	CTL, proliferation, IgG, IgM, protection	[111]

ALVAC: Strain of canarypox virus; BCG: Bacille Calmette-Guérin; BPL: β-propiolactone; CD69: T-cell transmembrane activation marker; CTL: Cytotoxic T-cell assay; DCD4: *In vivo* depletion of CD4+ lymphocytes; DCD8: *In vivo* depletion of CD8+ lymphocytes; E-M: Edmonston-Moraten strain; E-Z: Edmonston-Zagreb strain; F: Measles virus fusion protein; H: Measles virus hemagglutinin; ib.: Transepithelial injection reaching the buccal mucosa; ic.: Intracutan; id.: Intradermal; ij.: Intrajejunal; im.: Intramuscular; in.: Intranasal; ip.: Intraperitoneal; ISCOM: Immune stimulating complex; it.: Intratracheal; L-16: Leningrad-16 strain; MVA: Modified vaccinia virus Ankara; N: Measles virus nucleoprotein; NYVAC: Highly attenuated strain of vaccinia virus; PIV3: Parainfluenza virus Type 3; sc.: Subcutaneous; VN: Measles virus-specific virus neutralizing antibody; VSV: Vesicular stomatitis virus.

referred to as DNA vaccination). Plasmid DNA is very stable and the possibility of transdermal delivery, avoiding the use of needles, may improve overall compliance rates. A point of concern with regard to DNA vaccination is the possibility that plasmid DNA integrates into genomic material of the host [62]. Whether or not plasmid DNA integration is a real safety issue remains elusive. The outcomes of different studies listed here are not unambiguous but generally it appears that the DNA measles candidate vaccines can efficiently prime the immune system. Although DNA vaccination against measles does not warrant protection, it may be further potentiated in heterologous prime-boost vaccination regimens as has recently been demonstrated in combination with an edible candidate measles vaccine [63].

A completely different approach to induce a broadly reactive immune response, including VN antibody and CTL responses, is the use of bacterial and viral vectors, each with their own advantages and disadvantages. Of this group, recombinant poxviruses have been studied most extensively and currently the most interesting vector is the replication-deficient poxvirus, modified vaccinia virus Ankara (MVA). Due to its application in hundreds of thousands of people as a smallpox vaccine in the end-phase of the eradication of variola virus and studies in immunocompromised laboratory animals, it has developed an impressive efficacy and safety record.

Finally, we may contemplate the global eradication of MV. Although the complete elimination of MV from whole continents was achieved with the currently used LAV, global eradication might demand alternative vaccination strategies, such as those being effective in the presence of maternal antibody and waning vaccine-induced immunity. Since the major burden of measles is in developing countries, the vaccination strategy must be able to overcome major logistical problems. A strategy that is based on two doses, or prime-boost regimens, will probably not be possible in certain areas. An initial immunization that only primes the immune system, which probably prevents severe disease, will be sufficient to reduce mortality and morbidity but may allow the virus to continue to circulate. Optimally, at a very young age, one vaccine dose should induce long-lasting protective immunity that may not require an additional booster later in childhood. Due to the HIV epidemic, which is ongoing in certain target populations, safety in immunocompromised individuals must be guaranteed, although recent data showed no evidence for increased adverse events during a measles vaccination campaign in millions of African children [64,65]. In this respect inactivated vaccine candidates, such as the Quil A-adjuvanted preparations, which show long-lasting high levels of VN antibody after one dose and the poxvirus vector MVA, which is safe and proved to induce protection in the presence of pre-existing VN antibody hold promise [53,54,66]. Since one-dose human neonatal vaccines have not been described before, future experiments should address the potential of such vaccine candidates in an immature immune system in the absence or presence of variable amounts of maternal-acquired measles antibody. Furthermore, the safety with regard to atypical measles can now be tested in the macaque model [9]. In addition the effectiveness and safety of this candidate measles vaccine should be addressed in LAV-vaccinated individuals and people that have had measles. Vaccination against measles is a neverending story because if measles is eradicated the human population should stay matched for reintroduction of MV *via* bioterroristic acts and other morbilliviruses *via* contacts with infected animals [67,68].

Expert opinion

There is a growing opposition against vaccination due to reports and noises about adverse events associated with vaccination. Furthermore, the public support for vaccination against measles may weaken with the disappearance of cases with severe disease. Upon eradication of MV the necessity for continuing vaccination against measles will be even more unclear. Therefore, important issues are to provide the public with information and to keep the total number of vaccinations restricted. Today, the LAV vaccine is available as part of the combination vaccine MMR but if the vaccine will be exchanged for a non-replicating vaccine, which would result in dismantling the MMR vaccine, vaccine developers will need to work on other vaccine cocktails such as combination with DTP or a recombinant MVA containing multiple foreign genes.

Five-year view

In the next 5 years, no new vaccine against measles will be licensed. There are at this moment some promising candidate vaccines. The focus will be on Quil A-adjuvanted preparations and MVA-MV recombinants, which will be further tested in the preclinical models and subsequently in Phase I/II clinical trials in humans. More effort should be invested into testing these vaccine candidates for their ability to induce protection in early life. After having shown the efficacy of a candidate vaccine in adolescent or adult macaques, in the absence and presence of passively transferred MV neutralizing antibody, the efficacy should also be tested in newborn macaques with true maternal antibody.

Despite global efforts to control measles, a satisfactory level of control has not been reached. Therefore, key players in the fight against measles including the following organizations and/or partnerships: International American Red Cross, The International Federation of Red Cross and Red Crescent Societies, The United Nations (UN) Foundation, Centers for Disease Control and Prevention (CDC), World Health Organization (WHO), United Nations Children's Fund (UNICEF), Pan American Health Organization (PAHO), Global Alliance for Vaccines and Immunization (GAVI), World Health Assembly, World Summit for Children, collectively have appointed strategic milestones for vaccination against measles. Currently, boosting vaccination coverage is attempted through massive 'catch-up', 'keep-up' and 'follow-up' campaigns, of which the effect will be evaluated between 2005–2010, depending on the country/region [112,113]. Application of this strategy has substantially reduced measles transmission in the industrialized world. For this approach the LAV were selected, postponing questions such as: 'do we need new measles vaccines' or 'can the present vaccine be used more efficiently' [114]. The global health partners will determine, on the basis of

regional results (interruption of transmission etc.), whether the goal in vaccination against measles will finally be the achievement of a sustainable reduction of measles mortality, maintaining measles elimination or the global eradication of measles. This decision may be compromised by the fact that the feasibility for global eradication of measles dimishes with time, due to the increasing proportion of the human population that has been vaccinated instead of having experienced natural measles as a child.

Information resources

Useful websites:

- www.measlesinitiative.org
- www.who.int/vaccines-documents/DoxNews/h4meas.htm
- www.who.int/vaccines-diseases/research/nva.shtml
- www.cdc.gov/health/measles.htm
- www.unicef.org/pubsgen/measles-statement
- www.measles.nl

Key issues

- Vaccination against measles can and will never be discontinued.
- In order to eliminate endemic circulation of measles virus (MV), a very high (≥95%) level of vaccination coverage should be achieved.
- The current live attenuated vaccine against measles is safe, cheap and effective but alternative application routes may increase effectivity (compliance, seroconversion rate, cold chain maintenance) and safety (injection safety and waste disposal).
- In the long run, the current live attenuated vaccine against measles should be replaced by a nonreplicating vaccine.
- A vaccine against measles that is effective when administered at a young age in the presence of MV-specific neutralizing antibody would add significantly to the control of MV.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1 Hinman A. Eradication of vaccine-preventable diseases. *Ann. Rev. Public Health* 20, 211–229 (1999).

2 Rauh LW, Schmidt R. Measles immunization with killed virus vaccine. *Am. J. Dis. Child.* 109, 232–237 (1965).

3 Fulginiti VA, Eller JJ, Downte AW, Kempe CH. Altered reactivity to measles virus. Atypical measles in children previously immunized with inactivated virus vaccines. *JAMA* 202, 101–106 (1967).

4 Nossal GJ. Inactivated measles vaccine and the risk of adverse events. *Bull. World Health Organ* 78, 224–225 (2000).

5 Nader PR, Horwitz MS, Rousseau J. Atypical exanthem following exposure to natural measles: eleven cases in children previously inoculated with killed vaccine. *J. Pediatr.* 72, 22–28 (1968).

6 • Brodsky AL. Atypical measles. Severe illness in recipients of killed measles virus vaccine upon exposure to natural infection. *JAMA* 222, 1415–1416 (1972).

7 • Fulginiti VA, Helfer RE. Atypical measles in adolescent siblings 16 years after killed measles virus vaccine. *JAMA* 244, 804–806 (1980).

8 Merz DC, Scheid A, Choppin PW. Importance of antibodies to the fusion glycoprotein of paramyxoviruses in the prevention of spread of infection. *J. Exp. Med.* 151, 275–288 (1980).

9 •• Polack FP, Auwaerter PG, Lee SH *et al* Production of atypical measles in rhesus macaques: evidence for disease mediated by immune complex formation and eosinophils in the presence of fusion-inhibiting antibody. *Nat. Med.* 5, 629–634 (1999).

10 Enders JF, Katz SL, Milovanovic MV, Holloway A. Studies on an attenuated measles-virus vaccine I. Development and preparation of the vaccine: technics for assay of effects of vaccination. *N. Engl. J. Med.* 263, 153–159 (1960).

11 McNair TF, Bonanno DE. Reactions to live-measles-virus vaccine in children previously inoculated with killed-virus vaccine. *N. Engl. J. Med.* 5, 248–251 (1967).

12 Clements CJ, Cutts FT. The epidemiology of measles: thirty years of vaccination. *Curr. Top. Microbiol. Immunol.* 191, 13–33 (1995).

13 • Aaby P. Assumptions and contradictions in measles and measles immunization research: is measles good for something? *Soc. Sci. Med.* 41, 673–686 (1995).

14 Shann F. Immunization-dramatic new evidence. *P. N. G. Med. J.* 43, 24–29 (2000).

15 • Hartter HK, Oyedele OI, Dietz K *et al.* Placental transfer and decay of maternally acquired antimeasles antibodies in Nigerian children. *Pediatr. Infect. Dis. J.* 19, 635–641 (2000).

16 Holt EA, Moulton LH, Siberry GK, Halsey NA. Differential mortality by measles vaccine titer and sex. *J. Infect. Dis.* 168, 1087–1096 (1993).

17 Berry S, Hernandez H, Kanashiro R *et al.* Comparison of high titer Edmonston-Zagreb, Biken-CAM and Schwarz measles vaccines in Peruvian infants. *Pediatr. Infect. Dis. J* 11, 822–827 (1992).

18 Garenne M, Leroy O, Beau JP, Sene I. Child mortality after high-titre measles vaccines:prospective study in Senegal. *Lancet* 338, 903–907 (1991).

19 Aaby P, Samb B, Simondon F *et al.* Sex-specific differences in mortality after high-titre measles immunization in rural Senegal. *Bull. World Health Organ* 72, 761–770 (1994).

20 Paunio M, Peltola H, Valle M *et al.* Explosive school-based measles outbreak: intense exposure may have resulted in high risk, even among revaccinees. *Am. J. Epidemiol.* 148, 1103–1110 (1998).

21 • Belyakov IM, Moss B, Strober W, Berzofsky JA. Mucosal vaccination overcomes the barrier to recombinant vaccinia immunization caused by preexisting poxvirus immunity. *Proc. Natl Acad. Sci. USA* 96, 4512–4517 (1999).

22 Belyakov IM, Wyatt LS, Ahlers JD *et al.* Induction of a mucosal cytotoxic T-lymphocyte response by intrarectal immunization with a replication-deficient recombinant vaccinia virus expressing human immunodeficiency virus 89.6 envelope protein. *J. Virol.* 72, 8264–8272 (1998).

23 Okuno Y, Ueda S, Hosai H *et al.* Studies on the combined use of killed and live measles vaccines. II. Advantages of the inhalation method. *Biken. J.* 8, 81–85 (1965).

24 Ueda S, Hosai H, Minekawa Y, Okuno Y. Studies on the combined use of killed and live measles vaccines. 3. Conditions for the 'take' of live vaccine. *Biken. J.* 9, 97–101 (1966).

25 Cutts FT, Clements CJ, Bennett JV. Alternative routes of measles immunization: a review. *Biologicals* 25, 323–338 (1997).

26 Sabin AB, Flores Arechiga A, Fernandez de Castro J *et al*. Successful immunization of infants with and without maternal antibody by aerosolized measles vaccine. II. Vaccine comparisons and evidence for multiple antibody response. *JAMA* 251, 2363–2371 (1984).

27 •• Dilraj A, Cutts FT, Fernandez de Castro J *et al*. Response to different measles vaccine strains given by aerosol and sc. routes to schoolchildren: a randomised trial. *Lancet* 355, 798–803 (2000).

28 LiCalsi C, Christensen T, Bennett JV, Phillips E, Witham C. Dry powder inhalation as a potential delivery method for vaccines. *Vaccine* 17, 1796–1803 (1999).

29 • LiCalsi C, Maniaci MJ, Christensen T *et al*. A powder formulation of measles vaccine for aerosol delivery. *Vaccine* 19, 2629–2636 (2001).

30 Nechaeva EA, Varaksin N, Ryabicheva T *et al*. Approaches to development of microencapsulated form of the live measles vaccine. *Ann. NY Acad. Sci*. 944, 180–186 (2001).

31 Stittelaar KJ, De Swart RL, Vos HW *et al*. Enteric application of a live attenuated measles vaccine does not induce protective immunity in a macaque model. *Vaccine* in Press (2002).

32 Falk LA, Ball LK. Current status and future trends in vaccine regulation - USA. *Vaccine* 19, 1567–1572 (2001).

33 Niewiesk S, Brinckmann U, Bankamp B *et al*. Susceptibility to measles virus-induced encephalitis in mice correlates with impaired antigen presentation to cytotoxic T-lymphocytes. *J. Virol.* 67, 75–81 (1993).

34 Brinckmann UG, Bankamp B, Reich A, Ter Meulen V, Liebert UG. Efficacy of individual measles virus structural proteins in the protection of rats from measles encephalitis. *J. Gen. Virol.* 72, 2491–2500 (1991).

35 Thormar H, Mehta PD, Barshatzky MR, Brown HR. Measles virus encephalitis in ferrets as a model for subacute sclerosing panencephalitis. *Lab. Anim. Sci.* 35, 229–232 (1985).

36 Johnson KP. Experimental subacute sclerosing panencephalitis (SSPE) in the hamster. *Adv. Exp. Med. Biol.* 134, 303–309 (1981).

37 Huppes W, Smit V. Efficient replication of human immunodeficiency virus Type 1 and measles virus in a human-to-mouse graft *versus* host disease model permits immunization research. *J. Gen. Virol.* 76, 2707–2715 (1995).

38 Wyde PR, Ambrose MW, Voss TG, Meyer HL, Gilbert BE. Measles virus replication in lungs of hispid cotton rats after intranasal inoculation. *Proc. Soc. Exp. Biol. Med.* 201, 80–87 (1992).

39 Wyde PR, Moore-Poveda D, De Clercq E *et al*. Use of cotton rats to evaluate the efficacy of antivirals in treatment of measles virus infections. *Antimicrob. Agents Chemother.* 44, 1146–1152 (2001).

40 Wyde PR, Moore-Poveda DK, Daley NJ, Oshitani H. Replication of clinical measles virus strains in hispid cotton rats. *Proc. Soc. Exp. Biol. Med.* 221, 53–62 (1999).

41 • Wyde PR, Stittelaar KJ, Osterhaus A, Guzman E, Gilbert BE. Use of cotton rats for preclinical evaluation of measles vaccines. *Vaccine* 19, 42–53 (2000).

42 Niewiesk S. Studying experimental measles virus vaccines in the presence of maternal antibodies in the cotton rat model (Sigmodon hispidus). *Vaccine* 19, 2250–2253 (2001).

43 Schlereth B, Rose JK, Buonocore L, TerMeulen V, Niewiesk S. Successful vaccine-induced seroconversion by single-dose immunization in the presence of measles virus-specific maternal antibodies. *J. Virol.* 74, 4652–4657 (2000).

44 Weidinger G, Ohlmann M, Schlereth B, Sutter G, Niewiesk S. Vaccination with recombinant modified vaccinia virus Ankara protects against measles virus infection in the mouse and cotton rat model. *Vaccine* 19, 2764–2768 (2001).

45 Goldberger J and Anderson JF. An experimental demonstration of the presence of the virus of measles in the mixed buccal and nasal secretions. *J. Am. Med. Assoc.* 57, 476–478 (1911).

46 Van Binnendijk RS, van der Heijden RWJ, Osterhaus ADME. Monkeys in measles research, In: Ter Meulen V, Billeter MA, editors. Measles virus. Springer-Verlag, Berlin, Germany, 135–148 (1995).

47 Kobune F, Takahashi H, Terao K *et al*. Nonhuman primate models of measles. *Lab. Anim. Sci*. 46, 315–320 (1996).

48 • Van Binnendijk RS, van der Heijden RWJ, Van Amerongen G, UytdeHaag FGCM, Osterhaus ADME. Viral replication and development of specific immunity in macaques after infection with different measles virus strains. *J. Infect. Dis.* 170, 443–448 (1994).

49 • McChesney MB, Miller CJ, Rota PA *et al*. Experimental measles I. Pathogenesis in the normal and the immunized host. *Virology* 233, 74–84 (1997).

50 Zhu Y, Heath J, Collins J *et al*. Experimental measles II. Infection and immunity in the rhesus macaque. *Virology* 233, 85–92 (1997).

51 • Van Binnendijk RS, Poelen MCM, Van Amerongen G, De Vries P, Osterhaus ADME. Protective immunity in macaques vaccinated with live attenuated, recombinant and subunit measles vaccines in the presence of passively acquired antibodies. *J. Infect. Dis.* 175, 524–534 (1997).

52 • Zhu Y-D, Rota P, Wyatt LS *et al*. Evaluation of recombinant vaccinia virus - measles vaccines in infant rhesus macaques with preexisting measles antibody. *Virology* 276, 202–213 (2000).

53 • Stittelaar KJ, Wyatt LS, De Swart RL *et al*. Protective immunity in macaques vaccinated with a Modified Vaccinia virus Ankara-based measles vaccine in the presence of passively acquired antibodies. *J. Virol*. 74, 4236–4243 (2000).

54 • Stittelaar KJ, Kuiken T, De Swart RL *et al*. Safety of modified vaccinia virus Ankara (MVA) in immune-suppressed macaques. *Vaccine* 19, 3700–3709 (2001).

55 Auwaerter PG, Rota PA, Elkins WR *et al*. Measles virus infection in Rhesus Macaques: altered immune responses and comparison of the virulence of six different virus strains. *J. Infect. Dis.* 180, 950–958 (1999).

56 Tishon A, LaFace DM, Lewicki H *et al*. Transgenic mice expressing human HLA and CD8 molecules generate HLA-restricted measles virus cytotoxic T-lymphocytes of the same specificity as humans with natural measles virus infection. *Virology* 275, 286–293 (2000).

57 Nossal GJ. The Global Alliance for Vaccines and Immunization-a millennial challenge. *Nat. Immunol.* 1, 5–8 (2000).

58 Jaye A, Magnusen AF, Sadiq AD, Corrah T, Whittle HC. *Ex vivo* analysis of cytotoxic T-lymphocytes to measles antigens during infection and after vaccination in Gambian children. *J. Clin. Invest.* 102, 1969–1977 (1998).

59 York IA and Rock KL. Antigen processing and presentation by the class I major histocompatibility complex. *Ann. Rev. Immunol* 14, 369–396 (1996).

60 El Kasmi KC, Muller CP. New strategies for closing the gap of measles susceptibility in infants: towards vaccines compatible with current vaccination schedules. *Vaccine* 19, 2238–2244 (2001).

61 El Kasmi KC, Theisen D, Brons NH *et al.* A hemagglutinin-derived peptide-vaccine ignored by virus-neutralizing passive antibodies, protects against murine measles encephalitis. *Vaccine* 17, 2436–2445 (1999).

62 Siegrist CA. Potential advantages and risks of nucleic acid vaccines for infant immunization. *Vaccine* 15, 798–800 (1997).

63 Webster DE, Cooney ML, Huang Z *et al.* Successful boosting of a DNA measles immunization with an oral plant-derived measles virus vaccine. *J. Virol.* 76, 7910–7912 (2002).

64 Goon P, Cohen B, Jin L,Watkins R,Tudor-Williams G. MMR vaccine in HIV-infected children - potential hazards? *Vaccine* 19, 3816–3819 (2001).

65 Biellik R, Madema S, Taole A *et al.* First 5 years of measles elimination in southern Africa: 1996–2000. *Lancet* 359, 1564–1568 (2002).

66 • Stittelaar KJ, Vos HW, Van Amerongen G *et al.* Longevity of neutralizing antibody levels in macaques vaccinated with Quil A -adjuvated measles vaccine candidates. *Vaccine* in press (2002).

67 Stittelaar KJ and Osterhaus ADME. MVA: a cuckoo in the vaccine nest? *Vaccine* 19, v–vi (2001).

68 • Osterhaus ADME. Catastrophes after crossing species barriers. *Philos. Trans. R. Soc. Lond. B* 356, 791–793 (2001).

69 Virtanen M, Peltola H, Paunio M, Heinonen OP. Day-to-day reactogenicity and the healthy vaccinee effect of measles-mumps-rubella vaccination. *Pediatrics* 106, E62 (2000).

70 Halsey NA, Boulos R, Mode R *et al.* Response to measles vaccine in Haitian infants 6 to 12 months old. *N. Engl. J. Med.* 313, 544–549 (1985).

71 Ko B, Roberts D, Begg N *et al.* Neutralising antibody responses to two doses of measles vaccine at 5 and 13 months of age in the United Kingdom. *Commun. Dis. Public Health* 2, 203–206 (1999).

72 Saraswathy TS, Sinniah M, Lee WS, Lee PC. The value of potency testing of poliomyelitis and measles vaccines as an integral part of cold chain surveillance. *Southeast Asian J. Trop. Med. Public Health* 24, 265–268 (1993).

73 Levine MM. Live-virus vaccines in pregnancy. Risks and recommendations. *Lancet* 2, 34–38 (1974).

74 Rota JS, Wang Z-D, Rota PA, Bellini WJ. Comparison of sequences of the H, F and N coding genes of measles virus vaccine strains. *Virus Res.* 31, 317–330 (1994).

75 Tsang SX, Switzer WM, Shanmugam V *et al.* Evidence of avian leukosis virus subgroup E and endogenous avian virus in measles and mumps vaccines derived from chicken cells: investigation of transmission to vaccine recipients. *J. Virol.* 73, 5843–5851 (1999).

76 Pedersen IR, Mordhorst CH, Glikmann G, Magnus von H. Subclinical measles infection in vaccinated seropositive individuals in arctic Greenland. *Vaccine* 7, 345–348 (1989).

77 •• Whittle HC, Aaby P, Samb B *et al.* Effect of subclinical infection on maintaining immunity against measles in vaccinated children in West Africa. *Lancet* 353, 98–102 (1999).

78 Cutts FT, Markowitz LE. Successes and failures in measles control. *J. Infect. Dis.* 170 (Suppl. 1), S32–S41 (1994).

79 • Stittelaar KJ, Hoogerhout P, Ovaa W *et al.* *In vitro* processing and presentation of a lipidated cytotoxic T-cell epitope derived from measles virus fusion protein. *Vaccine* 20, 249–261 (2001).

80 Partidos CD, Vohra P, Steward MW. Priming of measles virus-specific CTL responses after immunization with a CTL epitope linked to a fusogenic peptide. *Virology* 215, 107–110 (1996).

81 Hathaway LJ, Partidos CD, Vohra P, Steward MW. Induction of systemic immune responses to measles virus synthetic peptides administered intranasally. *Vaccine* 13, 1495–1500 (1995).

82 El Kasmi KC, Fillon S, Theisen DM *et al.* Neutralization of measles virus wild type isolates after immunization with a synthetic peptide vaccine which is not recognized by neutralizing passive antibodies. *J. Gen. Virol.* 81, 729–735 (2000).

83 Stittelaar KJ, Boes J, Kersten GFA *et al.* *In vivo* antibody response and *in vitro* CTL activation induced by selected measles vaccine candidates, prepared with purified Quil A components. *Vaccine* 18, 2482–2493 (2000).

84 De Vries P, Van Binnendijk RS, Van De Marel P *et al.* Measles virus fusion protein presented in an immune-stimulating complex (iscom) induces haemolysis-inhibiting and fusion-inhibiting antibodies, virus-specific T-cells and protection in mice. *J. Gen. Virol.* 69, 549–559 (1988).

85 Garnier F, Forquet F, Bertolino P, Gerlier D. Enhancement of *in vivo* and *in vitro* T-cell response against measles virus haemagglutinin after its incorporation into liposomes: effect of the phospholipid composition. *Vaccine* 9, 340–345 (1991).

86 Stittelaar KJ, De Swart RL, Vos HW *et al.* Priming of measles-specific humoral- and cellular immune responses in macaques by DNA vaccination. *Vaccine* 20, 2022–2026 (2002).

87 Fooks AR, Sharpe SA, Shallcross JA, Clegg JC, Cranage MP. Induction of immunity using oral DNA vaccines expressing the measles virus nucleocapsid protein. *Dev. Biol. (Basel)* 104, 65–71 (2000).

88 Polack FP, Lee SH, Permar S *et al.* Succesful DNA immunization against measles:neutralizing antibody against either the hemagglutinin or fusion glycoprotein protects rhesus macaques without evidence of atypical measles. *Nat. Med.* 6, 776–781 (2000).

89 Schlereth B, Germann P-G, TerMeulen V, Niewiesk S. DNA vaccination with both the haemagglutinin and fusion proteins but not the nucleocapsid protein protects against experimental measles virus infection. *J. Gen. Virol.* 81, 1321–1325 (2000).

90 Torres CA, Yang K, Mustafa F, Robinson HL. DNA immunization: effect of secretion of DNA-expressed hemagglutinins on antibody responses. *Vaccine* 18, 805–814 (1999).

91 Fennelly GJ, Khan SA, Abadi MA, Wild TF, Bloom BR. Mucosal DNA vaccine immunization against measles with a highly attenuated Shigella flexneri vector. *J. Immunol.* 162, 1603–1610 (1999).

92 Siegrist CA, Barrios C, Martinez X *et al.*
•• Influence of maternal antibodies on vaccine responses: inhibition of antibody but not T-cell responses allows successful early prime-boost strategies in mice. *Eur. J. Immunol.* 28, 4138–4148 (1998).

93 Hsu SC, Obeid OE, Collins M *et al.* Protective cytotoxic T-lymphocyte responses against paramyxoviruses induced by epitope-based DNA vaccines: involvement of IFN-gamma. *Int. Immunol.* 10, 1441–1447 (1998).

94 Etchart N, Buckland R, Liu MA, Wild F, Kaiserlian D. Class I-restricted CTL induction by mucosal immunization with naked DNA encoding measles virus haemagglutinin. *J. Gen. Virol.* 78, 1577–1580 (1997).

95 Yang K, Mustafa F, Valsamakis A *et al.* Early studies on DNA-based immunizations for measles virus. *Vaccine* 15, 888–891 (1997).

96 Fooks AR, Jeevarajah D, Warnes A, Wilkinson GW, Clegg JC. Immunization of mice with plasmid DNA expressing the measles virus nucleoprotein gene. *Viral Immunol.* 9, 65–71 (1996).

97 Martinez X, Brandt C, Saddallah F *et al.* DNA immunization circumvents deficient induction of T-helper Type 1 and cytotoxic T-lymphocyte responses in neonates and during early life. *Proc. Natl Acad. Sci. USA* 94, 8726–8731 (1997).

98 Kovarik J, Gaillard M, Martinez X *et al.* Induction of adult-like antibody, Th1 and CTL responses to measles hemagglutinin by early life murine immunization with an attenuated vaccinia-derived NYVAC(K1L) viral vector. *Virology* 285, 12–20 (2001).

99 Skiadopoulos MH, Surman SR, Riggs JM,
• Collins PL, Murphy BR. A chimeric human-bovine parainfluenza virus Type 3 expressing measles virus hemagglutinin is attenuated for replication but is still immunogenic in rhesus monkeys. *J. Virol.* 75, 10498–10504 (2001).

100 Wild TF, Bernard A, Spehner D, Drillien R. Construction of vaccinia virus recombinants expressing several measles virus proteins and analysis of their efficacy in vaccination of mice. *J. Gen. Virol.* 73, 359–367 (1992).

101 Drillien R, Spehner D, Kirn A *et al.* Protection of mice from fatal measles encephalitis by vaccination with vaccinia virus recombinants encoding either the hemagglutinin or the fusion protein. *Proc. Natl Acad. Sci. USA* 85, 1252–1256 (1988).

102 Bankamp B, Brinckmann UG, Reich A *et al.* Measles virus nucleocapsid protein protects rats from encephalitis. *J. Virol.* 65, 1695–1700 (1991).

103 Durbin AP, Skiadopoulos MH, McAuliffe
• JM *et al.* Human parainfluenza virus Type 3 (PIV3) expressing the hemagglutinin protein of measles virus provides a potential method for immunization against measles virus and PIV3 in early infancy. *J. Virol.* 74, 6821–6831 (2000).

104 Fooks AR, Jeevarajah D, Lee J *et al.* Oral or parenteral administration of replication-deficient adenoviruses expressing the measles virus haemagglutinin and fusion proteins: protective immune responses in rodents. *J. Gen. Virol.* 79, 1027–1031 (1998).

105 Fooks AR, Schadeck E, Liebert UG *et al.* High-level expression of the measles virus nucleocapsid protein by using a replication-deficient adenovirus vector: induction of an MHC-1-restricted CTL response and protection in a murine model. *Virology* 210, 456–465 (1995).

106 Sharpe S, Fooks A, Lee J *et al.* Single oral immunization with replication deficient recombinant adenovirus elicits long-lived transgene-specific cellular and humoral immune responses. *Virology* 293, 210–216 (2002).

107 Olszewska W, Erume J, Ripley J, Steward MW, Partidos CD. Immune responses and protection induced by mucosal and systemic immunisation with recombinant measles nucleoprotein in a mouse model of measles virus-induced encephalitis. *Arch. Virol.* 146, 293–302 (2001).

108 Maggi T, Oggioni MR, Medaglini D *et al.* Expression of measles virus antigens in Streptococcus gordonii. *New Microbiol.* 23, 119–128 (2000).

109 Spreng S, Gentschev I, Goebel W *et al.* Salmonella vaccines secreting measles virus epitopes induce protective immune responses against measles virus encephalitis. *Microbes. Infect.* 2, 1687–1692 (2000).

110 Verjans GM, Janssen R, UytdeHaag FG, van Doornik CE, Tommassen J. Intracellular processing and presentation of T-cell epitopes, expressed by recombinant Escherichia coli and Salmonella typhimurium, to human T-cells. *Eur. J. Immunol.* 25, 405–410 (1995).

111 Zhu Y D, Fennelly G, Miller C *et al.* Recombinant Bacille Calmette-Guerin expressing the measles virus nucleoprotein protects infant rhesus macaques from measles virus pneumonia. *J. Infect. Dis.* 176, 1445–1453 (1997).

112 WHO. Strategies for reducing global measles mortality. *Wkly Epidemiol. Rec.* 75, 411–416 (2000).

113 WHO. Measles: progress towards global control and regional elimination, 1990–1998. *Wkly Epidemiol. Rec.* 73, 389–394 (1998).

114 Davey S. Measles eradication still a long way off. *Bull. World Health Organ.* 79, 584–585 (2001).

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