

# Chapter 3

## Measles Studies in the Macaque Model

R.L. de Swart

### Contents

Introduction.....	56
Vaccination Studies in Macaques.....	57
Atypical Measles.....	57
Live-Attenuated Vaccines.....	59
New-Generation Vaccines.....	59
Alternative Routes of Administration.....	60
Pathogenesis Studies in Macaques.....	61
Cell Lines and Virus Strains.....	61
Differences Between Macaque Species.....	62
Immunity, Protection, and Immunosuppression.....	63
Pathology.....	64
Infections with MV Expressing EGFP.....	65
Concluding Remarks.....	67
References.....	68

**Abstract** Much of our current understanding of measles has come from experiments in non-human primates. In 1911, Goldberger and Anderson showed that macaques inoculated with filtered secretions from measles patients developed measles, thus demonstrating that the causative agent of this disease was a virus. Since then, different monkey species have been used for experimental measles virus infections. Moreover, infection studies in macaques demonstrated that serial passage of the virus in vivo and in vitro resulted in virus attenuation, providing the basis for all current live-attenuated measles vaccines. This chapter will review the macaque model for measles, with a focus on vaccination and immunopathogenesis studies conducted over the last 15 years. In addition, recent data are highlighted demonstrating that the application of a recombinant measles virus strain expressing enhanced green fluorescent protein dramatically increased the sensitivity of virus

---

R.L. de Swart

Department of Virology, Erasmus MC, University Medical Center, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands, e-mail: r.deswart@erasmusmc.nl

detection, both in living and sacrificed animals, allowing new approaches to old questions on measles vaccination and pathogenesis.

## Introduction

At the beginning of the twentieth century, it was demonstrated that measles virus (MV) could be transmitted from humans to non-human primates. One to 2 weeks after inoculation with blood collected from measles patients, macaques developed fever and skin rash (Anderson and Goldberger 1911). Disease transmission could also be achieved using filtered respiratory secretions, demonstrating that the causative agent of measles was a virus (Goldberger and Anderson 1911). A decade later, Blake and Trask followed up on these studies by experimentally infecting macaques with nasopharyngeal washings of measles patients, thereby successfully inducing measles in 16 animals (Blake and Trask 1921a). The virus could be passaged from monkey to monkey by intratracheal inoculation of tissue homogenates or by intravenous injection of citrated blood (Blake and Trask 1921a). The authors carefully documented incubation time, fever, leukopenia and pathology of enanthem and exanthem (Blake and Trask 1921b). In addition, they demonstrated that experimental MV infection resulted in complete immunity against reinfection (Blake and Trask 1921c). In the 1950s, it was demonstrated that experimental infections with MV isolated in cell culture also caused measles in macaques (Peebles et al. 1957). The authors were able to reisolate the virus and detect MV-specific serum antibody responses, thus bringing the model close to its current state.

Although experimental MV infection of macaques had been demonstrated successfully, a number of other studies yielded negative results. Retrospectively, it can be concluded that in many of these cases animals were immune to measles due to prior exposure to the virus. Peebles et al. detected MV-specific serum antibodies in 22 out of 24 macaques tested from US laboratories, whereas animals screened immediately after capture all proved to be serum antibody-negative (Peebles et al. 1957). This illustrates that measles is a disease of humans and normally does not affect non-human primates unless they are brought in close contact with humans. Monkeys used as experimental animals were at that time in most cases wild-caught animals. Before and during transport they were housed under crowded conditions, and contacts with MV-infected humans could result in virus transmission (Meyer et al. 1962). On several occasions, measles outbreaks occurred at animal facilities after arrival of new animals, in some cases associated with significant morbidity (Potkay et al. 1966; Hime and Keymer 1975; Scott and Keymer 1975; Remfry 1976; MacArthur et al. 1979; Welshman 1989). Thus monkeys intended to be used for experimental MV infections needed to be transported using special precautions (Tauraso 1973). At present, all macaques used for experimental measles virus infections are purpose-bred animals, but prescreening for the absence of MV-specific antibodies remains imperative.

The high susceptibility of non-human primates to MV infection provided the first animal model for measles. The two species used most often are the rhesus

macaque (*Macaca mulatta*) and the cynomolgus macaque (*Macaca fascicularis*) (El Mubarak et al. 2007). As a result of the close similarity of clinical, virological, immunological and pathological parameters to those associated with measles in humans, the model continues to be used almost a century later. Although several rodent species and other small laboratory animals have been inoculated with MV, most of these do not or poorly replicate the virus (Stittelaar et al. 2002a; Pütz et al. 2003). Studies in cotton rats or transgenic animal species do reproduce certain aspects of measles, but none of these show the same level of similarity with the pathogenesis of measles in humans as the macaque model (see chapters 5 and 6).

New world monkeys proved to be even more susceptible to MV infection than old world monkeys, but developed a disease with a different pathogenesis than that of measles in humans, which was associated with high mortality (Levy and Mirkovic 1971; Albrecht et al. 1980). Due to their high susceptibility, marmosets (*Saguinus mystax*) were later used for encephalogenicity studies, as reviewed elsewhere (Van Binnendijk et al. 1995). The current review will address macaque models of measles, with a focus on studies published after 1995.

## **Vaccination Studies in Macaques**

The first isolation of MV in cell culture (Enders and Peebles 1954) was immediately followed by attempts to develop a vaccine against measles. Two different strategies were pursued in parallel: inactivated vaccines and live-attenuated vaccines. Whereas the first category of vaccines proved to predispose for enhanced disease, the second was highly successful. In the 1990s studies were initiated to develop new-generation vaccines as potential successors of the current live-attenuated vaccines, some of which showed promise in preclinical studies in macaques. However, none of these candidate new measles vaccines have been pushed forward towards licensing for human use. Studies in the 1980s had already demonstrated that administration of the current MV vaccine by aerosol held great promise (Sabin et al. 1982). However, regulatory authorities consider a vaccine and its route of administration as one entity, which means that before the aerosol route for measles vaccination can be implemented licensure will be required, for which preclinical studies were conducted in macaques.

## ***Atypical Measles***

In the 1960s, classical inactivated measles vaccines were manufactured by formalin inactivation of whole MV preparations, which were subsequently precipitated with aluminum salts (referred to as FI-MV). Initially, vaccination with FI-MV was shown to induce MV-specific serum antibody responses (Hilleman et al. 1962;

Norrby et al. 1964; Foege et al. 1965), and the vaccine was used in large-scale clinical trials in humans. However, after a number of years it turned out that vaccination predisposed for enhanced disease upon natural MV infection, which was referred to as atypical measles (Fulginiti et al. 1967) (see also chapter 10). During the same period, vaccination trials with formalin-inactivated respiratory syncytial virus (FI-RSV), another member of the family *Paramyxoviridae*, also proved to predispose for enhanced disease upon natural infection (Fulginiti et al. 1969; Kapikian et al. 1969; Kim et al. 1969). Whereas availability of small animal models for RSV allowed extensive studies on the pathogenesis of this vaccine-mediated enhanced disease, this remained difficult for measles. Because the pathogenesis remained subject to speculation, the risk of inducing atypical measles continued to form a major stumbling block for the development of new generation nonreplicating MV vaccines.

In 1999, Polack and Griffin were successful in reproducing atypical measles in macaques (Polack et al. 1999), thus providing an opportunity to study the pathogenesis of the disease as well as a model to test candidate new MV vaccines for predisposition of similar aberrant responses upon challenge infection. They immunized macaques with the original FI-MV preparation that had been manufactured in the 1960s. When challenged with pathogenic wild-type MV, two out of five rhesus macaques vaccinated with FI-MV developed atypical measles, characterized by a petechial rash and pneumonitis associated with the presence of eosinophils and immune complexes in their lungs. The authors concluded that atypical measles “results from previous priming for a nonprotective type 2 CD4 T cell response rather than from lack of functional antibody against the fusion protein” (Polack et al. 1999).

In follow-up studies, they further characterized immune responses in these animals, demonstrating *in vivo* impairment of interleukin (IL)-12 production and increased production of IL-4 by peripheral blood mononuclear cells (Polack et al. 2002). When characterizing the antibody responses, they found that these were not only transient but also lacked avidity maturation. Challenge infection resulted in anamnestic production of low avidity antibodies, which could explain the immune complex deposition in FI-MV-primed animals (Polack et al. 2003a). Before reproduction of atypical measles in the macaque model, it was speculated that inactivation with formalin had resulted in destruction of critical B cell epitopes on the fusion protein (Norrby et al. 1975). However, this hypothesis could be rejected on the basis of two macaque experiments: animals primed with FI-MV developed atypical measles in the presence of fusion-inhibiting antibodies (Polack et al. 1999), and macaques primed with a DNA vaccine encoding for the haemagglutinin gene that developed H- but not F-specific antibodies did not develop any of the clinical signs associated with atypical measles (Polack et al. 2000). Interestingly, vaccination of macaques with formalin-inactivated respiratory syncytial virus (RSV) or human metapneumovirus (hMPV) preparations also predisposed for hypersensitivity to challenge infection with the respective viruses, suggesting that these phenomena are characteristic for all members of the family *Paramyxoviridae* (De Swart et al. 2002, 2007c).

### ***Live-Attenuated Vaccines***

As mentioned above, the MV strain Edmonston isolated by Enders and colleagues in 1954 induced measles-like clinical signs in macaques (Peebles et al. 1957). Serial passage of this virus in vivo (in chicken embryos) and in vitro (in chicken embryo fibroblast [CEF] cells) resulted in virus attenuation: upon experimental infection of macaques, the virus still induced MV-specific immune responses but virtually no clinical signs (Enders et al. 1960). More than 30 years later, direct comparison of MV isolated in lymphoid cells (strain Bilthoven) with MV passaged in human and monkey kidney cells (strain Edmonston wild type) or live-attenuated MV passaged in CEF cells (strain Schwarz) demonstrated that these strains displayed high, intermediate and low pathogenicity in macaques, respectively (Van Binnendijk et al. 1994). Virus loads in peripheral blood mononuclear cells and in bronchoalveolar lavage cells were several log values lower in animals infected with MV strains of reduced pathogenicity. Whereas levels of MV-specific serum IgM antibodies seemed directly related to the magnitude of virus loads, levels of specific serum IgG and virus neutralizing (VN) antibodies induced by the three virus strains were on the same order of magnitude (Van Binnendijk et al. 1994).

Macaques and other non-human primate species have been used to assess the levels of attenuation of different candidate vaccine virus strains. In most cases, this was done by studying pathological lesions induced by intracerebral MV infection (Buynak et al. 1962; Nii et al. 1964b; Albrecht et al. 1981; Sharova et al. 1984). At present, vaccine manufacturers still use this method to assess appropriate attenuation of new MV vaccine virus seed stocks.

### ***New-Generation Vaccines***

Despite its documented safety and efficacy, live-attenuated MV vaccines also have drawbacks, most importantly their dependence on cold chain maintenance and their ineffectiveness in the presence of maternal antibodies (Stüttelaar et al. 2002a; Pütz et al. 2003). Live-attenuated MV vaccines are ineffective when used before the age of 9 months, resulting in a window of susceptibility in young infants between waning of maternal immunity and acquisition of vaccine-induced immunity. Since the 1980s, new-generation candidate MV vaccines have been developed to address these issues, including subunit vaccines, vectored vaccines and nucleic acid vaccines (see chapter 10). In the macaque model, passive transfer of MV-specific VN antibodies inhibited effectiveness of the live-attenuated MV vaccine, thus providing a model to evaluate the potential of candidate new vaccines in the presence of maternal antibodies (Van Binnendijk et al. 1997). The scientific steering committee of the World Health Organization (WHO) adopted the macaque model for pre-clinical comparison of the different candidates. A strategy was developed in which vaccine candidates were first used to immunize juvenile animals, either in the absence or presence of passively transferred MV-specific antibodies. This would

allow assessment of immunogenicity and longevity of the induced specific immune responses. Approximately 1 year after vaccination, animals were challenged with a pathogenic wild-type MV strain to assess levels of protection. Vaccine candidates that performed well in this evaluation were subsequently tested in infant macaques, to assess their potential effectiveness at an early age in the presence of true maternally derived VN antibodies. This strategy resulted in the identification of a number of promising candidate MV vaccines. However, further preclinical and clinical evaluation of a new MV vaccine will require huge financial investments. Moreover, improved coverage of the live-attenuated MV vaccine in recent years in combination with the implementation of a two-dose strategy has been highly successful in reducing measles mortality. As a result, it remains uncertain if any of the candidate new-generation MV vaccines will ever be licensed for human use.

### *Alternative Routes of Administration*

For regulatory purposes, a vaccine and the device used for administration form one integral entity: the current live-attenuated MV vaccines are licensed for injection only. However, alternative routes of administration have extensively been studied in humans. Whereas intradermal, conjunctival, oral or intranasal administration were not particularly successful, aerosol administration of nebulized MV vaccine proved highly effective (Cutts et al. 1997). The aerosol route closely mimics the natural route of MV infection and may result in both mucosal and systemic immunity (Valdespino-Gomez et al. 2006).

The WHO, in partnership with the American Red Cross and the Centers for Disease Control and Prevention and with funding from the Bill and Melinda Gates Foundation, has initiated a Product Development Group (PDG) for measles aerosol vaccination. The ultimate objective of the PDG is to achieve licensure of a combination of measles vaccine and nebulizer, which is equally safe, effective and cheap as the currently licensed measles vaccines administered by injection, but is easier to administer, less invasive, and could be administered by nonmedical personnel. The PDG evaluated two alternative strategies for measles aerosol vaccination: nebulization of a reconstituted vaccine (Dilraj et al. 2000) or inhalation of a dry powder aerosol (LiCalsi et al. 2001).

Both vaccination strategies were evaluated in immunocompetent and immunocompromised macaques and proved equally safe. However, vaccination with a nebulized aerosol proved more effective than inhalation of a dry powder vaccine (De Swart et al. 2006, 2007a). The animals used in these studies had body weights of 1.8–4.5 kg and consequently had much smaller tidal volumes than those of children. To mimic vaccination using a similar dose to that inhaled by a child in 30 s, the exposure time therefore had to be prolonged (De Swart et al. 2006). Before proceeding to clinical trials, a toxicology study was conducted using larger study groups than those used in the exploratory studies. These studies were also

conducted in macaques, to allow MV vaccine replication in all exposed tissues. This study revealed no adverse events, and measles aerosol vaccination is currently under investigation in clinical trials. The PDG aims to achieve licensure for this vaccination route in 2009.

## **Pathogenesis Studies in Macaques**

Experimental MV infections of macaques have been crucial for our understanding of the pathogenesis of measles. Infections with MV isolated in cell culture demonstrated the importance of passage history of the virus: MV strains exclusively cultured in lymphoid cells retained pathogenicity in macaques, whereas passage in other cell lines often resulted in virus attenuation. However, nonattenuated MV strains were in some cases also associated with subclinical infections, which seemed to be related to species differences between rhesus and cynomolgus macaques rather than to virus differences. A large number of studies have focused on the development of MV-specific immune responses, and the role of different arms of the immune system in protection from measles. Measles is usually not recognized before onset of rash, approximately 2 weeks after MV infection, making studies on the early events following virus transmission in humans difficult to perform. Pathological studies of experimentally infected macaques have provided important insights in the tissue distribution and cell tropism of the virus. Recently, infections with recombinant MV expressing enhanced green fluorescent protein (EGFP) resulted in improved sensitivity of virus detection, and provided new insights in the role of different target cells. This new approach to an old animal model provides alternative possibilities to further unravel the pathogenesis of measles and measles-associated immunosuppression.

### ***Cell Lines and Virus Strains***

The introduction of Epstein-Barr virus (EBV)-transformed marmoset B cell line B95a as a substrate for isolation of MV (Kobune et al. 1990) made it possible to isolate non-culture-adapted wild-type MV strains. Whereas isolation of wild-type MV from patient samples in Vero cells usually takes at least 2 weeks (and in many cases requires serial blind passage), virus isolation in B95a cells can be as rapid as 2–4 days (WHO 2007). Experimental infections with the Edmonston wild-type strain did not always result in detectable clinical signs (Enders et al. 1960; Hicks et al. 1977; Van Binnendijk et al. 1994), whereas non-cell culture-passaged virus usually induced more fulminant infections (Yamanouchi et al. 1973; Sakaguchi et al. 1986; McChesney et al. 1989). Kobune and colleagues demonstrated that MV isolated in B95a cells retained its pathogenicity in macaques, resulting in development of viremia, lymphopenia and rash (Kobune et al. 1996).

Van Binnendijk et al. isolated wild-type MV in human EBV-transformed B-lymphoblastic cell lines (BLCL) and demonstrated that this virus also retained pathogenicity (Van Binnendijk et al. 1994). These authors applied the infectious center assay to the model, thus quantifying the frequency of MV-infected cells during viremia. Intratracheal inoculation of macaques with a single infectious unit of wild-type MV strain Bilthoven could spark infection associated with similar viral load kinetics as infection with  $10^4$  infectious units, with the only difference that the peak of viremia shifted slightly backwards in time with decreasing infectious dosage (Van Binnendijk et al. 1994).

McChesney and colleagues developed a measles model in macaques using a MV strain isolated during an outbreak of measles in a primate facility (McChesney et al. 1997). The virus was isolated in Raji cells, a human BLCL isolated from a patient with Burkitt's lymphoma, a disease resulting from in vivo transformation of B lymphocytes by EBV infection. Subsequently, the virus was passaged in vivo in macaques, after which a challenge stock was produced in macaque mononuclear cells (McChesney et al. 1997). This virus was also fully pathogenic in macaques, as demonstrated by induction of clinical and pathological changes typical for measles (McChesney et al. 1997) and MV-specific immune responses (Zhu et al. 1997). Auwaerter and colleagues compared the pathogenicity in macaques of six different MV strains, demonstrating that the nonadapted Bilthoven strain was fully pathogenic while the other cell culture-adapted strains were not (Auwaerter et al. 1999).

Interestingly, a recent study addressing genetic changes that affect the virulence of MV in macaques demonstrated that wild-type MV isolated in Vero cells expressing CD150 also retained pathogenicity in macaques and induced skin rash (Bankamp et al. 2008). In contrast, the same MV isolated and passaged in normal Vero cells or in CEF cells did not induce rash. This suggests that expression of CD150 on the cells used for virus isolation is crucial, and that the cells are not required to be of lymphoid origin. Cell culture adaptation does not necessarily result in adaptation to the use of CD46 as a receptor, as the adapted MV isolates in the above-mentioned study did not infect Chinese Hamster Ovary cells expressing CD46 (Bankamp et al. 2008).

### *Differences Between Macaque Species*

Although experimental MV infections have been conducted both in rhesus and cynomolgus macaques, clinical signs such as rash and conjunctivitis were especially reported in rhesus macaques (Blake and Trask 1921b; McChesney et al. 1997; Auwaerter et al. 1999). Although skin rash has also been reported in cynomolgus macaques (Kobune et al. 1996), this symptom seemed to be less prominent in this species. The first direct comparison of MV infection in these two macaque species by experimental infection with two different non-culture-adapted wild-type MV strains indeed seemed to confirm this assumption: although animals of both species displayed similar virus replication curves in peripheral blood and broncho-alveolar



lavage cells as well as similar MV-specific immune responses, the appearance of skin rash was more prominent in rhesus macaques (El Mubarak et al. 2007).

Infection studies with a recombinant MV strain expressing EGFP conducted in parallel in rhesus and cynomolgus macaques showed that in both animal species MV-infected cells were detected in many different tissues, including the skin (De Swart et al. 2007b). Also with respect to other virological and immunological parameters, including infection of specific lymphocyte subsets, MV infection followed a virtually identical course in both animal species, suggesting that both macaque species can be used for measles pathogenesis studies.

### ***Immunity, Protection, and Immunosuppression***

Both in humans and macaques recovery from measles is accompanied by lifelong immunity (Blake and Trask 1921c). MV infection induces strong specific humoral and cellular immune responses, and many different assays have been developed to characterize these *ex vivo* in macaques. The most important serological parameter is the detection of VN antibodies in serum, of which levels above 0.1–0.2 IU/ml have been identified as a correlate of protection from measles in infants (Chen et al. 1990; Samb et al. 1995). In macaques, similar levels of passively transferred VN antibodies were shown to interfere with MV vaccination (Van Binnendijk et al. 1997). Measurement of specific cell-mediated immunity (CMI) is less well standardized, but several techniques including assessment of lymphoproliferation (Van Binnendijk et al. 1997; Pan et al. 2005), cytotoxicity (Van Binnendijk et al. 1997; Zhu et al. 1997, 2000), cytokine production (Auwaerter et al. 1999; Polack et al. 2002, 2003b; Stittelaar et al. 2002b; Premenko-Lanier et al. 2003; Pan et al. 2005; Pasetti et al. 2007) or flow cytometry-based stimulation assays (Stittelaar et al. 2000; Pahar et al. 2005; De Swart et al. 2006) have been employed as correlates of CMI.

VN antibodies can confer complete protection from measles, as also illustrated by the fact that infants born of a mother with adequate MV-specific antibody titers are protected from measles during their first months of life. In contrast, clearance of an established MV infection is largely dependent on CMI. Agammaglobulinemic patients recover normally from measles, while individuals with impaired CMI may succumb to MV infection (Burnet 1968). The role of specific lymphocyte populations in clearance of MV was addressed in the macaque model by depleting single or multiple populations using monoclonal antibodies to CD20 and/or CD8. Macaques depleted of all T lymphocytes or of CD8<sup>+</sup> T lymphocytes only at the moment of MV infection exhibited a more extensive rash, increased viral loads and delayed viral clearance (Hicks et al. 1977; Permar et al. 2003). In contrast, depletion of CD20<sup>+</sup> B lymphocytes did not result in alterations of clinical signs or kinetics of MV clearance (Permar et al. 2004). These studies demonstrate that CMI (and more specifically CD8<sup>+</sup> T lymphocyte responses), but not humoral immunity, plays a crucial role in MV clearance. These data also highlight a major strength of the

macaque model, correlating specific immune responses to protection from or clearance of experimental MV infection.

Measles is not only associated with the induction of strong MV-specific immune responses but also with a transient immunosuppression, leading to enhanced susceptibility to opportunistic infections (see also chapter 12). Many putative mechanisms have been suggested, but in vivo assessment of the relative importance of these hypotheses has remained difficult. MV infects lymphocytes in vitro and in vivo (Yamanouchi et al. 1973; McChesney et al. 1989), but may also alter functionality of noninfected lymphocytes (Okada et al. 2000) or antigen-presenting cells (Schneider-Schaulies et al. 2003). Furthermore, MV infection interferes with production of specific cytokines, thus changing the host response to invading pathogens (Griffin et al. 1994; Moss et al. 2002). Paradoxically, MV inhibits proliferation of lymphocytes in vitro (Hirsch et al. 1984), but recovery from measles is associated with extensive lymphocyte expansion in vivo (Mongkolsapaya et al. 1999).

Due to difficulties in standardization of CMI assays, it has been difficult to evaluate mechanisms of immunosuppression in macaques. Lymphopenia and reduced responses to mitogen stimulation have been described extensively (McChesney et al. 1989; Kobune et al. 1996; Zhu et al. 1997; Auwaerter et al. 1999). MV infection of macaques specifically alters the production of IL-10 and IL-12, thus affecting the balance between phenotypically different T lymphocyte populations (Polack et al. 2002; Hoffman et al. 2003a, 2003b). Perhaps the most functional assessment of immunocompetence of macaques during measles is the assessment of responses to immunization with other antigens, e.g., tetanus toxoid, which has been applied both to assess recall of previously primed immune responses (Bankamp et al. 2008) or as primary immunization during measles (Premenko-Lanier et al. 2004). Flow cytometry studies on lymphocytes of macaques infected with MV-EGFP suggest that infection of specific memory T lymphocyte subsets may also play an important role in measles-associated immunosuppression.

## ***Pathology***

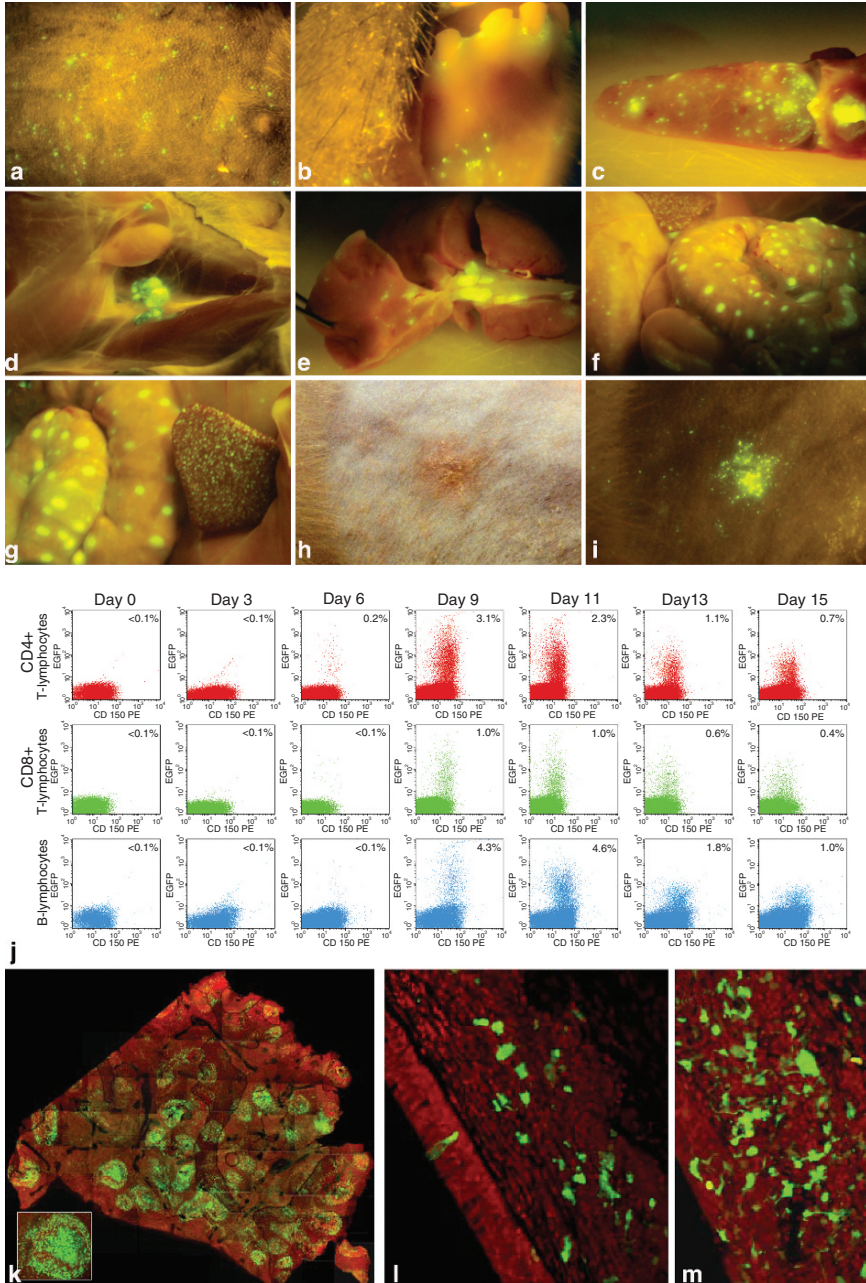
Early studies on the pathology of measles in macaques demonstrated the remarkable similarity to measles in humans (Blake and Trask 1921b). However, experimental infections in an animal model offer the possibility to evaluate pathological changes during different phases of the pathogenesis, whereas human tissue samples collected during the prodromal phase of measles are relatively rare. The classical Warthin-Finkeldey-type syncytial cells observed in human lymphoid tissues during the prodromal phase (Warthin 1931; Finkeldey 1931) were also observed in macaque tissues (Nii et al. 1964a; Yamanouchi et al. 1970, 1973; Hall et al. 1971; Sakaguchi et al. 1986; McChesney et al. 1997).

The major tissues affected by MV infection of macaques are the upper and lower respiratory tract, the gastrointestinal tract, the lymphoid system and the skin (Blake

and Trask 1921b; Sergiev et al. 1960; Nii et al. 1964a; Hall et al. 1971; McChesney et al. 1997). However, the cellular origin of the lesions has been debated for decades (Nii et al. 1964a; De Swart et al. 2007b). Both in human and macaque tissues, MV antigen is commonly detected in association with epithelial tissues. In addition, culture-adapted MV strains grow very well in epithelial cells, which has led to the assumption that respiratory epithelial cells were a primary target for MV infection. However, epithelial cells do not express CD150 and are not easily infected with non-culture-adapted wild-type MV strains *in vitro* (Takeuchi et al. 2003). These observations warrant a reevaluation of the pathogenesis of measles, which may be facilitated by the combination of modern immunohistochemistry and flow cytometry techniques for characterizing cell subsets with experimental infections of macaques with MV strains expressing EGFP.

### ***Infections with MV Expressing EGFP***

The development of reverse genetics techniques for non-culture-adapted MV strains resulted in the rescue of a MV strain from cloned cDNA that retained pathogenicity in macaques (Takeda et al. 2000). This molecular clone was used as a backbone for insertion of the gene encoding EGFP as an additional transcription unit upstream of the MV N gene. The resulting recombinant virus displayed similar *in vitro* replication characteristics as its parental strain, and infected cells produced high amounts of EGFP (Hashimoto et al. 2002). The MV-EGFP strain still proved to be virulent in macaques, and EGFP could be visualized macroscopically in both living and sacrificed animals, and microscopically by confocal microscopy and flow cytometry (De Swart et al. 2007b). As illustrated in Fig. 3.1, EGFP fluorescence was detected in skin, respiratory tract and digestive tract, but most intensely in lymphoid tissues. B and T lymphocytes expressing CD150 were the major target cells for MV infection. Highest percentages (up to more than 30%) of infected lymphocytes were detected in lymphoid tissues. In peripheral tissues, large numbers of MV-infected CD11c<sup>+</sup> MHC class-II<sup>+</sup> myeloid dendritic cells (DCs) were detected in conjunction with infected T lymphocytes, suggesting transmission of MV between these cell types. In the respiratory tract, the majority of MV-infected cells was detected in subepithelial tissues, but infected ciliated epithelial cells were also observed. In the T lymphocyte compartment, MV preferentially infected CD45RA<sup>-</sup> cells, which have a memory phenotype. This observation, which is in good accordance with recent data from MV infections in a human tonsillar explant model (Conrack et al. 2007), pointed to a possible role for depletion of specific lymphocyte subsets in the transient disappearance of recall responses, as described in the early twentieth century (Von Pirquet 1908). By using cell-sorting techniques, the model allows both identification and functional assessment of MV-infected cell populations. The macaque model using the pathogenic autofluorescent wild-type MV strain IC323/EGFP clearly opens new possibilities for measles pathogenesis



**Fig. 3.1 A–M** Imaging of MV-EGFP infection in macaques. **A–G** Macroscopic EGFP fluorescence in tissues of a cynomolgus macaque 9 days after experimental infection with MV-IC323-EGFP (close to the peak of virus replication): EGFP fluorescence in skin (**A**), gingiva and buccal mucosa (**B**), tongue and tonsils (**C**), inguinal lymph nodes (**D**), lungs with tracheobronchial lymph nodes

studies, including identification of the first target cells infected upon transmission of MV to a naive host.

## Concluding Remarks

In conclusion, the macaque model has proven to be of crucial importance for development of measles vaccines, as well as for our understanding of measles pathogenesis. Vaccination and challenge studies in macaques have provided information on immunogenicity and protective capacity of both new vaccines and old vaccines given via alternative routes of administration. Modeling atypical measles has provided a tool for safety assessment of nonreplicating candidate measles vaccines. This important lesson from the past should help us avoid making similar mistakes in the future with other viral vaccines intended for priming immunity to respiratory viruses (Marshall and Enserink 2004; Ruat et al. 2008). In those cases, preclinical studies should not only evaluate acute toxicity responses to the vaccination, but also potential immunopathological responses to challenge infection.

Further employment of the potential of reverse genetics holds the promise of new insights in measles pathogenesis in the coming years. Infection studies in macaques using pathogenic MV strains expressing EGFP may provide new insights in the pathogenesis of measles and measles-associated immunosuppression. In addition, by comparing pathogenic and vaccine strains expressing EGFP we may be able to learn more on the *in vivo* tropism of vaccine strains that can use either CD150 or CD46 as a receptor *in vitro*. Finally, manipulation of viruses and viral genes will enable direct assessment of the role of the different viral proteins in these processes.

---

**Fig. 3.1** (Continued) **(E)**, stomach (*left*), spleen (*upper left*) and large intestine with gut-associated lymphoid tissue (GALT) **(F)**, spleen (*right*) and large intestine with GALT **(G)**. **H, I** cynomolgus macaque 13 days after MV-IC323-EGFP infection (late stage of the infection): skin rash shown under normal light **(H)** or by EGFP fluorescence **(I)**. **J** Flow cytometric detection of EGFP<sup>+</sup> cells in peripheral blood mononuclear cell (PBMC) subpopulations of a cynomolgus macaque at different time points after infection. Freshly isolated PBMCs were stained with monoclonal antibodies, and analyzed in a FACScalibur measuring approximately 500,000 events per sample to allow detection of low-frequent MV-infected cell populations. Results are shown as dot plots, with EGFP expression on the y-axis and CD150 expression on the x-axis. EGFP expression in CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes is shown in *red*; in CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes in *green* and in MHC class-II<sup>+</sup>CD20<sup>+</sup> B lymphocytes in *blue*. **K** Confocal scanning laser microscopical image of EGFP<sup>+</sup> cells in lymphoid follicles of the spleen of a rhesus macaque 9 days after infection with MV-IC323-EGFP. MV infection was visualized in paraformaldehyde-fixed vibratome-cut tissue sections (100 μm) by direct detection of EGFP fluorescence. Propidium iodide (*red*) was used as a structural counter stain. **L, M** Confocal scanning laser microscopical image of EGFP<sup>+</sup> cells in the trachea of a rhesus macaque 9 days after infection with MV-IC323-EGFP. MV infection was visualized in formalin-fixed microtome-cut tissue sections (6 μm) by staining with anti-EGFP antibodies. Propidium iodide (*red*) was used as a structural counter stain. A single MV-infected ciliated epithelial cell can be seen in the mucociliary epithelium **(L)**, but the majority of infected cells is present in the lamina propria and submucosa of the epithelium. These include cells with the phenotype of lymphocytes and of dendritic cells. (From de Swart et al. 2007b)

**Acknowledgements** I thank Albert Osterhaus, Rob van Binnendijk, Teunis Geijtenbeek and Lot de Witte for critical comments on the manuscript.

## References

- Albrecht P, Lorenz D, Klutch MJ, Vickers JH, Ennis FA (1980) Fatal measles infection in marmosets pathogenesis and prophylaxis. *Infect Immun* 27:969–978
- Albrecht P, Lorenz D, Klutch MJ (1981) Encephalitogenicity of measles virus in marmosets. *Infect Immun* 34:581–587
- Anderson JF, Goldberger J (1911) Experimental measles in the monkey: a supplemental note. *Public Health Reports* 26:887–895
- Auwaerter PG, Rota PA, Elkins WR, Adams RJ, DeLozier T, Shi Y, Bellini WJ, Murphy BR, Griffin DE (1999) 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
- Bankamp B, Hodge G, McChesney MB, Bellini WJ, Rota PA (2008) Genetic changes that affect the virulence of measles virus in a rhesus macaque model. *Virology* 373:39–50
- Blake FG, Trask Jr JD (1921a) Studies on measles. I. Susceptibility of monkeys to the virus of measles. *J Exp Med* 33:385–412
- Blake FG, Trask Jr JD (1921b) Studies on measles. II. Symptomatology and pathology in monkeys experimentally infected. *J Exp Med* 33:413–422
- Blake FG, Trask Jr JD (1921c) Studies on measles. III. Acquired immunity following experimental measles. *J Exp Med* 33:621–626
- Burnet FM (1968) Measles as an index of immunological function. *Lancet* 292:610–613
- Buynak EB, Peck HM, Creamer AA, Goldner H, Hilleman MR (1962) Differentiation of virulent from avirulent measles strains. *Am J Dis Child* 103:290–303
- Chen RT, Markowitz LE, Albrecht P, Stewart JA, Mofenson LM, Preblud SR, Orenstein WA (1990) Measles antibody: reevaluation of protective titers. *J Infect Dis* 162:1036–1042
- Condack C, Grivel J-C, Devaux P, Margolis L, Cattaneo R (2007) Measles virus vaccine attenuation: suboptimal infection of lymphatic tissue and tropism alteration. *J Infect Dis* 196:541–549
- Cutts FT, Clements CJ, Bennett JV (1997) Alternative routes of measles immunization: a review. *Biologicals* 25:323–338
- De Swart RL, Kuiken T, Timmerman HH, Van Amerongen G, van den Hoogen BG, Vos HW, Neijens HJ, Andeweg AC, Osterhaus ADME (2002) Immunization of macaques with formalin-inactivated respiratory syncytial virus (RSV) induces interleukin-13-associated hypersensitivity to subsequent RSV infection. *J Virol* 76:11561–11569
- De Swart RL, Kuiken T, Fernandez de Castro J, Papania MJ, Valdespino JL, Minor P, Witham C, Yüksel S, Vos HW, Van Amerongen G, Osterhaus ADME (2006) Aerosol measles vaccination in macaques: preclinical studies of immune responses and safety. *Vaccine* 24:6424–6436
- De Swart RL, LiCalsi C, Quirk AV, Van Amerongen G, Nodelman V, Alcock R, Yüksel S, Ward GH, Hardy JG, Vos HW, Witham CL, Grainger CI, Kuiken T, Greenspan BJ, Gard TG, Osterhaus ADME (2007a) Measles vaccination of macaques by dry powder inhalation. *Vaccine* 25:1183–1190
- De Swart RL, Ludlow M, De Witte L, Yanagi Y, Van Amerongen G, McQuaid S, Yüksel S, Geijtenbeek TBH, Duprex WP, Osterhaus ADME (2007b) Predominant infection of CD150<sup>+</sup> lymphocytes and dendritic cells during measles virus infection of macaques. *PLoS Pathog* 3:e178
- De Swart RL, van den Hoogen BG, Kuiken T, Herfst S, Van Amerongen G, Yüksel S, Sprong L, Osterhaus ADME (2007c) Immunization of macaques with formalin-inactivated human metapneumovirus induces hypersensitivity to hMPV infection. *Vaccine* 25:8518–8528

- Dilraj A, Cutts FT, Fernandez de Castro J, Wheeler JG, Brown D, Roth C, Coovadia HM, Bennett JV (2000) Response to different measles vaccine strains given by aerosol and subcutaneous routes to schoolchildren: a randomised trial. *Lancet* 355:798–803
- El Mubarak HS, Yüksel S, Van Amerongen G, Mukhtar MM, De Swart RL, Osterhaus ADME (2007) Infection of cynomolgus macaques (*Macaca fascicularis*) and rhesus macaques (*Macaca mulatta*) with different wild-type measles viruses. *J Gen Virol* 88:2028–2034
- Enders JF, Peebles TC (1954) Propagation in tissue cultures of cytopathic agents from patients with measles. *Proc Soc Exp Biol Med* 86:277–286
- Enders JF, Katz SL, Milovanovic MV, Holloway A (1960) Studies on an attenuated measles-virus vaccine I. Development and preparation of the vaccine: techniques for assay of effects of vaccination. *N Engl J Med* 263:153–159
- Finkeldey W (1931) Über Riesenzellbefunde in den Gaumenmandeln, zugleich ein Beitrag zur Histopathologie der Mandelveränderungen im Maserninkubationsstadium. *Virchows Arch* 281:323–329
- Foege WH, Leland OS, Mollohan CS, Fulginiti VA, Henderson DA, Kempe CH (1965) Inactivated measles-virus vaccine. *Public Health Reports* 80:60–64
- Fulginiti VA, Eller JJ, Downte AW, Kempe CH (1967) Altered reactivity to measles virus. Atypical measles in children previously immunized with inactivated measles virus vaccines. *JAMA* 202:1075–1080
- Fulginiti VA, Eller JJ, Sieber OF, Joyner JW, Minamitani M, Meiklejohn G (1969) Respiratory virus immunization. I. A field trial of two inactivated respiratory virus vaccines; an aqueous trivalent parainfluenza virus vaccine and an alum-precipitated respiratory syncytial virus vaccine. *Am J Epidemiol* 89:435–448
- Goldberger J, Anderson JF (1911) 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
- Griffin DE, Ward BJ, Esolen LM (1994) Pathogenesis of measles virus infection: an hypothesis for altered immune responses. *J Infect Dis* 170 [Suppl. 1]:S24–S31
- Hall WC, Kovatch RM, Herman PH, Fox JG (1971) Pathology of measles in rhesus monkeys. *Vet Pathol* 8:307–319
- Hashimoto K, Ono N, Tatsuo H, Minagawa H, Takeda M, Takeuchi K, Yanagi Y (2002) SLAM (CD150)-independent measles virus entry as revealed by recombinant virus expressing green fluorescent protein. *J Virol* 76:6743–6749
- Hicks JT, Sullivan JL, Albrecht P (1977) Immune responses during measles infection in immunosuppressed rhesus monkeys. *J Immunol* 119:1452–1456
- Hilleman MR, Stokes J, Buynak EB, Reilly CM, Hampil B (1962) Immunogenic response to killed measles-virus vaccine. *Am J Dis Child* 103:274–281
- Hime JM, Keymer IF (1975) Measles in recently imported colobus monkeys (*Colobus guereza*). *Vet Rec* 97:392
- Hirsch RL, Griffin DE, Johnson RT, Cooper SJ, Lindo de Soriano I, Roedenbeck S, Vaisberg A (1984) Cellular immune responses during complicated and uncomplicated measles virus infections of man. *Clin Immunol Immunopathol* 31:1–12
- Hoffman SJ, Polack FP, Hauer DA, Griffin DE (2003a) Measles virus infection of rhesus macaques affects neutrophil expression of IL-12 and IL-10. *Viral Immunol* 16:369–379
- Hoffman SJ, Polack FP, Hauer DA, Singh M, Billeter MA, Adams RJ, Griffin DE (2003b) Vaccination of rhesus macaques with a recombinant measles virus expressing interleukin-12 alters humoral and cellular immune responses. *J Infect Dis* 188:1553–1561
- Kapikian AZ, Mitchell RH, Chanock RM, Shvedoff RA, Stewart CE (1969) An epidemiologic study of altered clinical reactivity to respiratory syncytial (RS) virus infection in children previously vaccinated with an inactivated RS virus vaccine. *Am J Epidemiol* 89:405–421
- Kim HW, Canchola JG, Brandt CD, Pyles G, Chanock RM, Jensen K, Parrott RH (1969) Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. *Am J Epidemiol* 89:422–434

- Kobune F, Sakata H, Sugiura A (1990) Marmoset lymphoblastoid cells as a sensitive host for isolation of measles virus. *J Virol* 64:700–705
- Kobune F, Takahashi H, Terao K, Ohkawa T, Ami Y, Suzaki Y, Nagata N, Sakata H, Yamanouchi K, Kai C (1996) Nonhuman primate models of measles. *Lab Anim Sci* 46:315–320
- Levy BM, Mirkovic RR (1971) An epizootic of measles in a marmoset colony. *Lab Anim Sci* 21:33–39
- LiCalsi C, Maniaci MJ, Christensen T, Phillips E, Ward GH, Witham C (2001) A powder formulation of measles vaccine for aerosol delivery. *Vaccine* 19:2629–2636
- MacArthur JA, Mann PG, Oreffo V, Scott GBD (1979) Measles in monkeys: an epidemiological study. *J Hyg* 83:207–212
- Marshall E, Enserink M (2004) Caution urged on SARS vaccines. *Science* 303:944–946
- McChesney MB, Fujinami RS, Lerche NW, Marx PA, Oldstone MBA (1989) Virus-induced immunosuppression: infection of peripheral blood mononuclear cells and suppression of immunoglobulin synthesis during natural measles virus infection of rhesus monkeys. *J Infect Dis* 159:757–760
- McChesney MB, Miller CJ, Rota PA, Zhu Y, Antipa L, Lerche NW, Ahmed R, Bellini WJ (1997) Experimental measles I. Pathogenesis in the normal and the immunized host. *Virology* 233:74–84
- Meyer HM, Brooks BE, Douglas RD, Rogers NG (1962) Ecology in measles in monkeys. *Am J Dis Child* 103:138–143
- Mongkolsapaya J, Jaye A, Callan MFC, Magnusen AF, McMichael AJ, Whittle HC (1999) Antigen-specific expansion of cytotoxic T lymphocytes in acute measles virus infection. *J Virol* 73:67–71
- Moss WJ, Ryon JJ, Monze M, Griffin DE (2002) Differential regulation of interleukin (IL)-4, IL-5, and IL-10 during measles in Zambian children. *J Infect Dis* 186:879–887
- Nii S, Kamahora J, Mori Y, Takahashi M, Nishimura S, Okuno Y (1964a) Experimental pathology of measles in monkeys. *Biken J* 6:271–297
- Nii S, Kamahora J, Okuno Y, Nishimura S, Kato S, Aoyama Y (1964b) Studies on the biological effects of live attenuated measles vaccines in monkeys. *Biken J* 7:65–69
- Norrby E, Carlstrom G, Lagercrantz R, Gard S (1964) Measles vaccination II. Antibody titers 8 to 9 months after vaccination with an inactivated vaccine. *Acta Paediatr* 53:533–540
- Norrby E, Enders-Ruckle G, Ter Meulen V (1975) Differences in the appearance of antibodies to structural components of measles virus after immunization with inactivated and live virus. *J Infect Dis* 132:262–269
- Okada H, Kobune F, Sato TA, Kohama T, Takeuchi Y, Abe T, Takayama N, Tsuchiya T, Tashiro M (2000) Extensive lymphopenia due to apoptosis of uninfected lymphocytes in acute measles patients. *Arch Virol* 145:905–920
- Pahar B, Li J, McChesney MB (2005) Detection of T cell memory to measles virus in experimentally infected rhesus macaques by cytokine flow cytometry. *J Immunol Methods* 304:174–183
- Pan CH, Valsamakis A, Colella T, Nair N, Adams RJ, Polack FP, Greer CE, Perri S, Polo JM, Griffin DE (2005) Modulation of disease, T cell responses, and measles virus clearance in monkeys vaccinated with H-encoding alphavirus replicon particles. *Proc Natl Acad Sci U S A* 102:11581–11588
- Pasetti MF, Resendiz-Albor A, Ramirez K, Stout R, Papania M, Adams RJ, Polack FP, Ward BJ, Burt D, Chabot S, Ulmer J, Barry EM, Levine MM (2007) Heterologous prime-boost strategy to immunize very young infants against measles: pre-clinical studies in rhesus macaques. *Clin Pharmacol Ther* 82:672–685
- Peebles TC, McCarthy K, Enders JF, Holloway A (1957) Behavior of monkeys after inoculation of virus derived from patients with measles and propagated in tissue culture together with observations on spontaneous infections of these animals by an agent exhibiting similar antigenic properties. *J Immunol* 78:63–74
- Permar SR, Klumpp SA, Mansfield KG, Kim WK, Gorgone DA, Lifton MA, Williams KC, Schmitz JE, Reimann KA, Axthelm MK, Polack FP, Griffin DE, Letvin NL (2003) Role of CD8<sup>+</sup> lymphocytes in control and clearance of measles virus infection of rhesus monkeys. *J Virol* 77:4396–4400



- Permar SR, Klumpp SA, Mansfield KG, Carville AA, Gorgone DA, Lifton MA, Schmitz JE, Reimann KA, Polack FP, Griffin DE, Letvin NL (2004) Limited contribution of humoral immunity to the clearance of measles viremia in rhesus monkeys. *J Infect Dis* 190:998–1005
- Polack FP, Auwaerter PG, Lee SH, Nousari HC, Valsamakis A, Leiferman KM, Diwan A, Adams RJ, Griffin DE (1999) 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
- Polack FP, Lee SH, Permar S, Manyara E, Nousari HC, Jeng Y, Mustafa F, Valsamakis A, Adams RJ, Robinson HL, Griffin D (2000) Successful 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
- Polack FP, Hoffman SJ, Moss WJ, Griffin DE (2002) Altered synthesis of interleukin-12 and type 1 and type 2 cytokines in rhesus macaques during measles and atypical measles. *J Infect Dis* 185:13–19
- Polack FP, Hoffman SJ, Crujeiras G, Griffin DE (2003a) A role for nonprotective complement-fixing antibodies with low avidity for measles virus in atypical measles. *Nat Med* 9:1209–1213
- Polack FP, Hoffman SJ, Moss WJ, Griffin DE (2003b) Differential effects of priming with DNA vaccines encoding the hemagglutinin and/or fusion proteins on cytokine responses after measles virus challenge. *J Infect Dis* 187:1794–1800
- Potkay S, Ganaway JR, Rogers NG, Kinard R (1966) An epizootic of measles in a colony of rhesus monkeys (*Macaca mulatta*). *Am J Vet Res* 27:331–334
- Premenko-Lanier M, Rota PA, Rhodes G, Verhoeven D, Barouch DH, Lerche NW, Letvin NL, Bellini WJ, McChesney MB (2003) DNA vaccination of infants in the presence of maternal antibody: a measles model in the primate. *Virology* 307:67–75
- Premenko-Lanier M, Rota PA, Rhodes GH, Bellini WJ, McChesney MB (2004) Protection against challenge with measles virus (MV) in infant macaques by an MV DNA vaccine administered in the presence of neutralizing antibody. *J Infect Dis* 189:2064–2071
- Pütz MM, Bouche FB, De Swart RL, Muller CP (2003) Experimental vaccines against measles in a world of changing epidemiology. *Int J Parasitol* 33:525–545
- Remfry J (1976) A measles epizootic with 5 deaths in newly-imported rhesus monkeys (*Macaca mulatta*). *Lab Anim* 10:49–57
- Ruat C, Caillet C, Bidaut A, Simon J, Osterhaus ADME (2008) Vaccination of macaques with adjuvanted formalin-inactivated influenza A (H5N1) vaccines: protection against H5N1 challenge without disease enhancement. *J Virol* 82:2565–2569
- Sabin AB, Fernandez de Castro J, Flores Arechiga A, Sever JL, Madden DL, Shekarchi I (1982) Clinical trials of inhaled aerosol of human diploid and chick embryo measles vaccine. *Lancet* 2:604
- Sakaguchi M, Yoshikawa Y, Yamanouchi K, Sata T, Nagashima K, Takeda K (1986) Growth of measles virus in epithelial and lymphoid tissues of cynomolgus monkeys. *Microbiol Immunol* 30:1067–1073
- Samb B, Aaby P, Whittle HC, Coll-Seck AM, Rahman S, Bennett J, Markowitz L, Simondon F (1995) Serologic status and measles attack rates among vaccinated and unvaccinated children in rural Senegal. *Pediatr Infect Dis J* 14:203–209
- Schneider-Schaulies S, Klagge IM, Ter Meulen V (2003) Dendritic cells and measles virus infection. *Curr Top Microbiol Immunol* 276:77–101
- Scott GBD, Keymer IF (1975) The pathology of measles in Abyssinian colobus monkeys (*Colobus guereza*): a description of an outbreak. *J Pathol* 117:229–233
- Sergiev PG, Ryazantseva NE, Shroit IG (1960) The dynamics of pathological processes in experimental measles in monkeys. *Acta Virol* 4:265–273
- Sharova OK, Rozina EE, Gordienko NM, Shteinberg LSH (1984) Effect of immunosuppression on morphological changes in CNS of monkeys infected with different measles virus vaccine strains. *Acta Virol* 28:144–147
- Stittelaar KJ, Wyatt LS, De Swart RL, Vos HW, Groen J, Van Amerongen G, Van Binnendijk RS, Rozenblatt S, Moss B, Osterhaus ADME (2000) Protective immunity in macaques vaccinated

- with a modified vaccinia virus Ankara-based measles virus vaccine in the presence of passively acquired antibodies. *J Virol* 74:4236–4243
- Stittelaar KJ, De Swart RL, Osterhaus ADME (2002a) Vaccination against measles: a neverending story. *Expert Rev Vaccines* 1:151–159
- Stittelaar KJ, Vos HW, Van Amerongen G, Kersten GFA, Osterhaus ADME, De Swart RL (2002b) Longevity of neutralizing antibody levels in macaques vaccinated with Quil A-adjuvanted measles vaccine candidates. *Vaccine* 21:155–157
- Takeda M, Takeuchi K, Miyajima N, Kobune F, Ami Y, Nagata N, Suzaki Y, Nagai Y, Tashiro M (2000) Recovery of pathogenic measles virus from cloned cDNA. *J Virol* 74:6643–6647
- Takeuchi K, Miyajima N, Nagata N, Takeda M, Tashiro M (2003) Wild-type measles virus induces large syncytium formation in primary human small airway epithelial cells by a SLAM(CD150)-independent mechanism. *Virus Res* 94:11–16
- Tauraso NM (1973) Review of recent epizootics in nonhuman primate colonies and their relation to man. *Lab Anim Sci* 23:201–210
- Valdespino-Gomez JL, De Lourdes Garcia-Garcia M, Fernandez de Castro J, Henao-Restrepo AM, Bennett J, Sepulveda-Amor J (2006) Measles aerosol vaccination. *Curr Top Microbiol Immunol* 304:165–193
- Van Binnendijk RS, van der Heijden RWJ, Van Amerongen G, UytdeHaag FGCM, Osterhaus ADME (1994) Viral replication and development of specific immunity in macaques after infection with different measles virus strains. *J Infect Dis* 170:443–448
- Van Binnendijk RS, van der Heijden RWJ, Osterhaus ADME (1995) Monkeys in measles research. *Curr Top Microbiol Immunol* 191:135–148
- Van Binnendijk RS, Poelen MCM, Van Amerongen G, De Vries P, Osterhaus ADME (1997) Protective immunity in macaques vaccinated with live attenuated, recombinant, and subunit measles vaccines in the presence of passively acquired antibodies. *J Infect Dis* 175:524–532
- Von Pirquet CE (1908) Das Verhalten der kutanen Tuberkulin-reaktion während der Masern. *Dtsch Med Wochenschr* 34:1297–1300
- Warthin AS (1931) Occurrence of numerous large giant cells in the tonsils and pharyngeal mucosa in the prodromal stage of measles. *Arch Pathol* 11:864–874
- Welshman MD (1989) Measles in the cynomolgus monkey (*Macaca fascicularis*). *Vet Rec* 124:184–186
- WHO (2007) Manual for the laboratory diagnosis of measles and rubella virus infection. WHO/IVB/07.01, Geneva
- Yamanouchi K, Egashira Y, Uchida N, Kodama H, Kobune F, Hayami M, Fukuda A, Shishido A (1970) Giant cell formation in lymphoid tissues of monkeys inoculated with various strains of measles virus. *J Med Sci Biol* 23:131–145
- Yamanouchi K, Chino F, Kobune F, Kodama H, Tsuruhara T (1973) Growth of measles virus in the lymphoid tissues of monkeys. *J Infect Dis* 128:795–799
- Zhu Y, Heath J, Collins J, Greene T, Antipa L, Rota PA, Bellini W, McChesney MB (1997) Experimental measles II. Infection and immunity in the rhesus macaque. *Virology* 233:85–92
- Zhu Y-D, Rota P, Wyatt LS, Tamin A, Rozenblatt S, Lerche N, Moss B, Bellini W, McChesney M (2000) Evaluation of recombinant vaccinia virus – measles vaccines in infant rhesus macaques with preexisting measles antibody. *Virology* 276:202–213