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SPECIAL ARTICLE

Atypical Measles and Enhanced Respiratory Syncytial Virus Disease (ERD) Made Simple

FERNANDO P. POLACK

Department of Pediatrics, Johns Hopkins University School of Medicine Baltimore, MD 21205

ABSTRACT: Atypical measles and enhanced respiratory syncytial virus disease (ERD) were serious diseases that resulted from exposure of children immunized with inactivated vaccines against measles virus (MV) and respiratory syncytial virus (RSV) to the respective wild-type agents in the 1960s. Although the clinical manifestations of both illnesses were different, the immune responses elicited and primed for by the vaccines shared important similarities. Both vaccines failed to elicit long-lived protective antibody and to promote cytotoxic T lymphocyte responses. In both cases, postvaccination exposure to wild type virus during community outbreaks was associated with immune complex deposition in affected tissues, vigorous $CD4^+$ T lymphocyte proliferative responses, and a Th2 bias of the immune response. No relapses of atypical measles or ERD were ever reported.

In this manuscript, the pathogeneses of both enhanced diseases and the requirements for the generation of protective antibodies against MV and RSV are discussed, to contribute to the development of newer safe and effective vaccines against these important pathogens. **(*Pediatr Res* 62: 111–115, 2007)**

Paramyxoviruses are important agents of diseases in children. Among them, measles virus (MV) and respiratory syncytial virus (RSV) have been recognized for decades as causes of pediatric illnesses associated with significant morbidity (1,2). While a protective live attenuated vaccine against MV (LAV) is available for older infants and children, seroconversion rates are lower in young infants and no vaccine has been licensed against RSV. Protecting young infants against MV and RSV is important. In the 1960s, formalin-inactivated vaccines against these agents were developed and administered to infants and children in the United States (3–8). The vaccines were not protective, and primed for severe forms of disease in individuals exposed to the respective wild-type viruses (3–12).

Although the pathogeneses of the enhanced illnesses elicited by MV and RSV have been studied separately for decades, both diseases share important similarities in their mechanisms of illness. In this manuscript, we discuss the similarities and specific differences between atypical measles and enhanced RSV disease (ERD). Understanding the pathogeneses of these vaccine-enhanced diseases is important for the development of safe, newer vaccines against paramyxoviruses.

Measles virus. MV is responsible for hundreds of thousands of deaths every year in developing countries, despite the availability of a safe and effective LAV (1). The vaccine is immunogenic when administered to infants and young children 9–15 months of age, but seroconversion rates are lower in infants under the age of 9 months due to the presence of interfering transplacentally acquired maternal antibody and the immune immaturity of the host (13,14). In developing countries with high rates of measles, infants are often exposed to the virus before the age of 9 months and represent an important number of the fatalities caused by the virus every year (1). For this reason, expanding vaccine coverage in affected areas and/or developing new immunization strategies for young infants is important if this "window of susceptibility" is to be closed.

In the 1960s, inactivated vaccines against MV were introduced in the United States and Europe (7–12). The formalin-inactivated MV vaccine (FIMV) licensed in the States was immunogenic, but antibody waned within months to a couple of years (7). Fifteen to sixty percent of immunized children subsequently exposed to wild-type MV during community outbreaks developed a severe form of disease called atypical measles (7–12). Atypical measles was characterized by high fever, a petechial or morbiliform rash that began on the extremities and a severe pneumonitis (7–12). Other clinical manifestations, including abdominal pain, eosinophilia and hepatic dysfunction were also described (7–12). The disease was severe enough to warrant hospitalization in many cases (7–12). The vaccine was withdrawn in 1967 because of these problems.

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Correspondence: Fernando P. Polack, M.D., Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe Street; E5202, Baltimore, MD 21205, phone: (443) 287-6407, FAX: (410) 955-0105; e-mail: fpolack@jhsph.edu

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Abbreviations: ERD, enhanced respiratory syncytial virus disease; **FIMV,** formalin-inactivated vaccine against measles virus; **FIRSV,** formalin-inactivated vaccine against respiratory syncytial virus; **LAV,** live attenuated vaccine against measles virus; **MV,** measles virus; **RSV,** respiratory syncytial virus

Respiratory syncytial virus. Respiratory syncytial virus (RSV) is the main viral respiratory cause of hospitalization in infants and young children worldwide (2). More than 50% of infants experience an RSV infection during their first seasonal encounter with the virus, and over 90% have become infected by the end of the second season of RSV exposure (2,15). Most of these primary infections are symptomatic and 30–70% of them manifest as lower respiratory illness (LRI) with bronchiolitis and/or pneumonia. Reinfections occur through life and are usually symptomatic, although they do not generally cause LRI in immunocompetent adults and healthy older children (2).

In 1961, a formalin-inactivated vaccine against RSV (FIRSV) was developed using the Bernett strain of RSV passaged in human embryonic kidney cells (×3) and vervet monkey kidney cells (×10) (3). RSV was inactivated by incubation with 0.4% formaldehyde for 72 h and adsorbed to 4 mg/mL of aluminum hydroxide. The vaccine was administered in 1–3 doses to RSV-seronegative and RSV-seropositive infants and children during 1966 (3–6). Control groups of children received a formalin-inactivated parainfluenza vaccine (3–5) or no vaccine (6). The vaccine was immunogenic, but elicited mainly nonprotective antibodies. During the winter of 1966–1967, immunized children were exposed to RSV in the community, and those that were seronegative for the virus before vaccination experienced a significant increase in the frequency and severity of LRI and a greater incidence of hospitalization compared with control children (3–6). The main clinical manifestations in these children included bronchoconstriction and pneumonia (3–6). Furthermore, two immunized infants died as toddlers as a consequence of subsequent RSV infection (3). Autopsy material showed bronchopneumonia, atelectases, and pneumothoraces (3). Histopathology was reported as a "peribronchiolar monocytic infiltration with some excess in eosinophils" (3). High titers of RSV were recovered from the lungs of the two children (3). No vaccine against RSV has been licensed since.

HYPOTHESES

Atypical measles. Several hypotheses were advanced to explain the pathogenesis of atypical measles, including a MV-derived delayed type hypersensitivity response and a generalized Arthus reaction (16–20). Perhaps the most widely accepted hypothesis early on was that atypical measles resulted from an imbalance in the antibody response to the MV glycoproteins hemagglutinin (HA) and fusion (F) elicited by the inactivated vaccine (21,22). Based on tissue culture experiments with a related paramyxovirus, simian virus 5, it was suggested that low levels of antibody against MV F – following a postulated disruption of the protein during formalin inactivation- allowed extensive spread of the virus *via* cell-to-cell fusion leading to more severe disease (23).

Enhanced respiratory syncytial virus disease. The question about the mechanism of illness in ERD has dominated the RSV literature for decades. Given the histopathology described in lung sections from affected children (3), a number of models of ERD have focused on the development of pulmonary eosinophilia and Th2 responses (24–27). The eosinophilia has been ascribed –as in the case of atypical measles- to an imbalance in the RSV glycoproteins present in the formalin-inactivated vaccine. A dominant immune response against the RSV attachment protein (G), associated again with the presumptive disruption of the fusion (RSV F) protein during formalin inactivation, was postulated to prime for lung eosinophilia and Th2 bias in affected individuals (25–27).

CLINICAL AND IMMUNOLOGIC MANIFESTATIONS COMMON TO ATYPICAL MEASLES AND ERD

Several similarities are apparent upon examination of the immune manifestations that characterized atypical measles and ERD.

First, *both FIMV and FIRSV failed to elicit long-lived protective antibodies in children and* – as expected for inactivated vaccines- *did not elicit a detectable virus-specific cytotoxic T lymphocyte (CTL) response* (3–8,28–31). Early determinations of the neutralizing capacity of sera from children immunized with FIMV using Vero cells suggested that these antibodies were protective against MV. However, the clinical manifestations of children and macaques with atypical measles demonstrated that the abundant anamnestic antibody response observed early after challenge was not protective (28,29) and that transient protection after vaccination was probably explained by steric hindrance of critical epitopes. In ERD, antibodies in mice and humans had high anti-RSV F EIA/anti-RSV neutralization ratios, also suggesting poor protective efficacy (live RSV infections elicit antibody responses of low EIA/neutralization ratios) (46). Furthermore, high titers of RSV were recovered from lung sections of affected children, clearly establishing the lack of protection (3).

Second, *exposure to wild type viruses led to strong proliferative $CD4^+$ T lymphocyte responses* (32,33) *and a Th2 polarization of the immune response in both diseases*. In rhesus macaques with atypical measles, this Th2 bias was characterized by early suppression of IL-12 (IL-12) secretion by monocytes, followed by pulmonary eosinophilia and late production of IL-4 (34). In animal models of ERD, pulmonary eosinophilia and production of Th2 cytokines have been frequently reported (35–37). In fact, formalin inactivation has also been noted to favor a Th2 bias (38). Yet, it is important to highlight that certain mouse strains, cotton rats, and cattle with ERD present pulmonary infiltrates dominated by neutrophils, and not eosinophils (39–41). Furthermore, revision of the autopsy reports and original slides from affected children revealed a clear predominance of neutrophils and macrophages in the lungs accompanied by the occasional presence of eosinophils in smaller bronchioles (these eosinophils somehow gained a disproportionate relevance in the original manuscript) (40).

Third, *both mechanisms of illness were associated with immune complex deposition in affected tissue*. Rhesus macaques with atypical measles had evidence of immune complex deposition on dermal vessels (28). In addition, individu-

als immunized with FIMV were subsequently re-immunized with LAV to prevent the development of atypical measles, and had significant local reactions to vaccination (16–20), also presenting with deposition of immune complexes around dermal vessels (16). As for ERD, a role for immune complexes was first suspected by authors of the original manuscript given the abundance of nonprotective antibody in sera from infected vaccine recipients and the bibasal distribution of pulmonary infiltrates in affected children (3). More recently, peribronchiolar and perivascular deposition of immune complexes has been demonstrated in the lungs of affected mice (30). Antibody deposition did not result in bronchoconstriction during murine ERD in the absence of complement activation and complement did not elicit bronchoconstriction in the absence of antibodies (30). A similar pattern of immune complex deposition was observed in cotton rats (G. Prince, personal communication). Further, staining of the lungs of children who died of ERD in 1967 demonstrate immune complex-mediated activation of the classic complement cascade, evidenced by peribronchiolar deposition of complement component C4d (30).

Fourth, *abundant evidence suggests that the glycoprotein imbalance postulated early on as the explanation for both diseases, and ascribed to formalin disruption of the fusion proteins in MV and RSV, is incorrect*. Rhesus macaques developed atypical measles in the presence of fusion-inhibiting antibodies (28), and DNA vaccines encoding only the HA glycoprotein (and therefore not the fusion protein) did not prime for atypical measles (42). In RSV, the G protein was postulated to elicit ERD in the theoretical absence of RSV F. But inoculation of BALB/c mice with a formalin-inactivated recombinant RSV that does not encode the G protein elicited ERD of identical severity as that induced by inactivated wild type virus (42,43). In fact, the G protein – often postulated as an important mediator of ERD pulmonary inflammation - has recently been shown to decrease the degree of pulmonary mononuclear cell infiltration during RSV infection (44,45).

Finally, *no child ever experienced atypical measles or ERD twice*. In fact, exposure to wild-type virus (or in the case of some of the children immunized with FIMV, administration of LAV) reestablished a normal immune response to subsequent exposures in all immunized individuals (33).

ANTIBODIES AND PARAMYXOVIRUSES

Perhaps, the most pressing question about the pathogeneses of atypical measles and ERD is why antibodies failed to confer protection and how was the problem "corrected" by subsequent exposure to live virus. In other words, what is required of specific antibodies to protect against these agents? The inability of several other nonreplicating MV and RSV vaccines to elicit long-lived protective antibody responses in subsequent experiments (including purified RSV F and G proteins, tween ether-inactivated MV, Baculovirus-expressed RSV F protein, among others) stress that lack of protection in atypical measles and ERD cannot be solely attributed to the poor preservation of specific antigens during formalin inactivation (21,22,46–48). Development of aberrant immune manifestations after administration of a tween-ether inactivated MV vaccine to children in Europe (21,22), and the failure of a variety of nonreplicating immunogens against RSV in animal models (46–48) illustrate the difficulties of developing protective and safe nonreplicating vaccines against these two agents.

Should FIMV or FIRSV had elicited protective antibody, it is likely that exposure to MV or RSV in the community would not have caused these serious illnesses (7,49). In fact, once antibodies fail to protect, ERD and atypical measles can come in different flavors. Different animal models of these enhanced diseases display a varying predominance of neutrophils, macrophages, or eosinophils in affected tissues that depend on the strain of mouse or the species chosen by the investigators (35–41). These findings suggest that a variety of $CD4^+$ T cells primed by vaccination to secrete differing cytokines and/or chemokines may elicit enhanced MV or RSV diseases when exposed to abundant wild type virus in the absence of protective antibody (35–41). Furthermore, complement activation through immune complex deposition enhances the $CD4^+$ T lymphocyte response and augments disease severity (50).

As for the requirements for the development of protective antibody responses, and also as a clue to the lack of relapses in both diseases, the avidity of antibody for wild-type virus may be important (29). MV-specific antibody elicited by FIMV was of low avidity (29). Changes in antibody avidity after MV challenge correlated with changes in neutralizing capacity (29). Affinity maturation of antibody following MV exposure established a long-lived protective antibody response (29).

Affinity maturation of antibody may be equally important to protect against RSV. In fact, a role for affinity maturation may help to clarify why none of the children who were RSV-seropositive before immunization with FIRSV developed ERD. It is likely that their preexistent RSV-specific antibodies against wild type RSV were of high affinity (low EIA/neutralization ratio) and "outcompeted" the pathogenic antibodies elicited by the vaccine (3). Similarly, exposure of immunized individuals to wild-type infection (21,22,28–30) [or LAV remedial administration (19,20)] elicited antibodies of high affinity that also "outcompeted" the pathogenic humoral response generated earlier on by FIMV or FIRSV, and ensured that no relapse of these diseases ever occurred. These observations suggest that characterization of antibody avidity is an important consideration in evaluating vaccines against these paramyxoviruses.

CONCLUSIONS

Atypical measles and ERD were serious diseases that resulted from immunization of children with inactivated vaccines against MV and RSV. Both vaccines failed to elicit protective antibody and, in both cases, postvaccination exposure to wild type virus led to immune complex deposition in affected tissues, vigorous anamnestic $CD4^+$ T lymphocyte proliferative responses, and a Th2 bias of the immune re-

sponse. No relapses of either illness were ever reported. Although the clinical manifestations of both illnesses were different, and obeyed primarily to the individual tropism of each virus (1,2), the similarities in immune responses elicited and primed for by the vaccines suggest that atypical measles and ERD share a common general mechanism of illness. Furthermore, these diseases resulted from a disproportionate response of a primed immune system exposed to wild-type virus in the absence of protective antibody. These experiences highlight the importance of understanding the requirements for the production of protective antibodies against these agents to develop new safe and effective vaccines to protect young infants.

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