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Short Communication

Maternal antibodies by passive immunization with formalin inactivated respiratory syncytial virus confer protection without vaccine-enhanced disease

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ABSTRACT

Maternal immunization of mice with formalin inactivated respiratory syncytial virus (FI-RSV) resulted in the passive transfer of RSV antibodies but not cellular components to the offspring. The offspring born to FI-RSV immunized mothers showed serum RSV neutralizing activity, effectively controlled lung viral loads without vaccine-enhanced disease, did not induce pulmonary eosinophilia, and cytokine producing cells after live RSV infection. Therefore, this study provides evidence that maternal immunization provides an *in vivo* model in investigating the roles of antibodies independent of cellular components.

1. Introduction

Respiratory syncytial virus (RSV), a family *Paramyxoviridae*, is the single most important viral cause of lower respiratory tract infection in infants and young children (Paramore et al., 2004; Thompson et al., 2003). The greatest hospitalization rates are in children within first 3 months of life and most children are infected during their first RSV season, for which maternal immunization could theoretically be beneficial (Glezen et al., 1986; Ochola et al., 2009; Siegrist et al., 1998). Formalin inactivated RSV vaccines (FI-RSV) were known to cause vaccine-enhanced disease in immunized children upon natural infection (Kapikian et al., 1969; Kim et al., 1969). However, the possible roles of antibodies induced by FI-RSV immunization in protection and disease have not been well understood yet although licensed drugs against RSV are based on antibodies prescribed for high-risk young infants (Alan et al., 2012; Hoopes et al., 2012; Resch et al., 2012). We hypothesized that maternal antibodies in pups born to vaccinated mothers would provide a proof-of-concept to investigate the roles of antibodies in inducing protection and vaccine-enhanced disease.

2. Immunization and challenge experiments

RSV (A2 strain) was harvested from infected HEp-2 cells, inactivated by a 1/10 volume of a 1:400 diluted 37% formaldehyde, purified by ultracentrifugation, and then absorbed onto aluminum hydroxide (4 mg/ml) as previously described (Prince et al., 2001). Female mice were intramuscularly primed and boosted with 2 and 1 μg FI-RSV (A2 strain) respectively in a 4-week interval ($n = 5$), mated and used as vaccinated mother mice. RSV specific antibodies were determined in immune sera of mother mice by using FI-RSV as an ELISA plate coating antigen as described previously (Quan et al., 2011). For RSV challenge, mice were intranasally infected with 1×10^6 plaque forming units (PFU).

3. Experimental methods

RSV plaque reduction and lung viral titer assays were performed to determine neutralizing activity and protective efficacy for viral clearance respectively as described (Quan et al., 2011). Cytokine secreting cell spots (ELISpot) were developed after simulation of lung cells (1×10^5 cells/well) or spleen cells (5×10^5 cells/well) with inactivated RSV, and counted by an ELISpot reader (Song

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et al., 2010). Lung samples were fixed in neutral buffered formalin, embedded with paraffin blocks, thin-sectioned, and stained with hematoxylin and eosin (H&E), periodic acid-Schiff stain (PAS) or hematoxylin and congo red (H&CR) as described (Murawski et al., 2010). We used the scoring system for evaluating the histopathological severity of pneumonia, bronchioles, blood vessels and interstitial space on a scale of 0–3 by blinded observers (Mok et al., 2007). Stained bronchoalveolar lavage cells with CD45, CD11c, CD11b, and SiglecF antibodies were analyzed using LSRFortessa flow cytometer (BD Biosciences) and FlowJo software (Tree Star Inc.). Significant differences among treatments were evaluated by 1-way or 2-way ANOVA where appropriate. *P*-values of less than or equal to 0.05 were considered statistically significant.

4. Effective transfer of maternal antibodies but not cellular components to the offspring

FI-RSV immunized mother mice induced high levels of RSV specific total IgG as well as IgG1 and IgG2a isotype antibodies (Fig. 1A, B, and C). Pups ($n = 5–10$) during the 3-week period of milk suckling also showed high levels of serum IgG, and isotype antibodies comparable to those in mothers (Fig. 1A, B, and C). This breast feeding is important because maternal IgG is transferred to infant mice via milk sucking in mice (Van de Perre, 2003). A progressive decline in the levels of maternal antibodies was observed in pups and antibody levels were decreased to half approximately 2–3 weeks after weaning (Fig. 1A, B, and C). By age of 12 weeks old, all mice born to immunized mothers showed no detectable levels of antibodies, similar to naïve mice. In contrast, all mother mice maintained high levels of RSV specific antibodies for over 19 weeks (Fig. 1A, B, C). As expected, these results suggest that antibody-secreting cells were not transferred to pups.

5. Passively transferred maternal antibodies from FI-RSV immunized mother mice confer protection

The levels of RSV plaques were significantly lowered in sera from 3 weeks old pups, similar to those from immunized mothers (3wk pups, Fig. 1D). The sera from pups with 8 weeks old showed substantial reductions in the plaque forming units at lower dilutions but no reduction with over 160 dilutions.

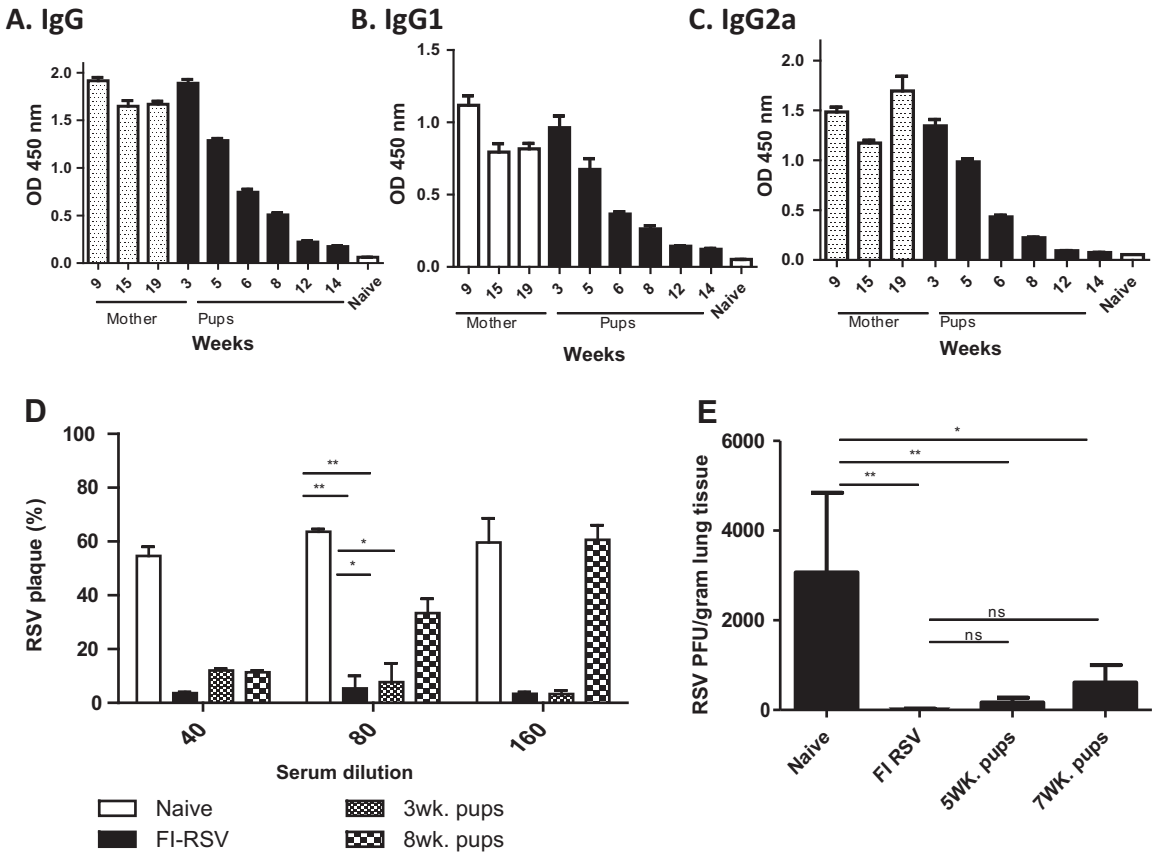

Fig. 1. Decay kinetics and protective roles of passively transferred maternal antibodies. (A–C) Decay kinetics of passively transferred maternal antibodies. Levels of RSV specific IgG and isotype antibodies are presented at different ages of pups ($n = 5–10$) born to FI-RSV immunized mice ($n = 5$). For comparison of antibody maintenance, antibody levels of immune mothers were included at 9, 15, and 19 weeks post immunization. The ages of pups are indicated as weeks (3, 5, 8, 12, and 14). (A) Total IgG antibodies specific to RSV. (B) IgG1 isotype antibodies specific to RSV. (C) IgG2a isotype antibodies specific to RSV. (D) RSV neutralizing activity of immune sera ($n = 5–10$). Serially diluted sera after heat-inactivation were used to determine their capacity to reduce RSV plaque formation. Naïve: sera of naïve mice, FI-RSV: immune sera of FI-RSV immunized mice (8–10 weeks old, $n = 5$). 3wk pups: sera of 3-week old pups born to FI-RSV immunized mothers, 8wk pups: sera of 8-week old pups born to FI-RSV immunized mothers. Statistical significances (GraphPad InStat software) are indicated between naïve sera and FI-RSV immune sera or passively transferred antibody sera (3wk.pus or 8wk pups). ($^*P < 0.05$; $^{**}P < 0.01$). (E) RSV loads in lungs after challenge. Lungs from individual mice in a different set of experimental groups ($n = 5–10$) from the panel D were collected on day 5 post challenge (1×10^6 PFU/mouse i.n.), and lung virus loads (PFU/g lung tissues) in each mouse were determined in HEp2 cells. Naïve: Unimmunized mice infected with RSV, FI-RSV: FI-RSV prime boost immunized mice born to naïve mothers at day 5 post RSV challenge, 5wk pups: 5-week old pups at day 5 post RSV challenge, 7wk pups: 7-week old pups at day 5 post RSV challenge. Each value represents the mean ± SD (standard deviation) in triplicates. Statistical significances (GraphPad InStat software) are indicated between FI-RSV and FI-RSV pups and between FI-RSV and FI-RSV pups. Bars indicate significant differences between groups (ns; not significant; $^*P < 0.05$).