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Age-Specific Profiles of Antibody Responses against Respiratory Syncytial Virus Infection

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ABSTRACT

Respiratory syncytial virus (RSV) is one of the most prevalent causative agents of lower respiratory tract infections worldwide, especially in infants around 3 to 4 months old. Infants at such a young age have maternally-transferred passive antibodies against RSV but do not have active immune systems efficient enough for the control of RSV infection. In order to elucidate age-specific profiles of immune responses against RSV protection, antibody responses were examined by using blood samples in both acute and convalescent phases obtained from child patients and adult patients. In addition to the serum neutralization activity, antibody responses to the RSV fusion protein (F protein) were dissected by analyzing levels of total IgG, IgG subclasses, the binding stability, and the levels of antibody for the neutralization epitopes. It was suggested that children's antibody responses against RSV are matured over months and years in at least 5 stages based on 1) levels of the neutralization titer and IgG3 for F protein in the convalescent phase, 2) geometric mean ratios of the neutralization titers and levels of IgG1 and IgG2 for F protein in the convalescent phase compared to those levels in the acute phase, 3) the affinity maturation of IgG for F protein and the cross reactivity of IgG for RSV glycoproteins of groups A and B, 4) levels of neutralization epitope-specific IgG, and 5) augmentation of overall antibody responses due to repetitive RSV infection.

1. Introduction

Respiratory syncytial virus (RSV) causes severe lower respiratory tract infection (LRTI), especially in infants <6 months old and the elderly, posing public health concerns worldwide. The results of a recent study based on the global mortality surveillance from 1990 to 2010 suggested that the cause of the highest mortality rate in infants is LRTI and that the most frequent causative agent of LRTI in infants is RSV (Lozano et al., 2012). It is also suggested that among contagious pathogens RSV is the second largest cause of mortality in infants next to the Plasmodium parasite (Lozano et al., 2012). As a disease burden associated with RSV infection, high risk for the development of asthma in children who experience RSV bronchiolitis in infancy has been demonstrated (Bacharier et al., 2012). In addition, results of recent clinical research in the UK estimated that the year-to-year mortality rate in the elderly, and the number of elderly outpatients associated with RSV infection are more consistent than those of influenza (Flu), and that the average mortality rate of RSV and the number of RSV patients over the years are comparable with or higher than those of Flu (Fleming et al., 2015). Results of the research also suggested that rates of both the mortality and the hospitalization attributable to RSV infection are higher in the high risk group of the elderly, defined as chronic conditions indicative of risk for severe influenza as per UK recommendations for influenza vaccination, than those in the non-risk group of the elderly (Fleming et al., 2015). Thus, prophylactic measures, i.e., vaccines especially for infants and elderly, are required for controlling RSV infections worldwide, but no approved vaccines are currently available. According to WHO reports and a PATH program, there are a number of different types of RSV vaccine candidates in a variety of development stages, whereas one of the greatest regulatory issues on RSV vaccines is that there are no consolidated immunological biomarkers. Also, an international serum

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standard is required to normalize data between different tests and different laboratories for the clinical evaluation of vaccine efficacy. The difficulty lies in the fact that infants' active antibody responses are hardly discriminated from pre-existing passively transferred maternal antibodies in infants. It is also suggested that regulatory authorities should harmonize and standardize the evaluation methods of biomarkers by using standard reagents and reference materials with common procedures (Higgins et al., 2016).

Palivizumab, an antibody drug targeting antigenic site II of the RSV fusion protein (F protein), is available for the prevention of severe RSV illness in certain infants and children who are at high risk. The drug can help prevent development of serious RSV diseases, yet with limited efficacy (Groothuis et al., 1993; Parnes et al., 2003; The IMpact-RSV Study Group, 1998). The results of palivizumab's clinical trials in preterm infants showed that 40 μg/mL of serum palivizumab corresponds to 7.3 $\log_2$ of the neutralization titer, enabling a 55% reduction of the hospitalization associated with RSV infections (The IMpact-RSV Study Group, 1998). The results of the past clinical research showed that participants with naturally acquired serum neutralization titers of 6.0 $\log_2$ or more to RSV group A and 8.0 $\log_2$ or more to RSV group B resulted in approximately 70% reduction in not having an RSV related-hospitalization (Piedra et al., 2003). The results of clinical studies on the prophylactic intravenous administration of RSV immune globulin to high risk infants and on serological assessment of RSV patients <6 months old have suggested that the sera with neutralization titers >1:200 or 8.0 $\log_2$ confer protection against LRTI (Blaser and Valentine, 2008; Groothuis et al., 1993; Wright et al., 2002). Another clinical study focused on the RSV-specific antibody kinetics in mother-infant pairs in Bangladesh, and demonstrated a strong correlation between maternal RSV antibody titers at the third trimester and at birth with efficient trans-placental antibody transfer to the fetus (Chu et al., 2014). Reduced risk of LRTI was associated with higher cord blood neutralization titers above 8.0 $\log_2$ (Chu et al., 2014). Taken together, these findings suggest that serum neutralization titers of >8.0 $\log_2$ or serum palivizumab-like neutralization antibodies (PLNA) of >40 μg/mL correlate with protection against RSV.

Accumulating scientific evidence suggests different profiles of each IgG subclass in protection against pathogens. Human IgG1 is the most dominant subclass in human sera and generally plays an important role in the protection against a variety of viral infections. In addition to inactivation and/or inhibition of the pathogenicity by direct interaction with target molecules, IgG1 and IgG3 have the ability to activate subsequent host immune responses, such as complement pathways, Fcγ receptor-mediated immune responses, and antibody-dependent cellular cytotoxicity. Such arms of responses are shown to significantly contribute to the elimination of pathogens and inactivation of pathogenic factors and some reports suggest that IgG3 has the strongest effector functions among all IgG subclasses (Cao et al., 2013; Hofmeister et al., 2011; Irani et al., 2015; Scharf et al., 2001; Natsume et al., 2008; Stapleton et al., 2011). With regard to the kinetics of the trans-placental transfer of maternal antibodies to the fetus, the efficiency of the IgG transport by neonatal Fc receptor (FcRn) in the placenta seems to marginally depend on its subclasses, i.e., a little higher efficiency for IgG1 and a little lower efficiency for IgG2, but is significantly reduced due to maternal diseases such as placental malaria or hyperglobulinemia, in which the transfer of IgG1 and IgG2 but not that of IgG3 is impaired (Hamilton, 1987; Okoko et al., 2001). A past clinical study focused on the comparison of levels of RSV-specific IgG1 and IgG3 in patients, and its results suggested that the avidity of IgG1 against the whole RSV protein correlates with the protection against RSV infection in infants <3 months old. The correlation between avidity of IgG3 and protection against RSV infection was hardly characterized because of lower concentrations of IgG3 as compared with IgG1 (Freitas et al., 2011).

Here, in order to examine the dynamics of human immune responses against acute RSV infections, we designed a clinical study targeting 2 different age segments (child patients <3 years old and adult patients) and obtained blood samples in both the acute and convalescent phases. Based on quantitative and qualitative multivariate analyses of the immune response against RSV, age-specific profiles and maturation of antibody responses were elucidated. Such results will significantly contribute to the design of RSV vaccines, i.e., adding adjuvants in the formulation and/or selection of the optimal route of administration to overcome the immaturity of immune responses in infants, and to their clinical evaluation by harnessing such parameters as biomarker candidates for determining efficacies of vaccines containing F protein antigen.

2. Materials and Methods

2.1. Design of Clinical Research

This clinical research was conducted throughout Japan from October 2014 to April 2016 at 6 regional base hospitals having pediatric departments, and 3 pediatric clinics in Tokyo and Kanagawa prefectures in Japan, and 13 medical clinics belonging to the Influenza Study Group of the Japan Physicians Association. In order to confirm RSV infections in medically attended patients having acute respiratory illness, nasal swab samples were obtained by investigators from those who were suspected of having been infected with RSV, and those consenting to the testing were examined by using an immunochromatographic test kit for RSV (Imunoace RSV Neo®). The exclusion criteria included: 1) any history of palivizumab administration, 2) any infection with HBV, HCV, syphilis, or HIV, 3) any participation in another clinical research or clinical trial, 4) any other conditions that were judged by investigators as inadequate for joining this clinical research. After enrollment, acute phase blood samples were taken from participants on the first day visiting the medical institutions where they were diagnosed as having been infected with RSV for the first time in the RSV season. The data of acute serum samples at <6 days after onset of fever were analyzed, since 6 days or greater after illness onset will likely have RSV-specific responses from the infection, which was not suggested to represent a true baseline response. For child participants, blood was again taken as samples of the convalescent phase at 2 to 3 weeks after the first visit, whereas for adult participants, blood was again taken at 2 weeks after the first visit. Nasal swab samples collected by flocked swabs were taken on the day of the first visit, the RSV genome was extracted from them, and then RSV group A and B were determined based on cDNA sequences of the G protein gene. This study was approved by the institutional review boards of Yokohama City University, Japan Physicians Association, National Institute of Biomedical Innovation, Health and Nutrition, and Daiichi Sankyo Co., Ltd. The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all parents of child participants and all adult participants before enrollment.

2.2. Materials

Hep-2 cells (ATCC) were maintained in MEM supplemented with 10% FBS, 1 mM sodium pyruvate, 1% non-essential amino acids solution and 1% penicillin-streptomycin solution at 37 °C with 5% CO_2. RSV strains, A2, and 18,537 were purchased from ATCC. Palivizumab (Synagis®) was purchased from AbbVIE Inc. A neutralization mouse monoclonal antibody against antigenic site I (131-2A) was purchased from Millipore, whereas that against antigenic site IV (6H6-2B4) was obtained by selecting hybridoma clones produced from rats immunized with the recombinant F protein. A recombinant neutralization antibody, palivizumab (rat Fc) recognizing antigenic site II, was genetically engineered by fusion of the $F(ab')_2$ region of palivizumab to the rat Fc region, and expressed in HEK293F cells.

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