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[Review]

## Neutralizing epitopes of RSV and palivizumab resistance in Japan

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### Abstract

Respiratory Syncytial Virus (RSV) is one of the most important viral pathogen related to acute lower respiratory infection in young children. The virus surface envelope contains the G, F, and SH proteins as spike proteins. The F protein is considered to be a major antigenic target for the neutralizing (NT) epitope as only the F protein is essential for cell infection among the three viral envelope proteins, and it is more highly conserved than the G protein. Recently, four antigenic targets related to NT activity have been reported; site I, site II, site IV, and site zero (0). Site II is the target for palivizumab used throughout the world to suppress severe RSV infection as passive immunity in high-risk children since 1998. Under the recent conditions in which indications for palivizumab administered subjects are being expanded, palivizumab-resistant mutations have been confirmed overseas in children with RSV infection, although they remain infrequent. Therefore, continuous genetic analysis of the palivizumab-binding region of the F protein is necessary. In addition, as vaccine development progresses, RSV infection control is expected to improve greatly over the next decade.

**Key words :** RSV, neutralizing epitope, palivizumab, resistance, Japan

### Introduction

Respiratory Syncytial Virus (RSV) is one of the most important viral pathogen related to acute lower respiratory infection (ALRI) in young children<sup>1</sup>. RSV infection accounts for 1/3 of deaths from ALRI in infants aged under 1 year and is the second largest cause of death from infectious diseases worldwide after malaria, with up to 200 thousand children, mainly in developing countries, losing their lives due to RSV infection each year<sup>2</sup>. In addition, the severity of infection in the high-risk group<sup>3,4</sup> as well as the onset of airway hyperresponsiveness/asthma after RSV infection are problems<sup>5,6</sup>. Patients with RSV infection usually receive symptomatic treatment, including oxygen administration, fluid replacement, and respiratory management<sup>7</sup>. RSV monoclonal antibodies have been administered to high-risk children as a prophylactic measure<sup>8</sup>. RSV monoclonal antibodies have been administered to high-risk children as a prophylactic measure, on the

other hand, there are several ongoing trials in pre-clinical, Phase-I, Phase-II, or Phase-III clinical stages for RSV vaccine development<sup>9</sup>. Analysis of the RSV epitope is important for the development of more effective RSV monoclonal antibodies and vaccines.

### Virological structure

RSV is a single-stranded (–) RNA virus classified as a member of the genus Orthopneumovirus in the family Pneumoviridae, and is composed of 10 genes including NS1, NS2 encoding nonstructural protein, N encoding nucleoprotein, P encoding phosphoprotein, M/M2 encoding matrix protein, SH encoding small hydrophobic protein (SH protein), G encoding large glycoprotein (G protein), F encoding fusion protein (F protein), and L encoding large polymerase protein. The virus surface envelope contains the G, F, and SH proteins as spike proteins. RSV is classified into two subgroups, A and B, due

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<https://www.jstage.jst.go.jp/browse/fms> <http://www.fmu.ac.jp/home/lib/F-igaku/>

to differences in reactivity with monoclonal antibodies to the G protein<sup>10</sup>.

### F protein

The F protein of RSV is a 574 amino acids class I fusion glycoprotein similar to influenza virus hemagglutinin and HIV-1 envelope glycoprotein<sup>11,12</sup>. It is synthesized as a precursor F<sub>0</sub> and cleaved by the furin-like host protease to produce F<sub>1</sub> and F<sub>2</sub> subunits<sup>13,14</sup>. The mature protein contains three copies of two polypeptides (F<sub>1</sub> and F<sub>2</sub>). After initially folding into a metastable prefusion conformation (pre-F), RSV F undergoes a structural change naturally or in the process of infection, and eventually acquires a stable postfusion conformation (post-F)<sup>12</sup>. Recently, cryoelectron tomography of cell culture-grown RSV has revealed that pre-F and post-F are present on the virion surface. In addition, The pre-F and post-F are about 11 nm and 16 nm high, with the distribution dependent on the age and state of the virus<sup>15,16</sup>.

### Neutralizing epitope

Of the 11 proteins that comprise RSV, only the F and G proteins are targeted by neutralizing anti-

bodies<sup>17</sup>. The F protein is considered to be a major antigenic target for the neutralizing (NT) epitope as only the F protein among the three viral envelope proteins is essential for cell infection, and F protein is more highly conserved than G protein<sup>18</sup>. Four antigenic targets related to NT activity have been reported. Site I is an antigenic target for monoclonal antibodies (mAbs), such as 2F, 44F, or 45F<sup>19</sup>, with weak or negligible NT activity. Site II is recognized by murine 47F and 1129<sup>20,21</sup>, with humanization of the latter resulting in palivizumab<sup>22</sup> and its more potent derivative motavizumab<sup>23</sup>. Palivizumab is licensed and widely used throughout the world to suppress severe RSV infection in high-risk children<sup>24</sup>. Site IV is recognized by mAb 19<sup>25</sup> or 101 F<sup>26</sup> with moderate NT activity. A recently isolated human mAb, 54G10, shows NT activity for human metapneumovirus (hMPV), which belongs to genus paramyxovirus as RSV<sup>27</sup>. The 54G10 mAb is predicted to recognize antigenic site IV. Of these three epitopes, site I exists only in post-F, and site II and site IV exist in both pre-F and post-F. All the mAbs that recognize these three sites can bind to the stable post-F conformation<sup>28</sup>. Studies using RSVIG, an immunoglobulin product enriched for high RSV NT activity, showed that adsorbing the product with post-F did not remove the NT activity

Table 1. Conformation of the F protein and epitopes

Conformation	Stability	Epitope	Ab(s) <sup>a)</sup>	Remarks regarding Ab <sup>b)</sup>
Pre-F <sup>c)</sup>	metastable	site 0 (zero)	D25, AM22, 5C4	✓ 10- to 100-fold more potent than palivizumab
		site II	murine 47F, 1129	✓ Mouse monoclonal antibody that was the basis of palivizumab development.
			palivizumab	✓ a licensed mAb administered prophylactically to infants at high risk of severe RSV-related disease
			motavizumab	✓ a more potent derivative of palivizumab
		site IV	101F, Mab19	✓ moderate NT activity <sup>b)</sup>
			54G10	✓ neutralizes hMPV and RSV
Post-F <sup>c)</sup>	stable	site I	2F, 44F, 45F	✓ weak or negligible NT activity <sup>b)</sup>
		site II	47F, 1129 palivizumab motavizumab	(same as Pre-F remarks)
			site IV	101F, Mab19
		54G10		(same as Pre-F remarks)

a) Ab(s) shows antibody or antibodies.

b) NT activity means neutralizing activity.

c) Pre-F, and Post-F indicate prefusion conformation F, and post-fusion conformation F, respectively.

from the immunoglobulins elicited by natural infection<sup>17</sup>), suggesting that there are other NT-sensitive targets on the pre-F. Site zero (0) was recently revealed to exist in the pre-F as the major fourth antigenic target<sup>28</sup>). Monoclonal antibodies against site 0 include D25, AM22, and 5C4, all of which are pre-fusion-specific and have NT activity 10–100 fold greater than that of palivizumab<sup>28,29</sup>). MPE8 is an additional pre-fusion-specific antibody that which cross-competes with palivizumab for binding to pre-F<sup>30</sup>). MPE8 can neutralize not only human and bovine RSV, but also hMPV and pneumonia virus in mice, suggesting that the MPE8 epitope is particularly well-conserved among pneumoviruses<sup>30</sup>) (Table 1). Based on the recently revealed characteristics of neutralizing epitope against RSV, not only new monoclonal Abs but also vaccines as the final goal of overcoming infectious diseases are expected in the future.

### Palivizumab

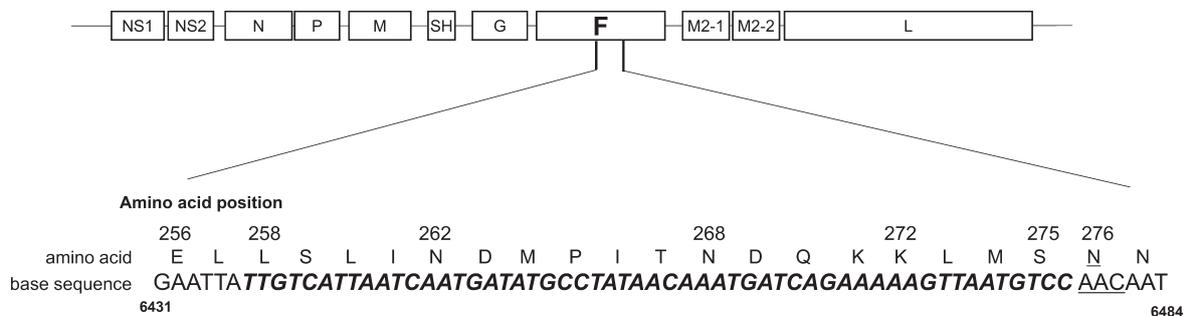
For use in providing passive immunity to RSV infection, a humanized monoclonal antibody palivizumab (Synagis<sup>®</sup>) targeting the antigenic site II (also called site A) region of the RSV F protein has been developed by genetic recombination technology<sup>22,31</sup>) and is now used throughout the world. There are many antigenic variations in the G protein among subtypes, whereas there are few for the F protein. Therefore, an antibody targeting the F protein was created. On the other hand, the G protein is the most variable RSV protein, making it useful for RSV evolution studies<sup>12</sup>). Palivizumab has been approved and used in the United States since 1998 with the aim of suppressing the severity of RSV infection in high-risk children, such as preterm infants, and those with congenital heart disease or immunodeficiency<sup>32</sup>). It is currently used in more

than 60 countries and has been shown to be effective in preventing severe infection in the high-risk group and subsequently alleviating airway hyperresponsiveness and asthma episodes<sup>33–35</sup>).

The indications for palivizumab administration of USA is different from that of Japan. The American Academy of Pediatrics guidance for palivizumab use has become more restrictive against otherwise healthy preterm infants born at or after 29 weeks of gestation<sup>36</sup>). In Japan, palivizumab was listed as a prescribed drug from 2002, with the subjects for administration being preterm infants (newborns and infants under 12 months of age with preterm birth from 29 weeks to 35 weeks or newborns and infants under 6 months of age with premature birth of 28 weeks or less of gestation), neonates, infants and young children under 24 months of age who have received treatment for bronchopulmonary dysplasia within the past 6 months. In 2005, palivizumab dosing indications were added for newborns, infants and young children with congenital heart disease with abnormal hemodynamics below 24 months of age. Furthermore, indications for neonates, infants and young children with immunodeficiency or Down's syndrome less than 24 months of age were added in 2013<sup>37–39</sup>).

### Palivizumab resistance

Recently, there have been reports on the detection of viruses showing palivizumab neutralizing-resistant amino acid mutations. Clinical isolates N262D<sup>40</sup>), K272E/M/Q<sup>41,42</sup>), and S275F/L<sup>40,42,43</sup>) were reported as possessing mutations in the F protein region palivizumab-binding site (amino acids 258–275) and exhibiting resistance to palivizumab neutralization, while laboratory induced isolates N268I, and K272N/T/M/Q have also been reported<sup>43–46</sup>). The N276S mutation adjacent to the palivizumab-



binding site in RSV-A was confirmed in Israel and Turkey in the 2007–2008 season<sup>40</sup>) and was dominant in Canada between 2008 and 2010<sup>41</sup>). Adams *et al.* showed that the N276S mutation in RSV-A leads to a decrease in NT activity against palivizumab<sup>47</sup>). This mutant virus was isolated from preterm infants 4 months old who were administered palivizumab but were treated in an intensive care unit for RSV infection. Furthermore, when the isolate virus was cultured in the presence of palivizumab, a second amino acid mutation, K272E, occurred in the palivizumab-binding site abolishing its ability to bind to palivizumab. On the other hand, Papenburg *et al.* conducted F gene analysis of 23 RSV strains isolated from patients with palivizumab and RSV 100 strains isolated from children without palivizumab during the 4 seasons from 2006 to 2010<sup>41</sup>). The N276S mutation was detected in RSV-A at a rate of 44.4% in 2008–2009 and 100% in 2009–2010. Furthermore, they reported that the N276S mutation was observed in children both with and without a history of palivizumab treatment<sup>41</sup>). In addition, Zhu *et al.* demonstrated that the N276S mutation in RSV-A and the S276N mutation in RSV-B did not result in palivizumab neutralization resistance based on neutralization tests using clinical isolates and recombinant virus<sup>40</sup>). However, in a mouse experiment, mutation of the 276th amino acid in the F protein was shown to induce MARMs (Monoclonal Antibody-Resistant Mutants) showing resistance to the mouse monoclonal antibody against the antigenic site II region<sup>20,44</sup>), indicating that the 276th amino acid is susceptible to selection pressure by the antibody.

Zhu *et al.* investigated natural gene polymorphisms involving the F protein in RSV isolates from patients with respiratory disease without palivizumab treatment<sup>40,43</sup>). The detection frequency of palivizumab neutralization resistant mutations was 0.7%, and these mutations were reported to be random mutations that occurred due to selective pressure by maternal or the patient's own NT antibodies against site II of the RSV F protein. On the other hand, they also reported that the detection frequency of palivizumab-resistant mutations in RSV isolated from patients receiving palivizumab treatment was 5.4%<sup>43</sup>). The amino acid at position 276 of the F protein is generally asparagine (N) for RSV-A and serine (S) for RSV-B<sup>40,47</sup>). It is possible that the N276S mutation in RSV-A and S276N mutation in RSV-B appeared due to hybridization between the subgroups, natural mutation and so on. However, since the N276S mutation has not been reported in

RSV isolated before the use of palivizumab, the predominance of the N276S mutation may be related to some selective pressure attributable to palivizumab<sup>20,44</sup>). Since the palivizumab binding ability is completely lost when a second amino acid mutation is introduced following the N276S mutation, the N276S mutation is considered to be the first amino acid mutation inducing palivizumab neutralization resistance.

Recently, the ON1 strain of RSV-A<sup>48,49</sup>) and BA strain of RSV-B<sup>50</sup>) have spread to various parts of the world over several seasons. These strains possess mutations in the G protein associated with adsorption to host cells, indicating that the immunogenicity of the G protein is involved in the epidemic expansion. On the other hand, the N276S mutation is a mutation in the F protein. The global dominance of the N276S mutation in the short term is thought to be due to some factors positively affecting virus growth. Viruses having palivizumab neutralization resistance mutations (K272 E/Q and S275 F/L) have a lower proliferative capacity than do wild type strains<sup>40</sup>). It is necessary to investigate the relation of virus proliferation ability and infectivity to amino acid mutations in segments other than antibody recognition segments, such as the N276S mutation.

#### The situation in Japan

Apart from our previous report<sup>51</sup>), there have been no reports on palivizumab resistance in Japan. We analyzed palivizumab resistance using clinical isolates or PCR products derived from 116 airway secretion specimens from patients diagnosed with RSV infection in Miyagi prefecture between 2004 and 2008, and in Fukushima prefecture between 2008 and 2013. Only one RSV patient in 2013 received prophylaxis with palivizumab. The nucleotide sequence and amino acid sequence of amino acids 215–313 including the F protein region palivizumab-binding site were analyzed for all 116 strain. A comparison of the RSV-A and RSV-A Long strain revealed 23 silent mutations and 9 mutations (N228I/T, E236A, V239I, L258I, N276S, V281L, R282S, S290P, and D310H). A comparison of the RSV-B and RSV-B 9320 strain revealed 35 silent mutations and 4 mutations (S228N/T, L247V, S276N, and I305L). Among the detected amino acid mutations, the only mutation in the palivizumab-binding site was the L258I mutation in RSV-A, which was detected from a specimen from a patient not receiving palivizumab. The frequency of muta-

Table 2-1. Mutations and detection rates in the F protein of RSV-A

RSV-A ( <i>n</i> =106)	
Amino acid mutation <sup>a)</sup>	Number of mutants (%) <sup>b)</sup>
228 (Asn → Ile)	1 (0.9)
228 (Asn → Thr)	2 (1.9)
236 (Glu → Ala)	6 (5.7)
239 (Val → Ile)	1 (0.9)
258 (Leu → Ile)	2 (1.9)
276 (Asn → Ser)	87 (82.1)
281 (Val → Leu)	1 (0.9)
282 (Arg → Ser)	1 (0.9)
290 (Ser → Pro)	3 (2.8)
310 (Asp → His)	3 (2.8)

- a) Amino acid mutations were identified in comparison with the Long strain (Accession No. AY911262) of RSV-A.
- b) The detection frequency of mutants was obtained by dividing the number of mutant strains by the total number of strains analyzed.
- c) The palivizumab-binding site is in region of amino acid 258-275.
- d) The table was cited from the reference 51.

Table 2-2. Mutations and detection rates in the F protein of RSV-B

RSV-B ( <i>n</i> =10)	
Amino acid mutation <sup>a)</sup>	Number of mutants (%) <sup>b)</sup>
228 (Ser → Asn)	9 (90)
228 (Ser → Thr)	1 (10)
236 (Glu → Ala)	2 (20)
247 (Leu → Val)	10 (100)
276 (Ser → Asn)	5 (50)
305 (Ile → Leu)	10 (100)

- a) Amino acid mutations were identified in comparison with the 9320 strain (Accession No. AY353550) of RSV-B.
- b) The detection frequency of mutants was obtained by dividing the number of mutant strains by the total number of strains analyzed.
- c) The palivizumab-binding site is in region of amino acid 258-275.
- d) The table was cited from the reference 51.

tions other than N276S in RSV-A was 6% or less (0.9 to 5.7%). The frequency of N276S was 82.1% (82 out of 106 strains) in RSV-A (Table 2-1), and the frequency of the S276N mutation in RSV-B was 50% (5 out of 10 strains) (Table 2-2). The frequency of the N276S mutation in RSV-A is 0% (0/2) in 2004, 0% (0/3) in 2005, 0% (0/3) in 2006, 60% (3/5) in 2007, 22.2% (2/9) in 2009, 95.6% (22/23) in 2009, 96.8% (31/32) in 2010, 100% (4/4) in 2011, 100%

Table 3. Annual change in N276S mutations in RSV-A

Year	Number of N276S mutations (%) <sup>a)</sup>
2004	0/2 (0)
2005	0/3 (0)
2006	0/3 (0)
2007	3/5 (60.0)
2008	2/9 (22.2)
2009	22/23 (95.6)
2010	31/32 (96.8)
2011	4/4 (100)
2012	7/7 (100)
2013	18/18 (100)

- a) One-hundred and sixteen RSV clinical strains or PCR products of RSV-A were analyzed to detect N276S. The detection frequency for N276S was obtained by dividing the number of strains with N276S by the total number of strains analyzed in each year.
- b) The table was cited from the reference 51 with slight modifications.

(7/7) in 2012, and 100% (18/18) in 2013. In other words, the N276S mutation began to appear around 2007-2008 and was found in over 90% of the analyzed strains after 2009 (Table 3). Thus, the results in Japan are similar to those reported overseas<sup>40)</sup>. Furthermore, the K272E mutation as second mutation was not reported in Japan and was not observed in our analysis. Analysis in children with RSV infection administered with palivizumab is necessary in the future.

### Conclusion

Under the recent conditions in which indications for palivizumab administration are being expanded, palivizumab-resistant mutations have been confirmed overseas in children with RSV infection, although they remain infrequent at present. In Japan, the N276S mutation thought likely to lead to palivizumab neutralization resistance is becoming dominant. Therefore, continuous genetic analysis of the F protein palivizumab-binding region is necessary. On the other hand, site 0, which leads to strong NT activity was also identified, and the development of monoclonal antibodies with site 0 as an epitope is certain to advance in the future. Vaccine development is also progressing, so that the control of RSV infection is expected to change markedly over the next decade.

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### Conflicts of interest

The authors have no conflict of interest to disclose with respect to this manuscript.

### References

- Nair H, Nokes DJ, Gessner BD, *et al.* Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children : a systematic review and meta-analysis. *Lancet*, **375** : 1545-1555, 2010.
- Lozano R, Naghavi M, Foreman K, *et al.* Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010 : a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*, **380** : 2095-2128, 2012.
- Hall CB, Simoes EA, Anderson LJ. Clinical and epidemiologic features of respiratory syncytial virus. *Curr Top Microbiol Immunol*, **372** : 39-57, 2013.
- Sommer C, Resch B, Simoes EA. Risk factors for severe respiratory syncytial virus lower respiratory tract infection. *Open Microbiol J*, **5** : 144-154, 2011.
- Kneyber MCJ, Steyerberg EW, de Groot R, Moll HA. Long-term effects of respiratory syncytial virus (RSV) bronchiolitis in infants and young children : a quantitative review. *Acta Paediatr*, **89** : 654-660, 2000.
- Mohapatra SS, Boyapalle S. Epidemiologic, experimental, and clinical links between respiratory syncytial virus infection and asthma. *Clin Microbiol Rev*, **21** : 495-504, 2008.
- Ralston SL, Lieberthal AS, Meissner HC, *et al.* Clinical practice guideline : the diagnosis, management, and prevention of bronchiolitis. *Pediatrics*, **134** : e1474-1502, 2014.
- Subramanian KN, Weisman LE, Rhodes T, *et al.* Safety, tolerance and pharmacokinetics of a humanized monoclonal antibody to respiratory syncytial virus in premature infants and infants with bronchopulmonary dysplasia. *MEDI-493 Study Group. Pediatr Infect Dis J*, **17** : 110-115, 1998.
- Rezaee F, Linfield DT, Harford TJ, Piedimonte G. Ongoing developments in RSV prophylaxis : a clinician's analysis. *Curr Opin Virol*, **24** : 70-78, 2017.
- Collins PL, Fearn R, Graham BS. Respiratory syncytial virus : virology, reverse genetics, and pathogenesis of disease. *Curr Top Microbiol Immunol*, **372** : 3-38, 2013.
- Collins PL, Huang YT, Wertz GW. Nucleotide sequence of the gene encoding the fusion (F) glycoprotein of human respiratory syncytial virus. *Proc Natl Acad Sci U S A*, **81** : 7683-7687, 1984.
- McLellan JS, Ray WC, Peeples ME. Structure and function of respiratory syncytial virus surface glycoproteins. *Curr Top Microbiol Immunol*, **372** : 83-104, 2013.
- Gruber C, Levine S. Respiratory syncytial virus polypeptides. III. The envelope-associated proteins. *J Gen Virol*, **64(Pt 4)** : 825-832, 1983.
- Day ND, Branigan PJ, Liu C, *et al.* Contribution of cysteine residues in the extracellular domain of the F protein of human respiratory syncytial virus to its function. *Virol J*, **3** : 34, 2006.
- Liljeroos L, Krzyzaniak MA, Helenius A, Butcher SJ. Architecture of respiratory syncytial virus revealed by electron cryotomography. *Proc Natl Acad Sci U S A*, **110** : 11133-11138, 2013.
- Graham BS, Modjarrad K, McLellan JS. Novel antigens for RSV vaccines. *Curr Opin Immunol*, **35** : 30-38, 2015.
- Magro M, Mas V, Chappell K, *et al.* Neutralizing antibodies against the preactive form of respiratory syncytial virus fusion protein offer unique possibilities for clinical intervention. *Proc Natl Acad Sci U S A*, **109** : 3089-3094, 2012.
- Melero JA, Moore ML. Influence of respiratory syncytial virus strain differences on pathogenesis and immunity. *Curr Top Microbiol Immunol*, **372** : 59-82, 2013.
- Calder LJ, Gonzalez-Reyes L, Garcia-Barreno B, *et al.* Electron microscopy of the human respiratory syncytial virus fusion protein and complexes that it forms with monoclonal antibodies. *Virology*, **271** : 122-131, 2000.
- Beeler JA, van Wyke Coelingh K. Neutralization epitopes of the F glycoprotein of respiratory syncytial virus : effect of mutation upon fusion function. *J Virol*, **63** : 2941-2950, 1989.
- Arbiza J, Taylor G, Lopez JA, *et al.* Characterization of two antigenic sites recognized by neutralizing monoclonal antibodies directed against the fusion glycoprotein of human respiratory syncytial

- virus. *J Gen Virol*, **73**(Pt 9) : 2225-2234, 1992.
22. Johnson S, Oliver C, Prince GA, *et al.* Development of a humanized monoclonal antibody (MEDI-493) with potent in vitro and in vivo activity against respiratory syncytial virus. *J Infect Dis*, **176** : 1215-1224, 1997.
  23. Wu H, Pfarr DS, Johnson S, *et al.* Development of motavizumab, an ultra-potent antibody for the prevention of respiratory syncytial virus infection in the upper and lower respiratory tract. *J Mol Biol*, **368** : 652-665, 2007.
  24. Rogovik AL, Carleton B, Solimano A, Goldman RD. Palivizumab for the prevention of respiratory syncytial virus infection. *Can Fam Physician*, **56** : 769-772, 2010.
  25. Taylor G, Stott EJ, Furze J, Ford J, Sopp P. Protective epitopes on the fusion protein of respiratory syncytial virus recognized by murine and bovine monoclonal antibodies. *J Gen Virol*, **73**(Pt 9) : 2217-2223, 1992.
  26. Magro M, Andreu D, Gomez-Puertas P, Melero JA, Palomo C. Neutralization of human respiratory syncytial virus infectivity by antibodies and low-molecular-weight compounds targeted against the fusion glycoprotein. *J Virol*, **84** : 7970-7982, 2010.
  27. Schuster JE, Cox RG, Hastings AK, *et al.* A broadly neutralizing human monoclonal antibody exhibits in vivo efficacy against both human metapneumovirus and respiratory syncytial virus. *J Infect Dis*, **211** : 216-225, 2015.
  28. McLellan JS, Chen M, Leung S, *et al.* Structure of RSV fusion glycoprotein trimer bound to a prefusion-specific neutralizing antibody. *Science*, **340** : 1113-1117, 2013.
  29. Kwakkenbos MJ, Diehl SA, Yasuda E, *et al.* Generation of stable monoclonal antibody-producing B cell receptor-positive human memory B cells by genetic programming. *Nat Med*, **16** : 123-128, 2010.
  30. Corti D, Bianchi S, Vanzetta F, *et al.* Cross-neutralization of four paramyxoviruses by a human monoclonal antibody. *Nature*, **501** : 439-443, 2013.
  31. Wu H, Pfarr DS, Losonsky GA, Kiener PA. Immunoprophylaxis of RSV infection: advancing from RSV-IGIV to palivizumab and motavizumab. *Curr Top Microbiol Immunol*, **317** : 103-123, 2008.
  32. Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. The IMPact-RSV Study Group. *Pediatrics*, **102** : 531-537, 1998.
  33. Simoes EA, Groothuis JR, Carbonell-Estrany X, *et al.* Palivizumab prophylaxis, respiratory syncytial virus, and subsequent recurrent wheezing. *J Pediatr*, **151** : 34-42, 42 e31, 2007.
  34. Yoshihara S, Kusuda S, Mochizuki H, *et al.* Effect of palivizumab prophylaxis on subsequent recurrent wheezing in preterm infants. *Pediatrics*, **132** : 811-818, 2013.
  35. Wegzyn C, Toh LK, Notario G, *et al.* Safety and Effectiveness of Palivizumab in Children at High Risk of Serious Disease Due to Respiratory Syncytial Virus Infection: A Systematic Review. *Infect Dis Ther*, **3** : 133-158, 2014.
  36. American Academy of Pediatrics Committee on Infectious D, American Academy of Pediatrics Bronchiolitis Guidelines C. Updated guidance for palivizumab prophylaxis among infants and young children at increased risk of hospitalization for respiratory syncytial virus infection. *Pediatrics*, **134** : 415-420, 2014.
  37. Kusuda S, Takahashi N, Saitoh T, *et al.* Survey of pediatric ward hospitalization due to respiratory syncytial virus infection after the introduction of palivizumab to high-risk infants in Japan. *Pediatr Int*, **53** : 368-373, 2011.
  38. Mori M, Kawashima H, Nakamura H, *et al.* Nationwide survey of severe respiratory syncytial virus infection in children who do not meet indications for palivizumab in Japan. *J Infect Chemother*, **17** : 254-263, 2011.
  39. Mori M, Morio T, Ito S, *et al.* Risks and prevention of severe RS virus infection among children with immunodeficiency and Down's syndrome. *J Infect Chemother*, **20** : 455-459, 2014.
  40. Zhu Q, Patel NK, McAuliffe JM, *et al.* Natural polymorphisms and resistance-associated mutations in the fusion protein of respiratory syncytial virus (RSV): effects on RSV susceptibility to palivizumab. *J Infect Dis*, **205** : 635-638, 2012.
  41. Papenburg J, Carbonneau J, Hamelin ME, *et al.* Molecular evolution of respiratory syncytial virus fusion gene, Canada, 2006-2010. *Emerg Infect Dis*, **18** : 120-124, 2012.
  42. Boivin G, Caouette G, Frenette L, Carbonneau J, Ouakki M, De Serres G. Human respiratory syncytial virus and other viral infections in infants receiving palivizumab. *J Clin Virol*, **42** : 52-57, 2008.
  43. Zhu Q, McAuliffe JM, Patel NK, *et al.* Analysis of respiratory syncytial virus preclinical and clinical variants resistant to neutralization by monoclonal antibodies palivizumab and/or motavizumab. *J Infect Dis*, **203** : 674-682, 2011.
  44. Crowe JE, Firestone CY, Crim R, *et al.* Monoclonal antibody-resistant mutants selected with a respiratory syncytial virus-neutralizing human antibody Fab fragment (Fab 19) define a unique epitope on the fusion (F) glycoprotein. *Virology*, **252** :

- 373-375, 1998.
45. Zhao X, Chen FP, Sullender WM. Respiratory syncytial virus escape mutant derived in vitro resists palivizumab prophylaxis in cotton rats. *Virology*, **318** : 608-612, 2004.
  46. Zhao X, Liu E, Chen FP, Sullender WM. In vitro and in vivo fitness of respiratory syncytial virus monoclonal antibody escape mutants. *J Virol*, **80** : 11651-11657, 2006.
  47. Adams O, Bonzel L, Kovacevic A, Mayatepek E, Hoehn T, Vogel M. Palivizumab-resistant human respiratory syncytial virus infection in infancy. *Clin Infect Dis*, **51** : 185-188, 2010.
  48. Agoti CN, Otieno JR, Gitahi CW, Cane PA, Nokes DJ. Rapid spread and diversification of respiratory syncytial virus genotype ON1, Kenya. *Emerg Infect Dis*, **20** : 950-959, 2014.
  49. Tsukagoshi H, Yokoi H, Kobayashi M, *et al.* Genetic analysis of attachment glycoprotein (G) gene in new genotype ON1 of human respiratory syncytial virus detected in Japan. *Microbiol Immunol*, **57** : 655-659, 2013.
  50. Trento A, Viegas M, Galiano M, *et al.* Natural history of human respiratory syncytial virus inferred from phylogenetic analysis of the attachment (G) glycoprotein with a 60-nucleotide duplication. *J Virol*, **80** : 975-984, 2006.
  51. Sato T, Hashimoto K, Sato M, Miyazaki K, Hosoya M. Amino Acid Substitutions-Associated Palivizumab-Resistant RSV in Japan, 2004-2013. *Clinical Virology (Japanese)*, **43** : 296-303, 2015.