

# Immunoglobulin A Antibody Response to Respiratory Syncytial Virus Structural Proteins in Colostrum and Milk

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**Immunoglobulin A (IgA) antibody response to respiratory syncytial virus (RSV) structural proteins in colostrum and milk was investigated by a radioimmunoprecipitation assay. By using [<sup>35</sup>S]methionine-labeled RSV-infected HEp-2 cells and antiserum to human IgA as the capture antibody, IgA antibody responses to large glycoprotein, fusion protein, nucleoprotein, phosphoprotein, and matrix protein were demonstrated in colostrum and milk. The IgA antibody response was mainly directed against fusion protein, whereas IgA activity against matrix protein was more variable and was not comparable to the antibody responses to other structural proteins. Maternal mammary IgA response after RSV infection in the infant was monitored in four cases, and the appearance of anti-RSV IgA activity against several RSV structural proteins was observed in convalescent-stage milk samples of two mothers in whom RSV infection was demonstrated.**

A beneficial effect of breast feeding on respiratory infections in the first months of life has been observed (1, 2). To what extent this works for a reduction in infection rates has not been clear, but breast feeding has been shown to have some protective effect against respiratory viruses, including respiratory syncytial virus (RSV), a major cause of respiratory infection during early infancy (4, 8). Furthermore, most mothers secrete colostrum immunoglobulin A (IgA) antibody specific for RSV (9), and the booster response of mammary IgA antibody specific for RSV has been demonstrated during lactation (3, 7). However, it is not yet clear how this passive protection is mediated. Moreover, IgA antibody responses to individual RSV-specific proteins have not been reported.

The present study was designed to characterize the colostrum and milk IgA antibody responses to different structural components of RSV.

## MATERIALS AND METHODS

**Colostrum and milk samples.** One colostrum sample was obtained randomly from each of seven women (no. 1 to 7) who gave birth in 1986 and 1987. Samples of human milk were obtained from four mothers (numbers 8 to 11) of breast-fed infants who were admitted to the hospital of Sapporo Medical College because of RSV infection. The mean age of these infants at admission was 3.5 months. RSV infection was confirmed by isolation of the virus from nasopharyngeal swab specimens. Nasal swabs were also collected from four mothers for RSV isolation irrespective of the presence or absence of clinical signs of respiratory tract infection. Colostrum and milk were obtained by manual expression and defatted by centrifugation (10,000 × g, 3 min), and the aqueous layer was stored at -20°C until the time of analysis. As controls, sera of two healthy adults were used.

**RSV antibody determination.** Class-specific antibodies to RSV were determined by indirect immunofluorescence (IF) analysis (10). Briefly, RSV (Long strain)-infected and -uninfected HEp-2 cells were trypsinized, air dried on glass slides, and fixed in acetone (4°C, 10 min). Colostrum and milk were titrated starting at a 1:8 dilution, and fluorescein

isothiocyanate-conjugated anti-human IgA or IgG (MBL, Nagoya, Japan) was used in the final phase of the assay. Nonspecific staining was not seen in these dilutions of specimens.

**RIP.** The methods for radioimmunoprecipitation (RIP) have been described previously (10). HEp-2 cells were infected with RSV Long strain, and viral antigens were labeled with [<sup>35</sup>S]methionine (Amersham International, Amersham, England) for 2 to 4 h. The monolayer was scraped, washed with phosphate-buffered saline, and dissolved on ice with a lysis buffer (0.5% Nonidet P-40, 10 mM Tris hydrochloride [pH 7.5], and 0.15 M NaCl) for 10 min at 4°C. The lysate was then spun in an Eppendorf centrifuge for 15 min at 4°C and brought to four times its volume with RIP buffer (1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 0.2 mM phenylmethylsulfonyl fluoride, 10 mM Tris hydrochloride [pH 7.5], and 0.15 M NaCl). Colostrum and milk samples were mixed with 100 to 200 μl of RSV antigen preparation at a final concentration of 1:20 and incubated overnight at 4°C. Then goat antiserum to human IgA or IgG (5 to 10 μl) (MBL) was added, and the mixture was incubated for 3 h at 4°C. The resultant immune complexes were precipitated with protein A-Sepharose CL-4B beads (Pharmacia AB, Uppsala, Sweden) and analyzed by sodium dodecyl sulfate-11% polyacrylamide gel electrophoresis under reducing conditions. After electrophoresis, the gels were fixed for fluorography, dried, and exposed to Kodak X-AR5 film.

## RESULTS

**Detection of IgA antibody to RSV in colostrum.** Human IgA does not bind appreciably to protein A (6). Therefore, in this study, a goat antiserum to human IgA was added to RSV antigen-IgA antibody complexes to enhance immunoprecipitation. Goat anti-human IgG was also used to demonstrate anti-RSV IgG antibodies. All samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. In Fig. 1 and 2, lanes a, RSV antigen-lactation product complexes are demonstrated. In the other two types of lanes, RSV antigens complexed with antibodies in human milk were precipitated with goat anti-human IgA (Fig. 1 and 2, lanes b) or with goat anti-human IgG (Fig. 1 and 2, lanes

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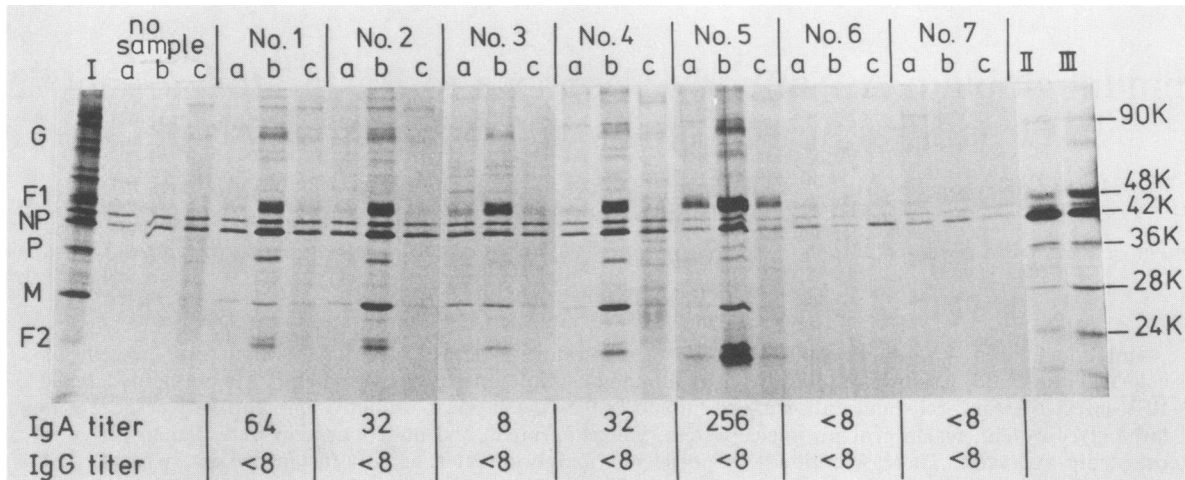


FIG. 1. [<sup>35</sup>S]methionine-labeled polypeptides immunoprecipitated with colostrum samples of seven mothers (no. 1 to 7). All a lanes were untreated, all b lanes were enhanced with anti-human IgA goat serum, and all c lanes were enhanced with anti-human IgG goat serum. Lane I, [<sup>35</sup>S]methionine-labeled RSV-infected cells. Lanes II and III, [<sup>35</sup>S]methionine-labeled polypeptides precipitated with adult human immunosera. IgA and IgG titers are class-specific anti-RSV antibody titers in colostrum samples determined by indirect IF analysis.

c). The specificities of these anti-human immunoglobulins were verified by immunoelectrophoresis.

To exclude a nonspecific reaction of protein A with the virus-infected cell lysate, radiolabeled viral antigen was incubated for 3 h at 4°C with or without anti-human immunoglobulin serum and then precipitated with protein A. As shown in Fig. 1 (no specimen), two fine precipitates (44 and 42 kilodaltons [kDa]) were observed. The 44-kDa polypeptide was precipitated also from uninfected HEP-2 cells (data not shown) and was therefore thought to be of HEP-2 cell origin as described previously (12). The 42-kDa polypeptide was considered to be an RSV nucleoprotein (NP). No

enhancement of these two precipitates was seen after treatment with goat anti-human IgA or IgG serum. Thus, these two polypeptides were precipitated nonspecifically by binding of protein A beads to the polypeptides, and these two bands were excluded from evaluation of the antibody activities in colostrum and milk.

Five colostrum samples from five mothers (no. 1 to 5) which were positive for anti-RSV IgA (1:16 to 1:256) and negative for anti-RSV IgG antibodies (<1:8) in the IF test were analyzed at a dilution of 1:20 (Fig. 1, no. 1 to 5). Without enhancement by the second antibody, only one sample (Fig. 1, no. 5, which had the highest anti-RSV IgA antibody titer in the IF test [1:256]) showed a moderate antibody response to 48-kDa fusion protein (F1). It is now generally thought that fusion protein (F) is made as precursor F0 (70 kDa), which is cleaved into two smaller glycoproteins, F1 (48 kDa) and F2 (24 kDa), which are held together by disulfide bonds (12). When anti-human IgA serum was used as the second antibody, significant amounts of IgA antibodies to five polypeptides (F1, NP, 36-kDa phosphoprotein, 28-kDa matrix protein [M], and F2) were detected in these specimens. However, treatment with antiserum to human IgG did not enhance the detection of RSV antibodies in human milk. A weak IgA response to 90-kDa large glycoprotein was also observed in these five samples (Fig. 1, no. 1 to 5) in spite of the low radiolabeling of this polypeptide by [<sup>35</sup>S]methionine (12). In these colostrum samples, IgA antibodies to F seemed to be the dominant response. IgA antibody to NP was observed consistently in these five samples, although the reactivity was generally weaker than that with F. Anti-phosphoprotein IgA activity was detected weakly in four of the five specimens (Fig. 1, no. 1, 2, 4, and 5). On the other hand, the anti-M activity was more variable. The reason for our failure to detect a strong reaction with NP antigen compared with that obtained with F antigen was not because of the methods used, because sera from healthy adults precipitated a larger amount of NP than F antigen (Fig. 1, lanes II and III) at a final concentration of 1:200 without enhancement by a second antibody.

Two colostrum samples (no. 6 and 7) which were negative for anti-RSV IgA and IgG antibodies in the IF test (<1:8)

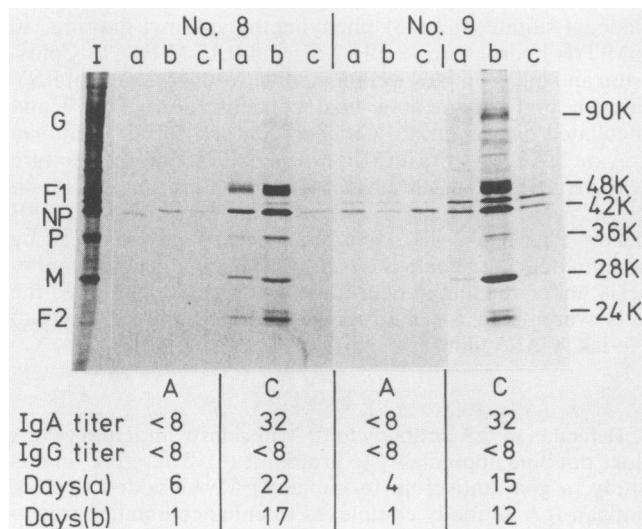


FIG. 2. [<sup>35</sup>S]methionine-labeled polypeptides immunoprecipitated with milk from two mothers (no. 8 and 9) in the acute (A) and convalescent (C) phases of infant RSV infection. All a, b, and c lanes were treated as described for Fig. 1. IgA and IgG titers are class-specific anti-RSV antibody titers in milk samples determined by indirect IF analysis. Lane I, [<sup>35</sup>S]methionine-labeled RSV-infected cells. Days (a), days after onset of infant's illness; days (b), days after onset of mother's illness.

were analyzed at a dilution of 1:20 (Fig. 1, no. 6 and 7). No significant amount of polypeptide was immunoprecipitated by these IF antibody-negative samples.

**Antibody in milk taken in the acute and convalescent stages of infant infection.** Milk samples were obtained from four mothers (no. 8 to 11) during the acute and convalescent stages of RSV infection in the recipient infant. Acute-phase samples were collected within 6 days after the onset of the infant's illness, and convalescent-phase samples were collected 15 to 22 days after the onset of the illness. Nasal swabs of mothers were also examined for RSV, and two of them (no. 8 and 9) were found to contain the virus. At that time, both mothers had mild rhinitis. All milk samples were analyzed in the same way as colostrum samples. No antibody responses to RSV were found in any acute-phase samples (Fig. 2; data not shown for no. 10 and 11). On the other hand, significant IgA activity against several RSV structural proteins appeared in convalescent-stage milk samples from only two mothers (no. 8 and 9) from whom RSV was isolated (Fig. 2; data not shown for no. 10 and 11). As in colostrum, antibody response to F was dominant in these specimens. Variation in anti-M antibody activity was also observed among the milk samples, as described for colostrum, i.e., weak activity in no. 8 and moderate activity in no. 9.

## DISCUSSION

The present study reports for the first time an anti-RSV IgA antibody response to RSV structural proteins in human colostrum and milk. By using RIP with [<sup>35</sup>S]methionine-labeled RSV antigens and anti-human IgA goat serum, we were able to characterize the anti-RSV polypeptide IgA antibody. Anti-RSV IgA activity in lactation products was mainly directed against F, although activity against other polypeptides was also observed. The antibody response to M varied among individuals and seems not to be comparable to the antibody responses to other structural proteins.

There have been no reports about IgA antibody responses to RSV proteins. However, some differences were observed in comparison with several studies of serum anti-RSV polypeptide antibody responses. Ward et al. (13) examined serum IgG antibody levels in women at or just before delivery by using purified <sup>125</sup>I-radiolabeled nucleocapsid or glycoprotein as the antigen. They observed almost identical antibody responses to F and NP, whereas antibodies to phosphoprotein and M were not detected. However, we could not compare these observations with ours easily because the degree of radiolabeling of RSV proteins with <sup>125</sup>I may be different from that of radiolabeling with [<sup>35</sup>S]methionine. Vainionpää et al. (11) have used [<sup>35</sup>S]methionine-labeled RSV-infected HEp-2 cells and observed that the serum antibody response in primary RSV infection was mainly directed against F and M and that the antibody response to M seemed to be quite comparable to the antibody response to F. Our results differed from those of Vainionpää et al. (11) in spite of the fact that almost the same RSV antigen preparation was used in the two studies. Therefore, the pattern of predominance of anti-F IgA activity may be one of the characteristic features in anti-RSV mammary immunity. F is now considered to be an important viral protein involved in virus infectivity (12). Thus, breast feeding provides abundant IgA antibodies to F which could play a role in suppressing replication of this virus at the surface of the infant upper respiratory tract.

RIP analysis of seven positive samples revealed differences among samples in the IgA antibody-binding pattern,

especially in an immune response to M. Gimenez et al. (5), using immunoblotting, also demonstrated differences among sera in antibody-binding patterns. They postulated that if antibodies with different protein specificities decayed at different rates, then that could account for the various antibody-binding patterns in samples obtained at different stages of infection. In our study, differences in immune response to M were observed not only between colostrum samples but also between two convalescent-stage milk samples which were collected at almost similar stages of RSV infection. Therefore, these differences may be explained by another possibility, i.e., that some patients had an immune response to a subset of the virus proteins (5). In other words, the pattern of sensitization of IgA-bearing lymphocytes by each RSV structural protein may differ among individuals.

In two mothers who had RSV infections concurrently with their infants during lactation, apparent natural boosting of RSV-specific IgA in milk (3, 7) was observed. However no obvious differences in clinical course could be found between their infants and infants of mothers who escaped RSV infection. This may be in part due to the small number of children evaluated. The implications of this apparent amnesic response and the role of colostrum IgA antibodies for the modification of RSV infection in breast-fed infants remains to be elucidated.

## LITERATURE CITED

1. Chandra, R. K. 1979. Prospective studies of the effect of breast feeding on incidence of infection and allergy. *Acta Paediatr. Scand.* **68**:691-694.
2. Cunningham, A. S. 1979. Morbidity in breast fed and artificially fed infants. *J. Pediatr.* **95**:685-689.
3. Fishaut, M., D. Murphy, M. Neiffert, K. McIntosh, and P. L. Ogra. 1981. Bronchomammary axis in immune response to respiratory syncytial virus. *J. Pediatr.* **99**:186-191.
4. Frank, A. L., L. H. Taber, W. P. Glezen, G. L. Kasel, C. R. Wells, and A. Paredes. 1982. Breast feeding and respiratory infection. *Pediatrics* **70**:239-245.
5. Gimenez, H. B., H. M. Keir, and P. Cash. 1987. Immunoblot analysis of the human antibody response to respiratory syncytial virus infection. *J. Gen. Virol.* **68**:1267-1275.
6. Lindmark, R., K. Thorén-Tolling, and J. Sjöquist. 1983. Binding of immunoglobulins to protein A and immunoglobulin levels in mammalian sera. *J. Immunol. Methods* **62**:1-13.
7. Nandapalan, N., C. Taylor, R. Scott, and G. L. Toms. 1987. Mammary immunity in mothers of infants with respiratory syncytial virus infection. *J. Med. Virol.* **22**:277-287.
8. Pullan, C. R., G. L. Toms, A. J. Martin, P. S. Gardner, J. K. B. Webb, and D. R. Appleton. 1980. Breast feeding and respiratory syncytial virus infection. *Br. Med. J.* **281**:1034-1036.
9. Toms, G. L., P. S. Gardner, C. R. Pullan, M. Scott, and C. E. Taylor. 1980. Secretion of respiratory syncytial virus inhibitors and antibody in human milk throughout lactation. *J. Med. Virol.* **5**:351-360.
10. Tsutsumi, H., M. Onuma, K. Suga, T. Honjo, Y. Chiba, S. Chiba, and P. L. Ogra. 1988. Occurrence of respiratory syncytial virus subgroup A and B strains in Japan, 1980-1987. *J. Clin. Microbiol.* **26**:1171-1174.
11. Vainionpää, R., O. Meurman, and H. Sarkkinen. 1985. Antibody response to respiratory syncytial virus structural proteins in children with acute respiratory syncytial virus infection. *J. Virol.* **53**:976-979.
12. Walsh, E. E., M. W. Brandriss, and J. J. Schlesinger. 1985. Purification and characterization of the respiratory syncytial virus fusion protein. *J. Gen. Virol.* **66**:409-415.
13. Ward, K. A., P. R. Lambden, M. M. Ogilvie, and P. J. Watt. 1983. Antibodies to respiratory syncytial virus polypeptides and their significance in human infection. *J. Gen. Virol.* **64**:1867-1876.