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Antibody Response to Respiratory Syncytial Virus Structural Proteins in Children with Acute Respiratory Syncytial Virus Infection

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The purified respiratory syncytial virus (RSV), Randall strain contained 10 polypeptides (72,000 molecular weight [72K], 66K, 48K, 42K, 40K, 36K, 30K, 23K, 18K, and 15K), 8 of which proved to be virus specific, and polypeptides 48K and 23K were glycosylated. In addition, a high-molecular-weight (150K), virus-specific glycopolypeptide was immunoprecipitated from RSV-infected cell lysate. The antibody response in human sera serially collected from children with primary RSV infection was mainly directed against the polypeptides 30K, 48K, and 72K. The immune response against the other viral proteins was also already detectable in the acute-phase sera. These results indicate that the immune response in RSV infection differs significantly from those for other diseases caused by paramyxoviruses.

The respiratory syncytial virus (RSV) has been classified to the pneumovirus subgroup of *Paramyxoviridae* (10), and the genome of RSV is presumed to be a single-stranded RNA molecule similar in coding capacity to those of paramyxoviruses. RSV is very labile, and as an enveloped virus, it can incorporate cellular material into its structure during the budding process. Therefore, the data concerning the polypeptide structure of the virion and the exact molecular weights (MWs) of the structural polypeptides are still conflicting (2, 17, 23, 24).

RSV is the major cause of lower-respiratory-tract illness during infancy, but the mechanism of immunity to RSV is still unclear. Maternal antibodies do not protect young babies against RSV infection during the first months after birth, and reinfections are common in early childhood. However, no clear evidence for strain variation has been reported. Live and killed viral vaccines have also failed to provide protection (1, 9). No reports have been published about antibody response against individual RSV-specific proteins, but neutralizing antibodies have been generally thought to have an important role in protective immunity. The purpose of our study was to characterize the antibody response against different structural components of RSV in serially collected sera from confirmed cases of RSV infection.

The structure of RSV was characterized by purifying the virus by two successive sucrose gradient centrifugations, radiolabeling in vitro, and analyzing the polypeptide composition by polyacrylamide gel electrophoresis. The Randall strain of the virus was grown in Vero cells. When the first signs of the virus infection were detected, the fresh medium was changed, and the virus was collected the next day. Infected culture fluid was concentrated by Amicon hollow-fiber ultrafiltration equipment. The virus in culture fluid was then centrifuged at 4°C for 4 h in a Spinco SW27 rotor through 30% sucrose onto a 60% (wt/wt) sucrose cushion in Hanks salt solution and finally purified by ultracentrifugation for 36 h at 25,000 rpm in an SW27 rotor in a 40 to 60% (wt/wt) linear sucrose gradient. The yield was about 1 mg of purified virus per 10 roller bottles of infected Vero cells. The purified virus was radiolabeled with [^{3}H]acetic anhydride (15) to a specific activity of ca. 10^7 dpm/mg of protein. The protein composition of the virus was analyzed in 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (11). After electrophoresis the proteins were fixed and treated for 60 min with 1 M sodium salicylate (4). Kodak RP-Royal X-Omat film was exposed to the dried gels at −70°C. Protein markers (and MWs) used in the estimation of the MWs of the structural polypeptides of RSV were bovine serum albumin (66,000 molecular weight [66K]) ovalbumin (46K), lysozyme (14K), and the proteins (79K, 70K, 60K, 40K, and 36K) of purified measles virus (20), all similarly radiolabeled with [^{3}H]acetic anhydride. Radiolabeled RSV polypeptides were later used as reference proteins in electrophoretic analysis of immunoprecipitates.

Purified RSV contained six major polypeptides (72K, 62K, 48K, 40K, 36K, and 30K) and three minor, small polypeptides (23K, 18K, and 15K). Also, a polypeptide with an MW of 42,000 was constantly detected (Fig. 1, lane a). In addition, some minor polypetides appeared at the very top of the gels, but their virus specificity could not be confirmed. However, one of them was probably the glycoprotein 150K, described below. Earlier, RSV was reported to contain 5 to 10 structural proteins, ranging in molecular weight from 200,000 to 10,000 (3, 5, 17).

The virus specificity of the polypeptides was confirmed by immunoprecipitating virus antigens from [^{35}S]methionine-labeled, infected Vero cell lysate with rabbit anti-RSV serum prepared by immunizing with RSV that had been purified as described above. Vero cell monolayers in petri dishes were infected with RSV at a multiplicity of 1 PFU per cell, and the next day, actinomycin D at a final concentration of 2.5 μg/ml was added. The cells were radiolabeled the next day, as described earlier (20). At the end of the 180-min labeling period, the cells were washed with ice-cold phosphate-buffered saline and lysed in phosphate-buffered saline containing 0.5% Nonidet P-40 and 1 mM tosylsulfonyl lysyl chloromethyl ketone. The lysates were sonicated for 1 min with a Branson sonifier cell disruptor B15 and centrifuged for 10 min at 8,000 rpm to remove insoluble proteins before the lysates were used for immunoprecipitation. In the immunoprecipitation assay, protein A–Sepharose CL-4B beads and

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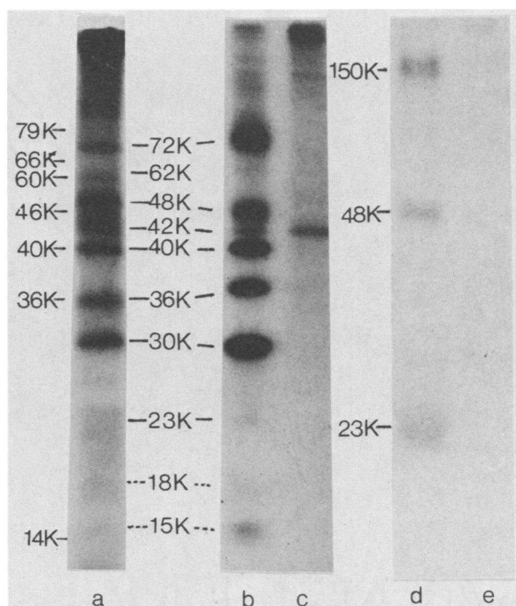

FIG. 1. Electrophoretic analysis of RSV polypeptides. Lane a, structural polypeptides of the purified [^{3}H]acetic anhydride-radiolabeled RSV; The MW markers have been marked at the left side of the lane. Lane b, [^{35}S]methionine-radiolabeled polypeptides immunoprecipitated with rabbit anti-RSV immunoserum from RSV-infected Vero cell lysate. Lane c, [^{35}S]methionine-labeled polypeptides immunoprecipitated with rabbit anti-RSV immunoserum from uninfected Vero cell lysate. Lane d, [^{3}H]glucosamine-radiolabeled polypeptides immunoprecipitated as described for lane b. Lane e, [^{3}H]glucosamine-radiolabeled polypeptides immunoprecipitated as described for lane c.

sera (100 μl of human sera or 10 μl of rabbit antiserum) were incubated at room temperature for 60 min, after which unreacted serum material was removed. The radiolabeled cell lysate (250 μl) was added, and the mixture was incubated at 37°C for 120 min, after which antigen-antibody complexes were washed three times with phosphate-buffered saline and once with 0.1 M Tris-HCl buffer, pH 6.8, eluted with a gel electrophoresis sample buffer (0.1 M Tris-HCl [pH 6.8], 2% sodium dodecyl sulfate, 5% β-mercaptoethanol) and heated at 100°C for 2 min.

The polypeptides 72K, 48K, 40K, 36K, 30K, 23K, 18K, and 15K were immunoprecipitated from RSV-infected Vero cell lysate (Fig. 1b), but none of them were precipitated from uninfected Vero cell lysate (Fig. 1c), indicating virus specificity. The virion polypeptide 42K was also immunoprecipitated from RSV-infected cell lysate, but the polypeptide with the corresponding MW was immunoprecipitated from uninfected Vero cell lysate (Fig. 1c) as well. Therefore, based on the earlier information concerning the structure of paramyxoviruses, this polypeptide was considered to be a cellular actin. The virion polypeptide 62K was immunoprecipitated with neither rabbit anti-RSV serum nor human RSV sera.

The glycoprotein nature of the RSV proteins was analyzed by radiolabeling the infected and uninfected cells with [^{3}H]glucosamine as described earlier (19) and immunoprecipitating RSV-specific proteins with rabbit anti-RSV serum from cell lysates, as described above. The polypeptides 48K and 23K and the high-MW polypeptide 150K were found to be glycosylated. However, the latter glycopolypeptide was immunoprecipitated only from the RSV-infected cell lysate (Fig. 1d) but was not clearly detectable in purified virion, probably due to the presence of other minor polypeptides in this region of the gel (Fig. 1a). None of these proteins were immunoprecipitated from [^{3}H]glucosamine-labeled, uninfected Vero cell lysate (Fig. 1e).

To characterize the antibody response against individual viral proteins in primary RSV infection, [^{35}S]methionine-labeled viral antigens were immunoprecipitated in serially collected human sera, and precipitates were analyzed by gel electrophoresis as described above. Serum specimens were collected from 17 patients with RSV infection. Acute-phase sera were collected within 9 days after onset of illness, and early and late convalescent sera were collected 16 to 32 and 50 to 140 days, respectively, after onset of illness. The diagnosis was verified by the detection of viral antigens in nasopharyngeal secretion by immunofluorescence or by radioimmunoassay (18) or by demonstration of a fourfold or greater titer increase in serum by enzyme immunoassay (EIA) (14). All patients except one (patient 7) obviously had a primary RSV infection, because either acute or early convalescent sera were immunoglobulin M (IgM) antibody positive. Antibodies to glycoprotein 48K and to polypeptide 30K dominated in the acute-phase sera, and the relative amounts of polypeptides 48K and 30K among the immunoprecipitates were higher than in the virion (Fig. 2, patients 1 through 4). The immune response against the other virus-specific proteins (72K, 40K, 36K, 23K, 18K, and 15K) was either weakly detectable or undetectable in the acute-phase sera. When compared with the polypeptide profiles of early- and late-convalescent-phase sera, acute-phase sera showed a clear increase against the major polypeptides 48K and 30K and against polypeptide 72K. Small amounts of all of the other virus-specific polypeptides were also immunoprecipitated by the convalescent-phase sera, but clear-cut changes in their polypeptide profiles were not seen. In general, polypeptide profiles of the early and late convalescent sera were similar, and the precipitation maximum correlated well with the highest EIA IgG antibody titers.

Three exceptions to the general immunoprecipitation pattern were seen. Patient 5, aged 4 months, had a weak response only against the polypeptides 30K and 48K, with no differences between acute- and convalescent-phase serum profiles. Correspondingly, no change was detected in his EIA IgG antibody titers, but a recent infection was confirmed by detection of IgM antibodies and antigen. Patient 6, aged 7 months, developed antibodies against polypeptide 48K but had an undetectable or very weakly detectable response against the other polypeptides. Patient 7, aged 3 years and 10 months, had an obvious secondary infection, with a very strong but unchanged polypeptide-specific antibody response in acute- and convalescent-phase sera, a very high EIA IgG antibody titer in the acute-phase serum, and negative EIA IgM response.

The results described are in general agreement with those of others (2, 7), with respect to the major structural proteins of RSV. However, great variations still occur in reports concerning the glycoprotein structure of RSV. We, as well as Peeples and Levine (17) and Bernstein and Hruska (2), found the polypeptides 48K and 23K to be glycosylated. In addition, a high-MW, virus-specific glycopolypeptide was immunoprecipitated from [^{3}H]glucosamine-labeled, RSV-infected Vero cells. Its MW (ca. 150,000) was larger than that of polypeptide 90K reported by others (21–23). The difference in MWs of this larger glycopolypeptide may be partly explained by the differences in glycosylation and processing of the polypeptides in different cell lines. Also, small changes

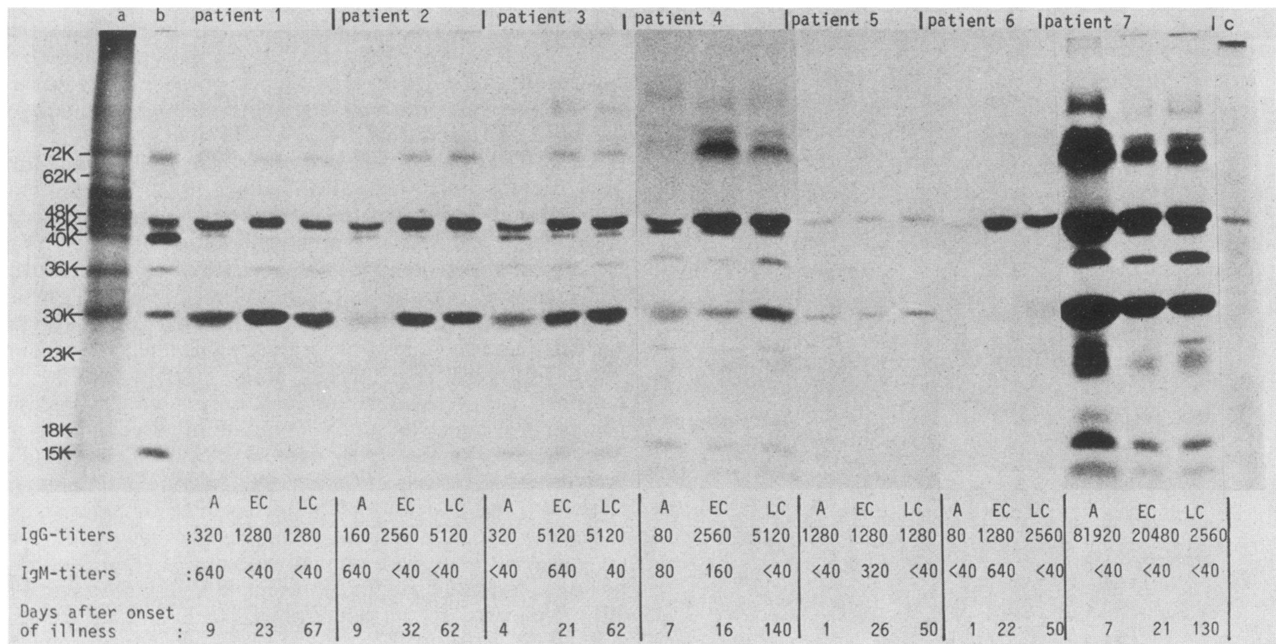

FIG. 2. [^{35}S]methionine-radiolabeled polypeptides immunoprecipitated with human acute (A), early convalescent (EC), or late convalescent (LC) sera of patients with RSV infection. Lane a, Purified, [^{3}H]acetic anhydride-radiolabeled RSV; lane b, [^{35}S]methionine-radiolabeled polypeptides immunoprecipitated with rabbit anti-RSV serum; lane c, [^{35}S]methionine-radiolabeled polypeptides precipitated with a human immunoserum from uninfected Vero cell lysate.

in glycosylation can cause strong variation in migration of the glycoproteins in sodium dodecyl sulfate-polyacrylamide gel.

The functions of many of the RSV-specific proteins are still unclear. Glycoproteins 50K and 20K (corresponding to our glycopolypeptides 48K and 23K) have been shown to be linked together with disulfide bonds (7), and it has been speculated that these glycoproteins form the fusion activity of RSV. Monoclonal antibodies to glycoprotein 70K neutralize the virus (6, 21). Glycoprotein 90K may be analogous to the hemagglutinin of the other paramyxoviruses, but its functional role has not been demonstrated. The polypeptides 40K and 30K have been reported to be the nucleocapsid protein and the matrix protein (17).

The antibody response in primary RSV infection was mainly directed against glycoprotein 48K and matrix protein 30K. The antibody response against RSV-specific nucleoprotein 40K was very weak in all tested serum specimens of patients with acute primary RSV infection. The reason for our failure to detect nucleocapsid antigens with human sera cannot be methodological, because high amounts of nucleocapsid antigen were precipitated with rabbit anti-RSV serum (Fig. 1b), indicating that the nucleocapsid was detectable by the present method. Here our results differ from those of Ward et al. (23), who detected antibody response to protein 48K but also to nucleoprotein 41K, whereas they could not detect antibody response against matrix protein (30K). However, Ward et al. (23) used purified, ^{125}I-radiolabeled nucleocapsids or glycoproteins as antigens with their patient sera. Also, an important difference was that the researchers studied maternal sera and sera collected from babies under 6 months of age. Maternal sera probably contain a variety of antibodies, including nucleocapsid antibodies, owing to repetitive RSV infections, and sera from infants may contain maternal antibodies.

The antibody response in acute primary RSV infection seems to also differ significantly from the antibody response in measles infection, in which the antibody response against the nucleoprotein dominates and the antibody level against the matrix protein is always very low, both in acute and chronic measles infection (8, 12, 16).

Antibodies against the fusion protein of paramyxoviruses have generally been thought to play an important role in protective immunity by preventing the spread of virus from cell to cell through the membrane. Merz et al. (13) have shown with simian virus 5, by using monoclonal antibodies against the fusion and hemagglutinating glycoproteins, that only antibodies against the fusion protein can prevent the spread of infection in vitro. In primary RSV infection, there was a strong antibody response against glycoprotein 48K, so the lack of antibodies against the fusion protein cannot be the reason for reinfections with RSV. In mild respiratory infection with RSV, the antibody response against intermembrane matrix protein 30K seems to be quite comparable to the antibody response against glycoprotein 48K. So the immune responses against the matrix proteins in measles and RSV infections are just opposite. Because no reports have been published about the immune response in diseases caused by other paramyxovirus, it is not possible to speculate on the importance of this finding. Ward et al. (23) have reported earlier that maternal antibodies against the RSV nucleocapsid protein gave the best protection against RSV infection during the first 6 months after the birth of the babies. Based on these findings, the antibodies against the nucleoprotein might have a more important role in protective immunity than it has been generally thought.

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