Antigen Mimicry Involving Measles Virus Hemagglutinin and Human Respiratory Syncytial Virus Nucleoprotein

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Intergenic antigenic relationships between measles virus and respiratory syncytial (RS) virus-specific structural components were studied by using monoclonal antibodies. Of 75 monoclonal antibodies against these components, only one, an anti-measles virus hemagglutinin monoclonal antibody, cross-reacted. Immunofluorescence analysis of measles virus- and RS virus-infected cells with this monoclonal antibody showed qualitatively different staining patterns which indicated that the antigen involved in cross-reaction was the RS virus nucleoprotein or phosphoprotein. A radioimmunoprecipitation assay showed the antigen to be the nucleoprotein.

The paramyxovirus group includes a number of medically important viruses. These viruses are parainfluenzavirus, morbillivirus, and pneumovirus (7). To evaluate intratypic, intragenic, and intergenic antigen relationships, libraries of monoclonal antibodies have been prepared against representative members, including measles virus (9, 11) and respiratory syncytial (RS) virus (8a). In the early phase of this comparative study, an unexpected reaction was observed between a monoclonal antibody against the measles virus hemagglutinin (H) and one of the internal components of RS virus. This reaction was further analyzed as described in this report.

Two different methods were used for the comparative studies. These were immunofluorescence with acetone-fixed cells and a radioimmunoprecipitation assay (RIPA) with [³⁵S]methionine-labeled infected cells. The details of the techniques used as well as the collection of measles virus-specific monoclonal antibodies were described previously (9, 11). Different RS virus-specific structural components were identified by monoclonal antibodies prepared in this laboratory (8a). These components included the large glycoprotein (G) of 90,000 molecular weight (90K), the cleaved glycoprotein (48K + 19K, the fusion [F] component analog), the 26K matrix (M), the 41K nucleoprotein (NP), and the 34K phosphoprotein (P) (5, 10, 13).

Of 44 different monoclonal antibodies against measles virus H, F, M, NP, and P components, only one reacted with RS virus-infected cells in immunofluorescence tests. This antibody, I41, reacted with one of nine different epitopes on the H component detectable by a group of 10 monoclonal antibodies (Sheshberadaran and Norrby, submitted for publication). None of 31 different monoclonal antibodies against RS virus G, F, M, NP, and P components reacted with measles virus-infected cells in immunofluorescence tests.

Immunofluorescence analysis with antibody I41 and measles virus- and RS virus-infected cells showed qualitatively different staining patterns (Fig. 1). Whereas the staining of measles virus-infected cells predominantly was diffuse and associated with the cytoplasmic membrane (Fig. 1a), the staining of RS virus-infected cells involved rounded cytoplasmic inclusions (Fig. 1b). The latter staining indicated

that the antigen involved in the cross-reaction most likely was either the NP or P RS virus component but not one of the envelope glycoproteins (Fig. 1c to e; cf. reference 13). The homotypic and heterotypic immunofluorescence titers of I41 were markedly different, 640 and 20, respectively. Antibody I41 did not stain uninfected cells.

In order to define the RS virus antigen involved in the reaction, RIPA tests were performed. It was seen that under the conditions for preparation of RIPA antigen generally used in this laboratory (11), antibody I41 did not show an identifiable reaction with RS virus proteins. Since this might reflect a modification of the epitope involved by the lysis buffer employed, a modified version of this buffer was tested. Instead of a molarity of 0.6 M KCl and 2% Triton X-100 as the only detergent, a physiological saline concentration and 0.1% sodium dodecyl sulfate and 0.5% sodium deoxycholate in addition to Triton X-100 were used. In the presence of this lysis buffer a precipitate was identified (Fig. 2). In the experiment shown in this figure three different monoclonal antibodies against the H component of measles virus were tested. All three antibodies precipitated the 79K glycoprotein of measles virus as anticipated (11), but only I41 gave a precipitate with RS virus antigen. The 41K viral antigen in this precipitate matched the polypeptide seen in RIPA with an RS virus NP-specific monoclonal antibody, B62. RS virus-specific monoclonal antibodies against the F component, hybridoma clone B46, and the P component, hybridoma clone C771, of RS virus precipitated some 41K polypeptide in addition to the protein of the specific precipitate. This was interpreted to represent coprecipitated material since in the presence of a lysis buffer with a different composition this precipitate was not seen (8a). Since the RS virus 90K glycoprotein labels poorly with methionine (8a), [³H]glucosamine-labeled material was used in RIPA tests with measles virus-specific anti-H monoclonal antibodies. No reaction was seen (data not shown).

Several studies of monoclonal antibodies against virus components have revealed the occurrence of antigen mimicry involving different host cell proteins. Antibodies against the simian virus 40 large T antigen (2), the vaccinia virus H (3), the herpes simplex virus type 1 146K protein, and the measles virus P (6) were found to react with cellular components, in some cases of cytoskeleton origin. Further, 3 of 11 monoclonal antibodies against the F protein of measles virus cross-reacted with a 79K inducible host cell stress

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FIG. 1. Immunofluorescence analysis of measles virus (a)- and RS virus (b to e)-infected cells with monoclonal antibodies against the measles virus H component (hybridoma I41) (a and b) and the RS virus NP (hybridoma B62) (c), P (hybridoma C771) (d), and H (hybridoma B16) (e) components.

FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of RIPA tests with RS virus (lanes a to f) and measles virus (lanes g to i) antigens and different monoclonal antibodies. The antibodies used were specific for the measles virus H-141 (lanes a and g), 144 (lanes b and h), and 16CD11 (lanes c and i) and the RS virus F (lane d), NP (lane e), and P (lane f) components.

protein (12). In the present case the observed cross-reaction involved nonhomologous structural components in two viruses belonging to the same family, the measles virus H and the RS virus NP components. There appears to be no logical reason for a sharing of amino acid sequences of these two components. The amino acid sequence of the NP antigen of RS virus strain A2 has already been deduced from the cloned virus gene (1, 4), but as yet there has been no published report on cloning of the measles virus H gene. Once the latter information is available, it will be of interest to compare the amino acid sequences. Eventually it may be possible to identify the I41-specific domain shared between the two structural components.

The absence of any other cross-reactions than the one seen with antibody I41 in tests with the comprehensive library of measles virus- and RS virus-specific monoclonal antibodies employed in this study indicates that morbilliviruses and pneumoviruses have been evolutionarily separated for a long time.

The present finding emphasized that it is imperative to carefully control reagents to be used for clinical virological purposes. The occurrence of fortuitous cross-reactions may be a source of erroneous results. The present data also may be considered in the evaluation of the findings of paramyxovirus antigens in bone tissue from patients with Paget's disease. In such material both measles and RS virus antigens have been identified by immunofluorescence with polyclonal and monoclonal antibodies (8).

This work was supported by grant B85-16x-00116-21B from the Swedish Medical Research Council.

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