Synthetic Oligonucleotide Probes Differentiate Respiratory Syncytial Virus Subgroups in a Nucleic Acid Hybridization Assay

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A new approach to respiratory syncytial virus subgroup differentiation has been developed on the basis of the hybridization of subgroup-specific synthetic oligonucleotides with viral mRNA. Two oligonucleotide probes were designed from the nucleotide sequences of an A and a B subgroup respiratory syncytial virus glycoprotein G gene. All 28 virus isolates tested were correctly classified by subgroup with these probes.

The recognition of two major antigenic subgroups (A and B) for human respiratory syncytial (RS) virus advanced our understanding of the epidemiology and immunology of this important pathogen (1, 11). Both subgroups may circulate concurrently in an epidemic period, although temporal and geographic clustering occur (4). In addition, in some years the two subgroups are equally represented, while in other years one or the other subgroup may predominate (3). The antibody responses of young children to RS virus infections have both subgroup-specific and subgroup-cross-reactive components, and neutralizing antibody titers are higher to a virus of the homologous subgroup (2). Young children infected with a subgroup A virus are relatively protected against subsequent subgroup A but not subgroup B infections (10). Subgroup A infections were more severe than subgroup B infections among children hospitalized in Rochester, N.Y., during a study that spanned two winters (8). Thus, knowledge of the subgroup of an infecting RS virus may have a role in clinical decisions such as whether to employ antiviral therapy. In addition, proposed components of subunit vaccines must be evaluated for their ability to confer protection against challenge by either the homologous or heterologous subgroup (6, 16). A simple technique for the subgroup classification of RS viruses would be of use to both clinical and epidemiological investigators.

The early descriptions of the two RS virus subgroups were based on the reactivities of individual viral isolates with monoclonal antibodies (1, 11). The greatest number of antigenic differences between subgroups were found on the attachment glycoprotein G. In agreement with this, the most extensive nucleotide sequence differences between the subgroups are found between the G protein genes (7, 17). We took advantage of these differences and developed previously an assay for the differentiation of RS virus subgroups which used cloned G protein cDNAs as probes in a nucleic acid hybridization assay (18). This method is restricted in its usefulness by the necessity of preparing the pure cDNAs to use as probes. In the present report, this approach has been simplified and made widely applicable by the use of synthetic oligonucleotides as RS virus subgroup-specific probes.

(This work was presented at the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy [19].)

The oligonucleotides chosen to differentiate the two subgroups were as follows. The subgroup A specific synthetic

oligonucleotide, 5' CTTGATC TGGCTTGTTCATC 3', was complementary to nucleotides 247 to 267 in the G mRNA of the A2 and Long subgroup A RS viruses (7, 20). The subgroup A probe was 21 nucleotides in length and contained 13 nucleotide mismatches relative to the G mRNA of the subgroup B 8/60 virus (17). The subgroup B-specific synthetic oligonucleotide, 5' CTTGTGTTTGGACATGGT TGC 3', was complementary to bases 10 to 30 in the G mRNA of the 8/60 and 18537 subgroup B RS viruses (7, 17). The subgroup B probe was 21 nucleotides long and contained two nucleotide mismatches relative to the G mRNA of the subgroup A A2 virus. For use as probes 10 pmol of these oligonucleotides was radiolabeled with [γ -³²P]ATP (Dupont, NEN Research Products) and T4 polynucleotide kinase and then purified with a spun column of Sephadex G50 (14).

The assay method consisted of direct fixation of cells infected with virus isolates to nylon membranes followed by hybridization with one of the subgroup-specific oligonucleotides. The cells and the RS viruses used were previously described and their subgroup classifications were previously determined with both monoclonal antibodies and the cDNA probes (18). Templates for hybridization were prepared by glutaraldehyde fixation of virus-infected cells onto a nylon membrane (GeneScreen Plus, NEN) by the method of Paeratakul et al. (12) as previously described (18). Prehybridization of templates was performed for 15 min in a 55°C shaking water bath in 4× SSC–5× Denhardt solution–50 µg of calf thymus DNA per ml–0.1% sodium dodecyl sulfate (SDS). (1×SSC is 0.15 M sodium chloride and 0.015 M sodium citrate dihydrate.) Hybridization was performed with 1 pmol of oligonucleotide with an activity of about 5 × 10⁶ cpm in 4× SSC–1× Denhardt solution–50 µg of calf thymus DNA per ml–0.1% SDS. After shaking in a 55°C water bath for 1 h, the hybridization solution was removed. The templates were washed at room temperature four times for 5 min each time in 6× SSC–0.1% SDS. A final wash of 5 min was performed at 55°C. The filters were dried and exposed to X-ray film (Fuji film) for 6 to 12 h at –70°C with an intensifying screen (Cronex Lighting Plus, Dupont).

The subgroup A probe produced a strong positive signal for all of the subgroup A samples, with no cross-reactivity to the subgroup B samples or to the mock-infected samples (Fig. 1, probe A). The subgroup B probe readily detected all of the subgroup B samples (Fig. 1, probe B), except for two samples for which the signal was faint but visible (column 8, row D) or easily discernible only with a longer exposure (column 7, row F). When these two viruses (2045-4 and

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FIG. 1. Nucleic acid hybridization of infected cells fixed to nitrocellulose with synthetic oligonucleotides which were either RS virus subgroup A (probe A) or subgroup B (probe B) specific and which were labeled with 32 P. Replicate templates were used for both probes so that the samples in columns 1 to 4 which were hybridized to probe A are the same samples which in columns 5 to 8 which were hybridized to probe B. The samples in columns 1, 2, 5, and 6 are subgroup A viruses, and those in columns 3, 4, 7, and 8 are subgroup B viruses with the exception that in columns 4 and 8, rows E through H are mock-infected samples. The samples in each column, rows A through H, are as follows: columns 1 and 5, A2, 961, 2045-16, 2045-25, 2045-33, 1836a, 2139-4, and 669; columns 2 and 6, 9324, 2045-1, 2045-32, 2045-12, 2012-8, 1935a, 2040-35, and 89-4928; columns 3 and 7, 8/60, 9320, 1776, 1842, 2040-23, 2045-4, 2045-5, 2045-41; columns 4 and 8, 2040-1, 2040-21, 18537, 89-1355, mock, mock, mock, and mock.

89-1355) were regrown and reanalyzed, they gave a clearly positive signal with the B probe (Fig. 2, probe B, rows C and D), indicating that these isolates grew poorly in the first assay. The difficulty with these two viruses may reflect the somewhat restricted replication in cell culture of some subgroup B viruses (5, 9).

The two synthetic oligonucleotides described here discriminated between the two subgroups of RS virus in a nucleic acid hybridization assay. The 28 viruses tested (16 A subgroup and 12 B subgroup) were all correctly classified by subgroup with these oligonucleotides. Cross hybridization between subgroups did not occur under the conditions described.

FIG. 2. Repeat hybridization of samples which gave weak signals upon first hybridization (Fig. 1). Subgroup B viruses 2045-4 and 89-1355 were regrown, and new replicate templates were prepared. Row A is a subgroup A control, A2; row B is a subgroup B control, 8/60; row C is 2045-4; and row D is 89-1355.

The viruses tested in this investigation are a heterogeneous group in terms of their temporal (1959 to 1989) and geographic (United States, Sweden, and Australia) origins and in their varied antigenicity as defined by testing with monoclonal antibodies (18). Thus, although we have tested only a small number of samples, it seems likely that these synthetic probes will be able to differentiate the subgroup classification of most human RS viruses.

Synthetic oligonucleotides have also been used to discriminate between herpes simplex virus types 1 and 2 (13) and to determine the serotype of human group A rotaviruses (15). Previous techniques for the differentiation of RS virus subgroups were dependent on obtaining reagents of limited availability (monoclonal antibodies and cloned cDNAs). Synthetic oligonucleotides are readily obtained, and the assay itself is easily performed.

We have described a new technique for the differentiation of RS virus subgroups by using synthetic oligonucleotides in a nucleic acid hybridization assay. The viruses we tested were isolated over a period of 3 decades on three continents and in each case hybridized with the correct oligonucleotide probe. These probes should prove to be useful tools in epidemiologic investigations, vaccine development efforts, and clinical situations in which RS virus subgroup diagnosis is desired.

Support was received from Public Health Service grants 1F32 AI07864 (W.M.S.) and R37 AI12464 and AI20181 (G.W.W.) from the National Institutes of Health and a grant from the World Health Organization Programme for Vaccine Development. Support for maintenance of the Wisconsin-GCG computer programs and the oligonucleotide synthesis facility was provided by Center for AIDS Research grant P30 AI27767.

We thank Larry Anderson (Atlanta, Ga.) and Leroy McClaren (Albuquerque, N.Mex.) for providing the viruses used in this investigation.

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