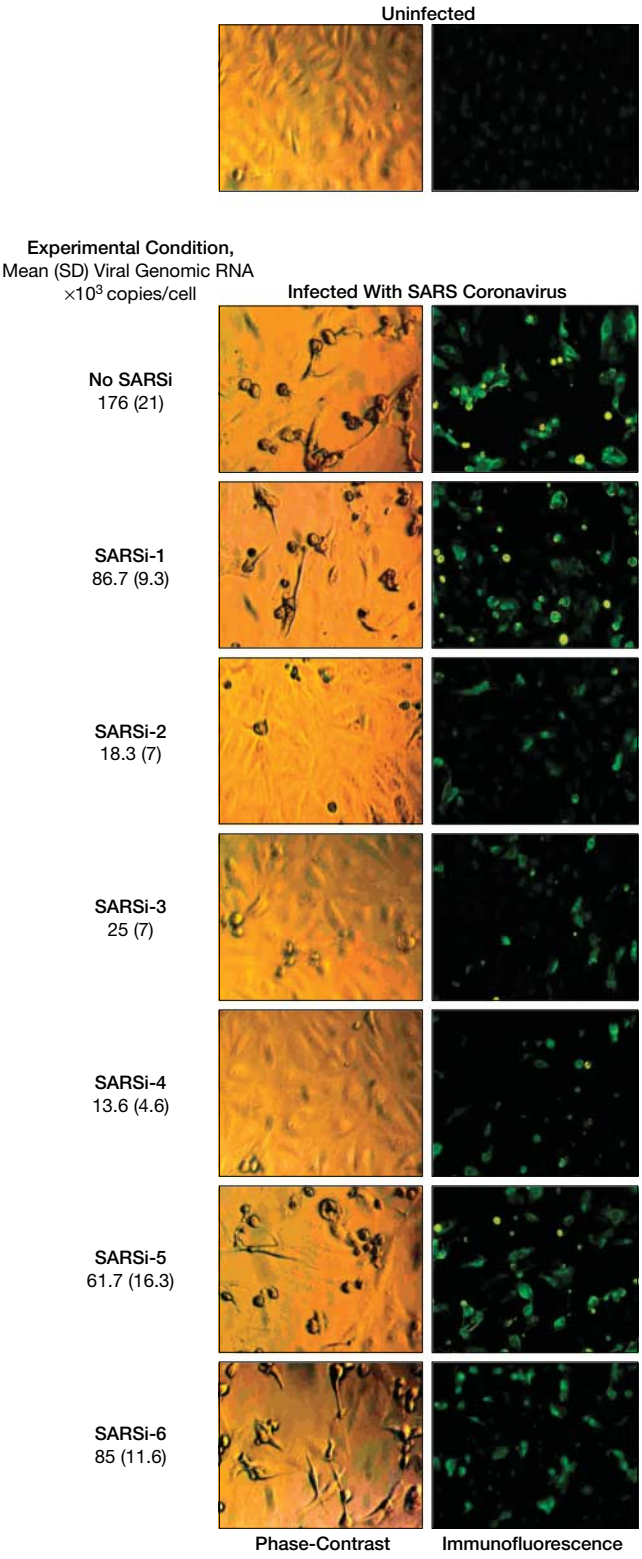
3. Peiris JSM, Lai ST, Poon LLM, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet*. 2003;361:1319-1325.
4. US Centers for Disease Control and Prevention (CDC). Updated interim U.S. case definition for severe acute respiratory syndrome (SARS). Atlanta, Ga: CDC; 2003. Available at: http://www.cdc.gov/ncidod/sars/casedefinition.htm. Accessibility verified September 30, 2003.
5. Ding JW, Ning Q, Liu MF, et al. Fulminant hepatic failure in murine hepatitis virus strain 3 infection: tissue-specific expression of a novel fgl2 prothrombinase. *J Virol*. 1997;71:9223-9230.
6. Kyuwa S, Shibata S, Tagawa Y, Iwakura Y, Machii K, Urano T. Acute hepatic failure in IFN-gamma-deficient BALB/c mice after murine coronavirus infection. *Virus Res*. 2002;83:169-177.

Inhibition of SARS-Associated Coronavirus Infection and Replication by RNA Interference

To the Editor: A novel coronavirus has been identified as the etiologic agent of severe acute respiratory syndrome (SARS),[1-3] for which there is no specific treatment. Small interfering RNAs (siRNAs) are double-stranded RNAs that direct sequence-specific degradation of messenger RNA in mammalian cells.[4] It is also possible, however, that siRNAs could specifically interfere with viral RNA.

Methods. We designed six 21-mer SARSis (siRNAs [GENSET SA Ltd, Paris, France] targeting different sites of the replicase 1A region of the SARS coronavirus [SARS-CoV] genome; siRNA sequences in the senses strands: GUGAACUCACUCGUGAGCUCdTdT [SARSi-1]; GUACCCUCUUGAUUGCAUCdTdT [SARSi-2]; GAGUCGAAGAGAGGUGUCUdTdT [SARSi-3]; GCACUUGUCUACCUUGAUGdTdT [SARSi-4]; CCUCCAGAUGAGGAAGAAGdTdT [SARSi-5]; and GGUGUUUCCAUUCCAUGUGdTdT [SARSi-6]). We then performed 3 in vitro experiments to test their antiviral effects. In the first, we transfected monkey kidney cells (FRhk-4) with 1 of the 6 siRNAs. In addition to these 6 groups of cells, we also created 2 groups of control cells—1 transfected with an unrelated siRNA targeting luciferase (GL2i),[5] and the other with the medium. OligoFectamine (Invitrogen Corp, Carlsbad, Calif) was the transfection reagent. All groups of cells were incubated for 8 hours before infection with SARS virus GZ50 strain. Thirty-six hours after viral infection, cytopathic effects were judged with phase-contrast microscopy. The cells were then fixed with –20°C ethanol for 10 minutes and immunostained with a SARS-CoV–specific antibody isolated from acute covalent sera of confirmed SARS patients. The coronavirus antigens were detected by indirect immunofluorescence assay using a fluoroscein isothiocyanate–coagulated antibody[1,2] (Inova Diagnostic Inc, San Diego, Calif). To quantify the viral genomic RNA, real-time polymerase chain reaction was performed as described previously.[2]

Figure. Inhibition of Severe Acute Respiratory Syndrome (SARS)-Associated Coronavirus Infection and Replication



Monkey kidney cells (Frhk-4) were uninfected or were infected with SARS-associated coronavirus and transfected either without or with 1 of 6 SARSis (ie, small interfering RNAs targeting different sites of the replicase 1A region of the SARS coronavirus genome). Photographs were taken under both a phase-contrast microscope (left panels; orange coloration due to filtration for better contrast; magnification, ×400) to show cytopathic effects , as well as a fluorescence microscope (right panels; magnification, ×400) to show coronavirus antigen expression after immunostaining.

In the second experiment, we transfected FRhk-4 cells with a combination of 2 or 3 SARSis (SARSi-2/3, SARSi-2/4, SARSi-3/4, SARSi-1/2/4, and SARSi-2/3/4), using GL2i as a control. In each combination, an equal amount of individual siRNA was used while the final concentration of total siRNAs remained the same (10 nM).

In the third experiment, we tested the inhibitory effect of SARSi-2/3/4 on the infection and replication of 3 other SARS-CoV strains isolated from SARS patients in Hong Kong and Guan Zhou (GZ34, HKR1, and HKR2 strains).

Results. Compared with uninfected cells, cells infected with SARS-CoV exhibited a marked morphologic change with cytopathic effects (FIGURE). The uninfected cells were flattened, whereas the SARS-CoV infected cells became refractile and rounded. Judged by morphologic changes, SARSi-2, SARSi-3, and SARSi-4 markedly inhibited the cytopathic effects caused by viral infection and replication, whereas SARSi-1, SARSi-5, and SARSi-6 were less effective. The results were further confirmed by immunostaining with antibody against SARS-CoV antigens. There was a consistent and marked 92.5%, 89.6%, and 85.8% reduction in the viral genomic RNA copies (as determined by quantitative real-time polymerase chain reaction) in cells transfected by SARSi-4, SARSi-2, and SARSi-3, respectively. The reduction was much less marked in cells transfected by the other 3 siRNAs (only 50%-65%).

In the second experiment, the combinations of SARSi-2, SARSi-3, and SARSi-4 also inhibited the infection and replication of different strains of SARS-CoV. No obvious synergistic effects were observed, however, from any of these combinations.

In the third experiment, we found that the efficacy of SARSi-2, SARSi-3, or SARSi-4 in inhibiting the infection and replication of the 3 other SARS-CoV strains were similar to that of the GZ50 strain. Transfection with SARSi alone or GL2i did not show any change in cell morphology or viral genomic RNA copies (data not shown).

Comment. siRNAs targeting the replicase 1A region of the SARS-CoV genome appear to be effective in vitro against the SARS virus. Their clinical usefulness, however, has yet to be demonstrated.

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1. Peiris JS, Lai ST, Poon LL, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet*. 2003;361:1319-1325.
2. Poon LLM, Wong OK, Luk W, et al. Rapid diagnosis of a coronavirus associated with severe acute respiratory syndrome (SARS). *Clin Chem*. 2003;49:1-3.
3. Rota PA, Oberste MS, Monroe SS, et al. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. *Science*. 2003;300:1394-1399.
4. McManus MT, Sharp PA. Gene silencing in mammals by small interfering RNAs. *Nat Rev Genet*. 2002;3:737-747.
5. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature*. 2001;411:494-498.

CORRECTION

Incorrect Number: In the Original Contribution entitled "Influence of Controllable Lifestyle on Recent Trends in Specialty Choice by US Medical Students" published in the September 3, 2003, issue of THE JOURNAL (2003;290:1173-1178), there was an incorrect number in a table. On page1174, in Table 1, in the "Radiology (diagnostic)" row, in the "Years of Medical Education Required" column, the number of years of education should have been 5, not 4.