Incubation Periods of Experimental Rhinovirus Infection and Illness

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Rhinovirus is the most commonly isolated virus from individuals with acute upper respiratory tract illness. The recognition of certain pathophysiological events may be of key importance in developing and applying treatments for rhinovirus colds. Ideally, to be most effective, treatment should be started as early in the infection as possible. For this to occur, the patient must be able to recognize the symptoms that announce the onset of the cold.

Careful monitoring of the onset of symptoms in the early period of rhinovirus infection has been limited, but one study [1] suggests that symptoms begin quite early in the infection. Likewise, the period from experimental rhinovirus deposition in the nose to the onset of viral shedding in nasal secretions has not been carefully studied.

In previous growth cycle experiments in cell cultures, newly produced rhinovirus was detectable in 5–7 hours, and a cycle of viral replication was completed in 10–12 hours, although the first appearance of new virus was sometimes as late as 9 hours (with completion of viral replication as late as 15–17 hours) [2]. The current study was done to determine the interval between intranasal rhinovirus inoculation and the onset of viral shedding and symptoms.

Materials and Methods

Subjects. Volunteer subjects were from the student and general populations of the University of Virginia in Charlottesville; they were 18 years of age or older and had given informed consent in a form approved by the University of Virginia Human Investigation Committee. Subjects were enrolled in the study if they had a serum titer of neutralizing antibody to rhinovirus type 39 of ≥2; had no history of asthma, allergies, or other chronic illness; and were not taking medications such as nasal sprays, antihistamines, steroids, or decongestants. Females could not be pregnant or lactating. A total of 18 subjects were enrolled. The study was conducted during three periods. For evaluation in the study, virus-challenged subjects had to be infected with rhinovirus type 39.

Virus challenge. Intranasal challenge was done with normal saline solution for controls and with an inoculum of rhinovirus type 39 that had been safety tested for extraneous microorganisms for cases [3]. The challenge was administered by a standard method in coarse drops in two inocula given ~10 minutes apart at 8 A.M. An inoculum of 0.25 mL was given per nostril, for a total of 1 mL per subject. Individual aliquots of the same viral inoculum pool were used during each period of the experiment.

On the basis of infectivity titrations, the concentrations of virus in the inoculum given during the three study periods were 300, 3,000, and 1,000 TCID₅₀/mL, respectively. The differences in the results of infectivity titrations were due to uncontrollable variations in the sensitivity of the different WI-38 cell culture lots in which the titrations were performed and do not represent true differences in viral concentration. The subjects were in the supine position when the inoculum was given, and each participant was asked not to blow his or her nose for at least 30 minutes after challenge.

Measures of infection. Nasal washings were collected at 2-hour intervals for the first 24 hours after viral challenge and daily at 8 A.M. thereafter for the next 4 days. Samples were obtained by placing 3 mL of normal saline solution into each nostril. After 10 seconds, the washing fluid containing nasal secretions was expelled into a waxed paper container. After mixing in a syringe, 3 mL of the fluid was pooled with 1 mL of virus-collecting broth for performing infectivity titrations.

An isolate from each case was identified as rhinovirus type 39.
by a standard neutralizing test [4]. Serial 10-fold dilutions of 0.1 mL of undiluted nasal washing specimens were placed in WI-38 cell cultures in screw-capped tubes, and the viral titers were calculated by the Karber method [5]. Samples in which virus did not grow were assigned a value of \(-0.5 \log_{10} \text{TCID}_{50}/0.1 \text{ mL}\); samples in which virus grew only in the undiluted specimen, 0; samples in which virus grew at a 10\(^{-1}\) dilution, 1 \(\log_{10} \text{TCID}_{50}/0.1 \text{ mL}\); and so on.

Serum samples were obtained 2 days before and 3 weeks after viral challenge to measure homotypic antibody to rhinovirus type 39 [4]. Infection was defined as recovery of rhinovirus type 39 from nasal washings on at least 1 day, a fourfold or greater rise in serum titer of neutralizing antibody to rhinovirus type 39, or both.

**Measures of illness.** The occurrence and severity of symptoms were assessed on a five-point scale as follows: 0, absent; 1, mild; 2, moderate; 3, severe; and 4, very severe. Symptoms were reported by the subjects every 2 hours for the first 48 hours and then daily at 8 A.M. Symptoms were recorded on prepared forms that were collected by study personnel.

Symptoms assessed were sneezing, rhinorrhea, nasal obstruction, sore or scratchy throat, cough, headache, malaise, and chilliness. This method of scoring and diagnosing illness is based on a modification [6] of a previously described method [7]. These criteria define illness as being present if a subject had a minimum total symptom score of 6 and had the subjective impression that he or she had a cold or that rhinorrhea was present on three or more of the 5 days of observation.

**Experimental design.** The dates of the experiments were 12–17 January, 13–18 May, and 14–19 October 1994. During the first and second periods, only virus-challenged subjects (cases) were studied; during the third period, both cases and saline-challenged subjects (controls) were studied. During the last period, subjects were randomly assigned to be inoculated with the virus or normal saline solution; they were blinded to their challenge status, as were the investigators collecting specimens and clinical information. During all three study periods, subjects were isolated in individual hotel rooms beginning 1 hour after challenge and remained in isolation for 5 days. Nasal washing specimens were collected and symptom scores were recorded every 2 hours after challenge for the first 24 hours and daily at 8:00 A.M. thereafter for the next 4 days.

**Data analysis.** Analysis was based on data for all study participants. Measures of illness and viral shedding were analyzed by the Student’s \(t\) test for significance. All reported \(P\) values were one-sided.

**Results**

**Subjects.** Of the 18 subjects enrolled in the study, 17 were evaluated. One subject was excluded because of infection with a wild strain of rhinovirus before experimental viral challenge. Of the evaluated subjects, 11 (four men and seven women) were challenged with rhinovirus type 39 (cases) and six (three men and three women) were challenged with normal saline solution (controls). The mean age of cases was 23 years, and the mean age of controls was 22.5 years.

**Occurrence of infection.** Of the 11 cases, all shed rhinovirus type 39 on at least 1 day. Only five of the cases had a fourfold or greater rise in serum titer of homotypic neutralizing antibody, a finding consistent with the known weak immunogenicity of rhinovirus type 39. Of the six controls, none shed virus on any day during the study or had a rise in titer of antibody to rhinovirus type 39.

Virus was first recovered from 2 cases at 8 hours, 5 cases at 10 hours, 1 case at 12 hours, 2 cases at 14 hours, and 1 case at 16 hours after challenge (figure 1A). The mean time to viral shedding was 11.3 hours (median, 10 hours; mode, 10 hours). The one instance of viral isolation at 18 hours could represent a situation in which two cycles of viral replication occurred and the first cycle of replication was missed. If this value is excluded from the data set, the mean time to viral shedding becomes 10.6 hours (median, 10 hours; mode, 10 hours).

Geometric mean titers of rhinovirus reached \(10^{1.2} \log_{10} \text{TCID}_{50}/0.1 \text{ mL}\) at 10 hours after viral challenge (figure 1B). Mean titers then remained relatively stable until 18 hours after challenge, when they rose to \(10^{1.5} \log_{10} \text{TCID}_{50}/0.1 \text{ mL}\); this occurrence suggests the effect of a second cycle of viral replication. Mean titers reached their highest point \((10^{1.4} \log_{10} \text{TCID}_{50}/0.1 \text{ mL})\) in the specimen collected on the morning of the second day (48 hours after viral challenge). Mean titers then began to decline by day 3 \((10^{1.3} \log_{10} \text{TCID}_{50}/0.1 \text{ mL})\) and remained low on day 4 \((10^{0.6} \log_{10} \text{TCID}_{50}/0.1 \text{ mL})\) and day 5 \((10^{0.4} \log_{10} \text{TCID}_{50}/0.1 \text{ mL})\), which is characteristic of rhinovirus type 39 infection [8, 9].

**Occurrence of illness.** Of the 11 cases, nine (82%) had colds. One (16.7%) of the six controls met the criteria for illness. The mean total symptom score for the cases rose by 2 hours and increased steadily over the initial 18-hour period (figure 2A). A second increase in the mean total symptom score followed shortly after the second peak in viral replication, occurring between 18 and 20 hours. The mean total symptom score for the controls rose at 4 hours, and thereafter this score remained relatively stable throughout the remainder of the 24 hours. All symptoms of the controls during the first 24 hours were reported by the one subject who met the criteria for illness.

Although the actual symptom scores were approximately twofold higher for the cases than for the controls during the early period, the scores for the two groups did not become significantly different until 16 hours after viral challenge \((P = .04)\). After the initial 24 hours, the mean total symptom score for the cases increased further to a peak on the second day compared with that for the controls \((P = .01)\). The mean total symptom score then declined, and by the fifth and final day of the study, it was not significantly different from that for the controls \((P = .4)\). The characteristics of illness and the individual symptom scores resembled those previously de-
Figure 1. A. Time to first positive viral culture for 11 rhinovirus-challenged subjects. Numbers on the top of the bars represent the number of subjects. B. Geometric mean titers (+ SE) of neutralizing antibody to rhinovirus in rhinovirus type 39–challenged subjects (●; n = 11) and saline-challenged subjects (□; n = 6).

scribed in detail for a large group of subjects with experimental colds due to rhinovirus type 39 [10, 11].

With the small sample sizes, it was not possible to optimally evaluate the individual symptom scores because of known variations in experimental rhinovirus colds. However, it is interesting that the complaint of sore or scratchy throat appeared in the cases at the time when virus was first detected in nasal secretions (figure 2B). On the other hand, nasal obstruction

Figure 2. Mean symptom scores (+ SE) for rhinovirus type 39–challenged subjects (●; n = 11) and saline-challenged subjects (□; n = 6). A. Total symptom score (based on the sum of all eight symptoms); B. sore or scratchy throat score; C. nasal obstruction score; and D. rhinorrhea score.
(figure 2C) and rhinorrhea (figure 2D) occurred in the cases shortly after viral challenge. Transient rhinorrhea also occurred in the controls at 4 and 6 hours and 18–22 hours after challenge (figure 2D).

Discussion

Colds are the most common acute infectious illnesses in humans. The magnitude of colds can be seen by looking at the incidence data for different populations. National Health Survey data for the United States indicate that acute respiratory tract illness accounts for about 53% of all acute conditions on an annual basis [12]. In the study of families in Cleveland [13], 60% of all illnesses over a 10-year period were classified as common respiratory tract diseases. Thirty-six percent of absenteeism of insurance company employees in Charlottesville, Virginia, was due to respiratory tract illness [14]. In a group of Seattle families with children, 86% of illnesses were respiratory tract diseases [15]. The most commonly isolated viruses from individuals with acute respiratory tract illness are rhinoviruses [16].

The predominant symptoms of the illness accompanying rhinovirus infection are sneezing, nasal obstruction, nasal discharge, and sore or scratchy throat [11]. Other symptoms are cough, headache, and malaise. Because of antigenic diversity, a vaccine approach to the control of rhinovirus infections does not appear feasible. Therefore, recent work has focused on treatment, especially with a combination of antiviral and anti-inflammatory compounds to block both viral replication and the inflammatory pathways that cause symptoms [17]. Since the period of active and progressive viral replication is short, treatment should be started early (ideally, at the first clinically recognizable indication of infection).

In the current study, the median time to the onset of viral shedding (10 hours) was similar to that observed in cell culture. After 48 hours, viral production declined, as has been found in earlier studies [8, 9]. Therefore, the optimum period for initiation of antiviral treatment is during the first five or six cycles of viral replication, before natural processes in the host begin to shut down viral production.

To begin treatment during this early period of infection, an individual must be able to recognize that an illness is starting. In the current study, mean total symptom scores became significantly higher for the cases than for the controls at 16 hours. It is surprising that mean nasal obstruction scores for the cases rose as early as 2 hours after viral challenge. Since no new virus can be assumed to have been released by 2 hours, these early symptoms may have resulted from a response to the viral inoculum pool that contained not only rhinovirus but also cell culture medium and constituents from the harvested cells. Mean nasal obstruction scores then rose progressively, while mean rhinorrhea scores remained stable. Sore or scratchy throat started at the time when virus was first detected, and these mean scores steadily progressed. There is a common perception that a “tickle in the throat” is an indication of an incipient cold.

This study shows that both viral replication and some cold symptoms occur early after intranasal challenge with rhinovirus type 39. This finding suggests that with appropriate education and motivation, individuals may be able to recognize some of the symptoms of early rhinovirus infection and begin treatment at a time when it would be most effective. Experiments with other rhinovirus immunotypes and with varying concentrations of viral inoculum would be useful to further examine these early events in rhinovirus colds.

References