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The Common Cold

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Introduction

The common cold is one of several potential syndromes related to infection of the cells of the respiratory epithelium by any of a group of viruses. All of the viral respiratory syndromes are characterized by nasopharyngeal inflammation as a host response, but the common cold is distinguished from other syndromes by the predominance of symptoms of nasal discharge and obstruction and by the lack of significant temperature elevation (71,73). Although other names such as acute coryza or afebrile respiratory tract viral infection are synonomous with the term "common cold," the latter persists and reinforces the erroneous lay notion that chilling of the body by exposure to moisture or cold temperatures contributes to the initiation of the illness. During the 20th century it has become clear that exposure of a susceptible person to respiratory virus can induce a cold regardless of the physical environment (3,38). The viruses associated with the common cold include the adenoviruses, the myxoviruses, and, most recently identified, the coronaviruses, but the majority of colds are associated with infection caused by one of the more than 100 serotypes of the rhinovirus (42,59–62,76,77,105,107,117). In spite of an understanding of the viral etiology, control measures are problematic. The ubiquity of the ailment and the susceptibility of virtually the entire population of the Earth to multiple respiratory viruses underscores the need for continued efforts in developing means and strategies for providing immunoprophylaxis, antiviral chemotherapy, and symptomatic relief.

Etiology

The viral etiology of the common cold was suspected for several decades before it was possible to define the causative agents. It was shown as early as 1914 that nasal secretions from a person with a common cold could transmit the illness to others by intranasal inoculation after filtration of the secretions to remove cellular and bacterial materials (83). However, the tissue culture techniques needed to permit reliable in-vitro replication of the respiratory viruses were not available for several more decades (4,21–23,62,63). Subsequent refinements of methodology encouraged large-scale investigation of naturally acquired and laboratory-initiated volunteer viral respiratory tract infection in order to provide specific information about the biology and epidemiology of the respiratory viruses.

The viruses associated with the common cold belong to several taxonomic families with diverse biophysical properties (Table 7.1). The respiratory viruses include both RNA and DNA viruses. Some are characterized by the presence of a naked protein capsid of icosahedral configuration to enclose the nucleic acid. Others include a complex lipid envelope around a pleomorphic helical nucleocapsid. The lipid envelope is derived from the membrane structures of the host cell with virus-specified proteins embedded in the membrane bilayer during the replicative cycle of the virus. A lipid envelope confers susceptibility to inactivation by organic solvents such as ether, chloroform, and trichlorfluorane to which the unenveloped viruses are resistant. All of the respiratory viruses may

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Table 7.1. Physical properties of viruses associated with the common cold.

Virus family	Representatives	Size (nm)	Genome	Symmetry	Envelope
Picorna-	Rhinoviruses 1A, 1B–89, coxsackie virus A 21, Echovirus 11	18–30	ss RNA	Cubic	No
Corona-	Coronaviruses 229E, OC39, OC43	80–130	ss RNA	Helical	Yes
Orthomyxo-	Influenza viruses A and B	80–120	ss RNA	Helical	Yes
Paramyxo-	Parainfluenza viruses 1–4, respiratory syncyntial virus	125–250 300–350	ss RNA	Helical	Yes
Adeno-	Adenoviruses 1,2,3,4,7,21	80–130	ds DNA	Cubic	No

be inactivated by various physical protein denaturing techniques such as exposure to formalin or glutaraldehyde or heating to temperatures of 50° to 100°C for minutes to hours. Neutralizing antibodies produced as a response to specific viral surface protein structures can also inactivate respiratory viruses and provide the host with protection from reinfection with the same virus.

Initiation of infection occurs when a virus contacts cell receptor structures. The chemical composition of the receptor(s) for the respiratory viruses on the ciliated epithelium is not yet known, but work is proceeding to that end. It is likely that several cell membrane structures serve the purpose for different viruses; however, unrelated viruses may share the same cellular receptor as defined by competitive binding assays (94). After penetration of the cell, virus replication begins at the expense of the host, and all resources are used to produce new progeny virus. The length of time required to complete a cycle of replication depends on the infecting virus but is on the order of several hours. The rhinovirus replicates with maximal efficiency at nasal temperature (33° to 34°C) and may be prevented from spread to other respiratory structures for that reason (29,142). All other respiratory viruses can replicate effectively at nasal temperatures, although maximal efficiency may occur at higher temperature (36° to 37°C).

The rhinoviruses have received much attention as they are the cause of 35% to 50% of all of the common colds in adults and children. Rhinoviruses were first isolated in the 1950s and named in the 1960s to recognize the fact that they appear to replicate only in the nasal passages (4,102, 113,120,123,145). The first strains were identified in tissue cultures not by the production of cytopathology but by the ability of the inoculated tissue cultures to reproduce infection when transferred to volunteers. Development of serologic techniques permitted a system of identification based on neutralizing antibodies that makes clear that the potential number of rhinovirus serotypes is unlimited and possibly driven by evolutionary pressures (24,25,43,106,133,139). Human rhinovirus strains have man as the only known resevoir and do not infect nonprimate animal species. Although nonhuman primates can be infected with a human rhinovirus strain, the illness noted in man is not reproduced (28).

Like other picornaviruses, the rhinovirus consists of a single strand of RNA surrounded by an icosahedral protein capsid made of 60 identical subunits or capsomeres (129). Each capsomere includes one strand of the four structural polypeptides. Neutralization by antibody occurs by reaction of immunoglobulins with specific sites on the exposed structural polypeptides (133). Rhinoviruses, again like other picornaviruses, are not affected by organic solvents because they are not enveloped (58). Acid inactivation of rhinoviruses differentiates them from the enteroviruses, which include three subspecies: coxsackieviruses, echoviruses, and polioviruses (143). In addition, while enteroviruses can replicate at 33°C, they are in general better suited to maximal replication at 37°C (98). Although most enteroviruses are associated with forms of systemic illness (pericarditis, aseptic meningitis, paralytic poliomyelitis) after enteric replication, some strains such as coxsackievirus A21 and echovirus 11 have a propensity possibly related to receptor affinity for the respiratory tract (94). Respiratory tract illness related to an enterovirus is clinically indistinguishable from rhinovirus infection.

The coronaviruses were initially isolated during the 1960s from a person with a cold (144). The name of the virus is derived from its appearance by electron micrography, which shows the nucleocapsid of the virus to be surrounded by a crown-

like array of club-shaped projections from the lipid membrane (1). Coronaviruses are single-stranded RNA viruses with an helical nucleocapsid configuration. Several strains of the coronavirus have been isolated and appear to fall into two serogroups represented by the original prototype strain 229E and the OC43 strain (107). The 229E and related strains replicate well in monolayer tissue cultures, but some strains require tissue explant organ cultures (human fetal trachea) on primary isolation attempts from clinical specimens. Coronaviruses probably account for 15% to 20% of common colds.

Among the myxovirus group are the orthomyxoviruses represented by influenza viruses A and B, which were first isolated in the laboratory in the 1930s (135) and early 1950s (44), and the paramyxoviruses represented by the parainfluenza viruses (19) and respiratory syncytial virus (109), which were initially recovered in the mid-1950s. These viruses are similar in symmetry and composition of the nucleocapsid. However, the envelopes of the viruses differ substantially in terms of composition and function of the virus-specified glycoproteins that are embedded in the envelope during viral maturation. The influenza viruses have two envelope glycoprotein spikes: the hemagglutinin (HA), which permits virus attachment to cellular receptor sites which contain sialic acid residues, and the neuraminidase (N), which cleaves sialic acid residues and may prevent clumping of progeny virus (135). The HA and N occur on a single glycoprotein spike in the case of parainfluenza viruses, which also have a second spike bearing the fusion (F) protein (20). The F protein has a role in the penetration of the host cell and in inducing fusion of neighboring susceptible cells. The F protein is found in the absence of HA and N on respiratory syncytial virus, which has a distinct tendency to induce syncytium formation in tissue cultures by cellular fusion (89). Changes in the HA and N proteins of the influenza viruses occur continuously by point mutation and by genetic recombination and result in minor (drift) and major (shift) changes in infectivity, since the modified proteins permit the virus to escape the immunologic controls of the host species (9,69,130). Similar changes in the parainfluenza viruses and respiratory syncytial virus have not yet been recognized. Although the influenza viruses are associated with febrile illness involving the lower respiratory tract, it has been noted that as many as 50% of the persons during influenza epidemics may have symptoms more suggestive of the common cold (75). A common cold is more likely to result from influenza B virus infection than influenza A. Likewise, respiratory syncytial virus, which commonly produces severe lower tract disease in young children, may produce a cold when infecting adults or older children (62).

The adenoviruses now include more than 40 distinct serotypes that can be isolated from the respiratory and enteric tracts of humans (46). The first adenoviruses were recovered in the 1950s from tissue explants of adenoids and tonsils, hence the name (127). The adenoviruses are DNA viruses with naked icosahedral capsids that each include 252 capsomeres. At the vertices of the icosahedron are groups of capsomeres (pentons) that carry an antigen shared by all adenovirus serotypes. Serotype-specific antigens are carried on the face groups of capsomeres (hexons). Like other double-stranded DNA viruses, adenoviruses may produce latent infection by incorporation of the virus genome in that of the host cell. Although adenoviruses cause a spectrum of illnesses including conjunctivitis, pharyngoconjunctival fever, and diarrhea, certain serotypes most commonly affect the upper respiratory tract. Strains most likely to be isolated in industrial countries and endemic in populations include types 1, 2, 3, 4, 7, and 21 (6). Frequently respiratory tract infection with an adenovirus presents as febrile pharyngitis, but a small percentage of infections is characterized by symptoms more suggestive of a cold.

Epidemiology

The common cold has a worldwide distribution in virtually all climates and populations (61, 70,103,111,147). It is absent only from certain completely isolated groups of people such as those in antarctic or arctic colonies. However, introduction of a viral respiratory tract infection into those communities from an outside source results in a rapid spread among the immunologically susceptible individuals. In the general population, outbreaks also occur with the introduction of viruses to which little herd immunity is present (49).

Colds occur sporadically throughout the year, but certain seasons of the year are associated with increased numbers of infections for reasons that

are not yet entirely clear. It has been suggested that the annual fall increase in colds may be attributed to the congregation and confinement of children in schools after the summer recesses (7,91,92). Other unknown factors may be more critical, since the respiratory viruses display other patterns of seasonality unrelated to social functions. Rhinovirus infection is associated with two peaks of activity during the fall and spring with one or the other season predominating in different locations during different years (42). The parainfluenza viruses peak in activity during the late summer to fall months, and different serotypes predominate in alternate years in a continuing pattern (97). The influenza viruses and respiratory syncytial virus are most important during the winter months (97,108). An illustration of the changing predominance of viruses may be seen in the types of viruses isolated from nasal washings from young adults with afebrile upper respiratory tract illness during sampling in winter and summer months at a university health service in Chicago during 1983 (Table 7.2). During January and February, influenza A, respiratory syncytial virus, and adenoviruses were the only viruses recovered. However, when rhinoviruses appeared in early March, the other respiratory viruses quickly receded. Later in the year during the summer and fall months, essentially all of the isolates from persons with colds were rhinoviruses. Although herpes simplex virus was occasionally isolated in the absence of overt herpetic lesions as previously reported (117), it is probable that herpes simplex is not responsible for the common cold, since latent herpes infection is common, and virus may be asymptomatically shed. In addition, herpes simplex replicates so readily in most tissue cultures that its presence may obscure other agents.

The relative proportions of the respiratory viruses isolated from persons with common colds vary by geographic location at any given time. Outbreaks associated with a single type of respiratory virus are common. Where rhinoviruses predominate, several serotypes are usually found simultaneously (49,103,104,147). From year to year some serotypes remain in a given community while others are replaced by new serotypes. When new rhinovirus serotypes appear, they are usually higher numbered or untypeable (and probably new) serotypes (17). Coronaviruses are usually second in frequency only to the rhinoviruses as the etiologic agent of colds, but their presence may be difficult to detect because of the requirement of some strains for organ culture to permit replication on primary isolation (107).

The basic unit of infection for most of the population appears to be the family (7,42,91,92). The index case is often a younger school-aged child who acquires infection and introduces the respiratory virus into the home by infecting other susceptible family members. Other siblings are most frequently afflicted, and mothers develop infections more frequently than fathers. The increased maternal susceptibility may be explained by the greater contact mothers have with their children than fathers. However, an increased susceptibility to infection with a common cold virus has been noted for women about the midpoint of the menstrual cycle (37). Although the workplace can also permit transmission of colds, it is not as efficient, perhaps because of less direct personal contact (49). The average for colds in industrial countries is approximately one cold per person per year. Some individuals experience several and others report none. The reason for some of the variability is undoubtedly related to the social factors listed above. The occurrence of asymptomatic and mildly symptomatic infections helps to obscure recognition of some respiratory virus transmission. Some persons are more readily symptomatic after viral challenge, particularly those with allergic rhinitis (37). However, exposure to cold or damp conditions results

Table 7.2. Common cold isolates from young adults at a university health service, Chicago, 1983.

Isolates	Jan–Mar	Aug–Oct	Total
Rhinovirus	7 (46%)	8 (89%)	15 (60%)
Respiratory syncytial virus	4 (24%)	0	4 (16%)
Influenza A	2 (12%)	0	2 (8%)
Adenovirus	2 (12%)	0	2 (8%)
Herpes simplex virus	1 (6%)	1 (11%)	2 (8%)

in no change in the pattern or severity of symptoms nor does susceptibility to infection increase, as demonstrated by several volunteer studies (3,38).

The major determinant of susceptibility to viral respiratory tract infection is the immune status of the host, with emphasis on previous exposures (2,72,100,110). In the case of most respiratory viruses, secretory IgA can be found in the nasal secretions of immune individuals (14,125,126), and serum IgG antibodies can be demonstrated in the same persons (44). In volunteers challenged with a common cold virus, 75% to 80% of unselected persons become infected. A second exposure to the same virus produces infection in only 10% to 20% (72,73). However, the protection to infection conferred by the presence of antibody is only relative (65). The occurrence of a second viral respiratory tract infection within 3 weeks (before protective antibody can develop) is unusual, possibly because of nonspecific protection against susceptible viruses by local nasal production of interferon (16,40,100).

Clinical

Common cold symptoms follow a familiar pattern that has been investigated with various respiratory viruses but most completely with the rhinovirus (3,13,15,32,34,37,38,71,73). Common cold symptoms begin within 24 to 72 hours of virus inoculation. Sneezing, sore or scratchy throat, and headache appear along with the characteristic nasal obstruction and nasal discharge as early indicators of a cold. The headache and sore throat usually begin to wane within a day or two while cough appears (with or without sputum production), and nasal obstruction and discharge worsen for several more days. Fever (temperature greater than 38°C) is uncommon with a simple cold, but many individuals experience a chilly sensation. True chills or rigors are distinctly unusual and suggest a process other than a common cold. The symptoms persist for seven to ten days in most instances, but cough and sputum production may continue for several weeks in persons with underlying lung disease. Tracheobronchitis has been produced in otherwise healthy persons by experimental administration of an infected small particle aerosol (13).

The nasal secretions change in character qualitatively and quantitatively throughout the duration of a cold. Prior to infection, nasal secretions are scanty, mucoid, and clear in appearance. Some cellular debris is present and includes epithelial cell fragments, rare functioning ciliated cells, squamous epithelial cells from the nasal vestibule, and a few granulocytes. At the onset of cold symptoms nasal secretions increase in quantity dramatically to as much as 15 g/d (34). The secretions are initially watery in consistency but rapidly assume a more mucopurulent appearance. An increase in cellular debris in the secretions accounts for the purulence, and all of the cells mentioned increase in number. Many of the leukocytes in the nasal secretions are lymphocytes morphologically, but polymorphonuclear leukocytes predominate. Functional, viable ciliated cells as well as dead and dying ciliated epithelial cells are shed, and some can be shown to be infected by immunologic staining (67,141).

The common cold is mild and without seqelae in the overwhelming majority of instances, but complications may occur in a few individuals. Persons with asthma or chronic bronchitis frequently experience an exacerbation of obstructive lung symptoms, with bronchoconstriction and increased sputum production (45,101,138). Changes in small airways function and mucociliary clearance mechanisms may appear during respiratory tract virus infection even in otherwise healthy persons (8,45,47,87,95). Sinusitis and otitis media occur in association with some colds and may be caused by viral replication in the epithelium in some cases. In other instances bacterial colonization and growth permitted by obstruction of the ostia into the nasopharynx may be responsible (57). However, colds do not change the bacterial flora of the nasal passages (71).

Several scoring systems have been developed to judge the severity of common cold symptoms based on objective measurements made by experienced observers and subjective responses of the infected persons (10,12,71,115). Objective criteria include weight of nasal secretions, number of paper handkerchieves required per day, and degree of nasal obstruction based on planimetry of condensation of exhaled water vapor on a chilled surface. Some assessments such as the degree of turgidity or the degree of erythema of nasal mucosa are not reproducible and unreliable (71). Subjective scoring of the severity of symptoms by infected persons permits a reliable semiquantitative means of determining the relative severity of a cold (71).

It is clear from volunteer studies that some virus-infected persons have symptoms so mild as to be no different from simultaneously examined uninfected controls. On the other hand 10% to 20% of those with symptoms have scores indicating a severe level of discomfort.

The symptom pattern is itself tied to an extent to the quantity and duration of virus shedding (32). For example, shedding of rhinoviruses in volunteer challenge studies is less in frequency, less in quantity, and relatively delayed in appearance in persons with fewer, less severe, and later-developing symptoms. The explanation for the difference in symptoms between persons is not clear, but selection of volunteers to ensure seronegativity and susceptibility to infection does not alter this feature. Additional factors determined by individual heredity and psychology may also influence the pattern and severity of symptoms.

The presence of seroimmunity to a respiratory virus is generally indicative of resistance to infection by that virus (33,41). However, the protection is only relative. Following an initial respiratory tract infection with a given virus, antibody appears in increasing titer in serum and nasal secretions. In the absence of repeat exposure to the same or a closely related virus, antibody titers begin to wane and in some instances may be negligible after 18 to 24 months (72). An increase in the circulating lymphocytes specific for the antigens of a respiratory virus (e.g., rhinovirus) may be found following infection, but may be shorter in duration than the antibody response as an indicator of preceding infection (85). Subtle changes may occur in viral antigenic composition over time so that neutralizing antibodies become less effective at binding to later isolates (137,139). In addition, the protection conferred by antibody in the nasal secretions and serum is only relative, and large inocula of rhinoviruses may permit excess free infectious virions to infect cells (65). In the case of coronaviruses, up to 80% of infections occur in persons with preexisting serum antibody (107).

In order to reach the receptors for attachment to the cells of the respiratory epithelium, virions must have a mode of transmission. Direct inoculation of contaminated secretions into the conjunctival sac or nasal vestibule via the fingers or inhalation of droplets of an infected aerosol through the nasal passages are the two routes available. The ability to produce infections by both routes has been documented for most viruses, but transmission under natural conditions primarily occurs by one or the other route, depending on the type of virus. Evidence suggests that myxoviruses (like influenza viruses A and B) and coronaviruses are transferred mainly by the aerosol route (75,107). Others such as rhinoviruses and adenoviruses appear to be most effectively transmitted by self-inoculation with contaminated secretions (50,51,66). In the case of coxsackievirus A21 and other enteroviruses, both routes may be common (98). Most viruses, if kept moist, can persist for hours on inanimate objects and surfaces, but rhinovirus infection does not appear to be efficiently acquired by most such exposures (123). However, person-to-person contact for as short as 10 seconds has been shown to transfer sufficient quantities of rhinoviruses from an infected donor to a susceptible recipient in as many as 75% of the donor-recipient pairs (51). Minimal quantities of rhinovirus are detectable in oral and enteric secretions, which may explain the apparent ineffectiveness of oral secretions at transferring rhinovirus infections (11,15,27,51).

The exact sequence of events that results in symptoms when respiratory tract infection is established remains speculative. Myxovirus infection may produce significant alterations in the structural integrity and functional ability of the ciliated respiratory epithelium (47,87). However, simple destruction of infected epithelial cells does not appear to account for the symptomatic response in the case of the rhinoviruses. Although rhinovirus-infected epithelial cells can be easily identified with immunologic-staining procedures, negligible damage to the ciliated epithelium has been found in nasal biopsy specimens and nasal polyp explant cultures after infection with a rhinovirus (34,56). This suggests that the host response plays an important role in the process and perhaps determines the apparent susceptibility of some individuals to more numerous or more severe viral respiratory tract infections. Cholinergically mediated reflexes have some part in the increase in nasal secretions during a cold, since treatment with an anticholinergic agent, ipratropium, can reduce the quantity of nasal discharge (10). Although arachidonate metabolites have the potential for acting as mediators of inflammation, prostaglandins are not clearly implicated since aspirin and other cyclooxygenase pathway inhibitors do not significantly al-

ter nasopharyngeal symptoms compared with no treatment (136; Levandowski, unpublished observation).

Cellular mechanisms appear to contribute to the host response and the inflammatory events. Mononuclear leukocytes increase in number in nasal tissues early in the symptomatic phase of common colds (68). A transient but significant reduction in the number of circulating lymphocytes concurrent with an overall increase in the WBC count has been demonstrated for a number of respiratory viruses within the first 24 to 48 hours of symptoms (12,31). In the case of acute infection with a rhinovirus, the reduction in lymphocytes is related to reduction of T lymphocytes, mainly of the helper population (88). A strong correlation between symptom severity and the magnitude of decrease in numbers of circulating T lymphocytes suggests that circulating lymphocytes become sequestered in infected nasal tissues and play a role in modulating the inflammatory response. Large numbers of viable leukocytes are exfoliated in nasal secretions within the first 48 hours of a symptomatic cold (Fig. 7.1). Cytologic preparations examined microscopically demonstrate that some of the exfoliated leukocytes have the appearance of large granular lymphocytes, which are known to be natural killer cells (140).

Laboratory

Identification of the etiologic agent of a common cold may be made by isolation of the infecting virus in tissue culture and/or by demonstrating a serologic response to the agent. A number of serologic tests have been developed to assist in identification, including assays for complement fixing antibodies, hemagglutination inhibition antibodies, neutralizing antibodies, and antibodies that can react in an enzyme-linked immunosorbent assay (ELISA). However, a single elevated antibody titer is insufficient to prove that a contemporary cold has been produced by an agent identified by antibody titer. It is necessary to document a fourfold or greater rise between sera obtained during the acute phase of illness and sera collected 3 to 6 weeks after the illness. Serologic testing as the sole means of identifying infectious agents is relatively insensitive. In volunteer studies with rhinoviruses when the challenge serotype virus is already known, only 50% to 75% of the volunteers infected have a rise in titer of neutralizing antibody with paired sera. In naturally occurring rhinovirus infection the task of identifying a serologic response becomes monumental, since the only serologic test readily available for rhinovirus detection

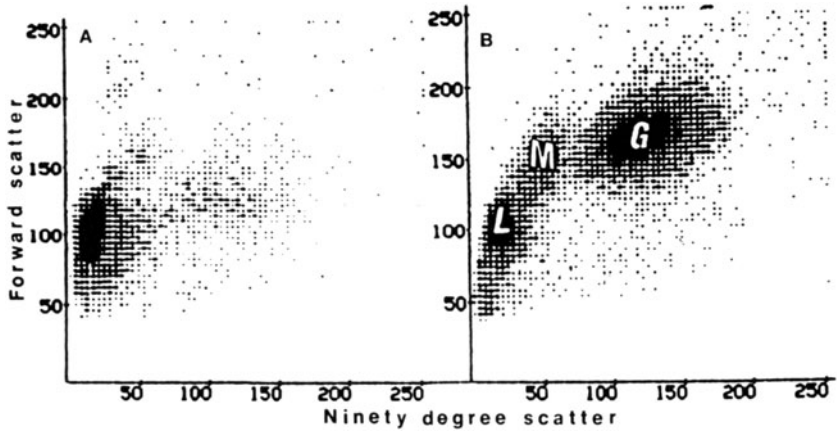

Figure 7.1. Flow cytometry of viable leukocytes in nasal secretions during the initial 48 hours of a common cold (panel A) compared with normal peripheral blood leukocytes (panel B). Forward light scatter relates to cell size; 90 degree light scatter relates to cell granularity. Leukocytes in peripheral blood have been labeled to demonstrate the light scatter pattern for lymphocytes (L), monocytes (M), and granulocytes (G). The majority of the leukocytes in the nasal secretions are lymphocytes with some increase in size and granularity. A few monocytes are also present. The cells with 90 degree light scatter similar to granulocytes (75–175 units) are probably chemotactically activated granulocytes that appear smaller on flow cytometry because of loss of spherical symmetry.

is neutralization, which is serotype specific. The task can be simplified if a viral isolate is obtained, since the isolate can be used as the substrate for antibody titrations.

The rate of respiratory virus isolation in a laboratory with experienced personnel is in the range of 50% of submitted specimens. Communication between the clinician and the laboratory can permit emphasis on inoculation and observation of appropriate tissue cultures to detect the type of virus indicated by the circumstances. Sample collection methods are important. Nasal secretions are the best source of virus-contaminated materials. Throat secretions may yield a number of viruses, but nasal secretions are more appropriate for recovering a virus during a common cold because rhinoviruses do not appear in significant quantities in oropharyngeal secretions (11,27,51). Collection of the nasal secretions by direct aspiration or cotton-tip applicator may be done for practicality in children, but nasal secretions obtained by washing with a buffered saline solution offer the best specimens for studies in adults. Collection of multiple daily samples can also increase the yield. Most persons tend to shed virus in the earlier days of maximal symptoms, and single samples obtained during that time are most likely to be positive during rhinovirus infection (Fig. 7.2). However, virus shedding associated with a common cold is variable in pattern and may be delayed in some persons.

The nasopharyngeal secretions collected should be inoculated into several types of tissue cultures simultaneously to maximize yield (42,53,79,90, 97,128). Each type of respiratory virus replicates best in certain tissue cultures (Table 7.3). For example, adenoviruses are maximally recovered in human embryonic kidney, whereas myxoviruses do best in primary monkey kidney cell lines. In addition, the recovery of individual viruses is maximized by inoculation of more than one type of tissue culture in which the specific virus replicates well. A reasonable battery of tissue culture types for the recovery of respiratory viruses would include a human diploid fibroblast cell line, HeLa or HEp2 cells, and a primary monkey kidney line, although additional tissue culture types would increase the probability of isolating the various viruses. The rhinoviruses replicate best at 33° to 34°C within a pH range of 6.8 to 7.2, and the viral cytopathic effect (CPE) is maximized by incubation of inoculated tubes on roller drum apparatus at 12 revolutions per hour (142). The same conditions permit excellent recovery of the other respiratory viruses. Viral CPE appears in tissue cultures within a few days to 2 weeks after primary inoculation, depending on the titer and type of virus. Subsequent tissue culture passages may reach total CPE in one to three days, and a blind passage of cultures without CPE increases the overall yield by amplification of virus titer.

The virus isolates are presumptively identified by the appearance of the initial CPE and the cell lines in which the virus has replicated. Rhinoviruses produce foci of CPE in diploid fibroblasts characterized by cellular disintegration with round

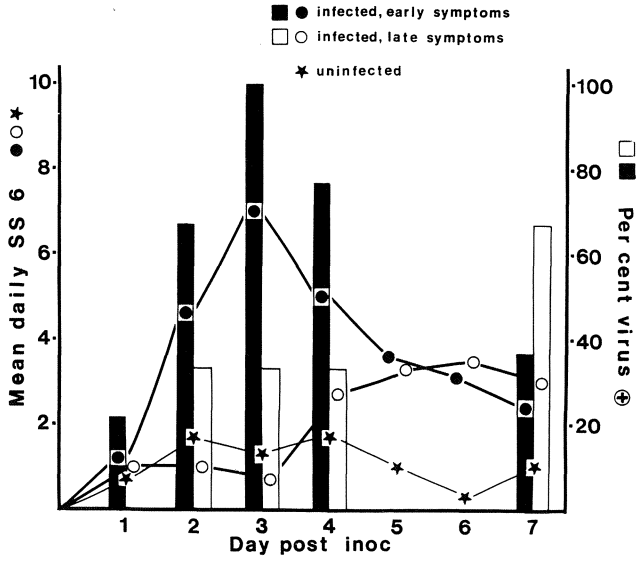

Figure 7.2. Mean daily scores for six common cold symptoms including headache, sneezing, nasal obstruction, nasal discharge, sore throat, and cough (SS6) and the frequency of virus shedding for the seven days following intranasal challenge of a group of volunteers with a rhinovirus. The closed circles and bars indicate scores and virus shedding for persons with early, relatively more severe symptoms. The open circles and bars indicate scores and virus shedding for persons with later, milder symptoms. Stars indicate the symptom scores for challenged volunteers who were not infected.

Table 7.3. Tissue cultures for the isolation of respiratory viruses from clinical specimens.

Virus	HDF	HeLa	HEp2	HEK	MK
Rhinoviruses	+++	+++	+	+	++
Coxsackie, Echo	+++	++	+	+	+++
Coronaviruses	++			++	
Influenza					+++
Parainfluenza		+	+	++	+++
Respiratory syncytial virus	+	++	+++	+	+
Adenoviruses	++	++	++	+++	

HDF = human diploid fibroblast, HEK = human embryonic kidney, Mk = primary monkey kidney.

fragments of variable size (86). Adenoviruses in the same cells produce rounded cells attached to neighboring cells by strands of cytoplasm (128). Respiratory syncytial virus characteristically produces syncytia that are easily recognized in HEp2 and HeLa cell lines (97). The influenza and parainfluenza viruses frequently produce little CPE on initial passage, but hemadsorption of inoculated cells with guinea pig RBCs will detect the presence of hemagglutinins embedded in the host cell membrane (78). With initial suspicions, further testing can definitively identify the viruses. Rhinoviruses are differentiated from the enteroviruses by inactivation at pH 3 to 5 for three hours (58). Coronaviruses are inactivated both by acid and by ether. Complement-fixing antibodies that recognize group antigens are generally used to identify potential adenoviruses and myxoviruses. Serotyping of viruses may be done by neutralization or by hemagglutination inhibition with specific reference sera, depending on the virus. In the case of rhinoviruses and enteroviruses the huge number of serotypes recognized only by neutralization antibodies makes identification of individual virus strains tedious. For that purpose intersecting pools of sera may be used to narrow the spectrum of possibilities to a few serotypes that can be tested individually, and microtiter techniques can be used to reduce the quantity of antiserum expended for each test (79,106).

Other techniques have been investigated and need to be developed in order to permit viral diagnosis within hours rather than the days required by tissue cultures, which are currently the most sensitive techniques. Nasopharyngeal epithelial cells can be examined after staining with fluorscein or peroxidase conjugated antibody in an attempt to identify suspected viruses, including rhinoviruses (if the serotype is suspected or known) and myxoviruses (93,141). Viral antigenic components can be identified via ELISA techniques in some instances (67). Influenza viruses are potentially detected by the metabolism of fluorescent substrates by the enzymatic activity of viral neuraminidase in secretions (148). Methods incorporating radiolabeled probes of complementary nucliec acids are also being investigated.

Treatment and Prevention

A single specific treatment for the common cold is not yet developed and may not be achievable. However, antiviral chemotherapy of respiratory viruses is in a stage of increasing potential. A number of chemical compounds that are potent inhibitors of rhinovirus replication have been identified but have been disappointing in limited clinical trials because of failure to prevent or eradicate infection or to alleviate symptoms (74,84,115,116, 122,124,149). If a cold occurs during an epidemic of influenza or during the winter months when influenza A is circulating in the community, treatment or prophylaxis with amantadine (or rimantadine, a related compound) could be considered, particularly for patients who might suffer severe consequences including the elderly or patients with cardiopulmonary disorders (30). Although amantadine is effective against all strains of influenza A, it has no effect against other myxoviruses. However, ribavirin is another antiviral agent that may be beneficial for the treatment of all types of myxovirus infection (18,54,80,96). When delivered as an aerosol it appears to speed recovery even in severe forms of illness such as tracheobronchitis

related to influenza A, influenza B, and respiratory syncytial viruses, and it could conceivably be used to treat milder illness expressed as a cold with an appropriate delivery system.

Ascorbic acid (vitamin C) deserves mention because of the continued interest in it as a preventive or therapeutic agent for the common cold. The rationale for its use is that serum ascorbate levels decline during acute infections like the common cold. It has been postulated that an increased intake (1 to 10 g/d) would increase the body's ability to resist the effects of infection and reduce the severity of symptoms. In spite of considerable enthusiasm, clinical trials have provided no evidence for a consistent benefit. Volunteer trials with rhinoviruses have yielded no significant effect, no specific antiviral activity has been defined, and many other benefits claimed appear to be best explained as statistical artifacts (26,131). A major problem with ascorbic acid studies is difficulty in eliminating observer bias because participants are able to detect the characteristic flavor of ascorbic acid.

A similar defect confronts a study of the effects of oral zinc gluconate on naturally acquired symptomatic common colds (39). Although it indicated a reduction in symptom severity and duration among the zinc recipients, the study can not be considered to be truly blinded since the participants were able to identify the zinc-containing preparation because of a distinct metallic taste. The use of zinc is based on the principle that zinc ions interfere with functions of virus-specified proteases in rhinovirus replication (82). However, the administration of zinc after the onset of symptoms has an unclear basis since several replication cycles are completed by that time and virus shedding has already peaked. Further study will be necessary to document a definite benefit.

Nonspecific measures are an imperfect form of treatment. Aspirin can relieve some of the systemic symptoms including malaise and chilliness but does nothing to prevent the nasal symptoms or to shorten the duration of symptoms overall. In a study of rhinovirus common colds, virus shedding appeared to be prolonged by the administration of aspirin (136). Other nonspecific measures include the use of antihistamines and vasoconstricting nasal sprays. The antihistamines provide minimal relief from nasal discharge and sneezing, the effect is short-lived, and drowsiness may be the most prominent result. The vasoconstricting sprays are associated with a rebound effect that often leads to a cycle of nasal congestion, necessitating use of the medication long after the cold has resolved. Nasal washing with physiologic saline is a simple procedure that relieves nasal obstruction by helping to remove tenacious secretions. A combination of aspirin for the early systemic symptoms plus saline nasal washes to remove later mucopurulent nasal secretions can provide a reasonable and inexpensive remedy. If anticholinergics prove safe and effective, these may also be useful for reducing early nasal discharge (10).

Prevention of the common cold is most simply accomplished by observance of careful personal hygiene to avoid person-to-person spread of virus-contaminated secretions. In this regard hand washing can be helpful. In the case of rhinovirus infection, inactivation of the virus in secretions carried by the fingers of infected persons may be achieved with paper handkerchieves soaked in mild iodine or acid secretions (52).

The antiviral state that is induced by interferon in neighboring cells confers a nonspecific resistance on those cells to infection by many viruses, including most of the respiratory viruses (16). Applications of leukocyte-derived interferon and inducers to promote local interferon production have been highly effective in reducing both infection rates and symptom severity in volunteers challenged with rhinoviruses (48,99,112,137). In addition, genetic engineering has yielded an abundant supply of preformed interferon that appears to be as effective in clinical trials as other interferons (36,132). Thus, interferon as a prophylactic regimen for the common cold meets several important criteria: (a) it is effective, (b) it has a broad spectrum of activity, and (c) it can be readily available. There are, however, also some strategic problems. Although recent studies have shown the efficacy of recombinant interferon given to household contacts with the first appearance of a cold in family members to reduce rhinovirus infections, the results with other viruses have not been as dynamic (134). Timing of administration may also be critical. Application of interferon at too late a time after viral contact may reduce effectiveness, since natural interferon may be induced in significant quantity by common cold viruses by the time symptoms begin (112,137). In addition, administration of the large amounts of recombinant interferon required to prevent infection results in symptoms mimicking the cold, including nasal obstruction, dis-

charge, and nosebleed in 10% to 20% of recipients (36,132).

Chemoprophylaxis is at present limited to compounds that are effective for influenza A viruses. Amantadine and rimantadine are both highly effective in reducing the risk of acquiring infection (30). Rimantadine appears to produce fewer adverse effects that include central nervous system symptoms such as insomnia and euphoria. Amantadine is usually given orally but may achieve higher local concentrations by the aerosolized route. Although amantadine has been shown to alter small airway functions in vivo after aerosol administration and to impair ciliary epithelial function in vitro (64,81), mucociliary clearance patterns do not appear to be altered by administration of the standard oral dose of medication in otherwise healthy adults (87). During the winter months of peak influenza A activity, amantadine could be considered for administration to those exposed to persons with a cold-like illness, since as many as 50% of influenza infections may have symptoms suggestive of a simple cold.

Immunoprophylaxis has been much investigated but is little available for viral respiratory tract infections at present. To be useful a vaccine must induce protective antibodies (secretory IgA) at the site of infection in the respiratory tract, with reasonable duration of action and few adverse effects. The exceptions to availability are the vaccines for influenza viruses A and B (108). Because of shift and drift in the influenza viruses, the vaccine must be administered yearly to ensure resistance to infection by circulating viral strains. Two types of inactivated influenza vaccines are available: a whole virus vaccine and a split-virus vaccine. Both may be recommended for adults, but the latter, with fewer febrile reactions, is used mainly for children. Vaccines have been prepared for most of the other respiratory viruses. A live oral vaccine with attenuated strains of adenovirus serotypes 4 and 7 has been effective in reducing infection in military recruits, but further development of the vaccine has not been pursued and no vaccine is available for use at present (118). Live attenuated vaccines are presently being developed for the myxoviruses including influenza viruses, parainfluenza viruses, and respiratory syncytial virus (119,145,146). These vaccines are administered by intranasal inoculation where the virus replicates but produces few symptoms because of diminished virulence.

Inactivated rhinovirus vaccines administered by intranasal or intramuscular routes have also been effective in reducing infection rate to homotypic viral serotypes (5,35,55,114,121). However, several problems remain to permit effective immunoprophylactic control of the common cold. Chief among these problems is the huge number of individual serotypes. A trial with two decavalent rhinovirus vaccines demonstrated the feasibility of inducing immunity to several serotypes simultaneously (55). However, the immunogenicity of strains may be variable, and cross-reactive protection to heterotypic strains not included in the vaccine may be infrequent. As mentioned, protection via secretory antibody is relative and may be overwhelmed (65). In addition, the duration of protection is not clearly defined but may be in the range of 1 to 2 years before protective effect wanes (72). Nevertheless, the ability to protect with vaccine is encouraging, and efforts to develop and implement vaccine strategies continue, with the hope of providing safe and effective regimens.

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