

# Nasal Cytokines As Mediators of Illness During the Common Cold

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Identification of a pharmacologically targeted mediator of the common cold is a desirable, but, to date, elusive goal of current research. The roles of various mediators, such as histamine, leukotrienes, bradykinin, and, more recently, chemokines and cytokines, in the pathophysiology and development of complications of the common cold are the subject of previous and current investigations. Establishing causality of a mediator in the common cold has been difficult for a number of reasons, including the limitations of our research tools and protocols and the complexity of the inflammatory and immune pathways that participate during the common cold. The available evidence for mediation of the common cold is the subject of this manuscript.

(*eg*, cytokines, chemokines) [6–9] have been performed by numerous laboratories, and the results related to extant symptom/sign expression. In turn, temporal correlations between signal (chemical) and response (symptom/sign) were often interpreted in terms of causal mediation and, therefore, as potential targets for pharmacologic modulation of the response. The most established success of this method is with its application to seasonal nasal allergy in which the identification of signal chemicals “causally” related to symptom/sign expression (*eg*, histamine) led to the development of efficacious “treatments.” However, that method fared less well in application to diseases with an underlying infectious etiology, such as otitis media [5], sinusitis [10], and viral upper respiratory illness [11•]. In this article, we discuss the limitations of applying this method to studies of the “common cold.” This review is organized to first define the impact and nosology of the common cold, then to present the critical evidences required for causal inference, and finally to review existing data with respect to causal pathways for disease expression.

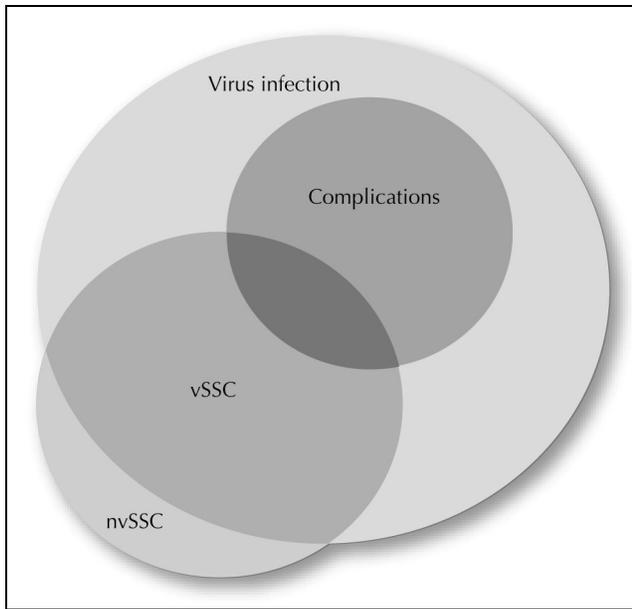
## Introduction

...if we have a proposition which contains the idea of necessity in its very conception, it is a judgement a priori; ...an empirical (a posteriori) judgement never exhibits strict and absolute, but only assumed and comparative universality (by induction); therefore, the most we can say is—so far as we have hitherto observed—there is no exception to this or that rule. If, on the other hand, a judgement carries with it strict and absolute universality, that is, admits of no possible exception, it is not derived from experience, but is valid absolutely a priori. (Kant, 1781)

A traditional method for studying the pathogenesis of upper respiratory diseases and their complications is the assay of secretions and/or other recovered fluids for chemicals that are expected to participate in the immune/inflammatory process. For example, assays of nasal secretions/washings [1–3], sinus fluids [4], and middle ear effusions [5] for “classic” inflammatory mediators (*eg*, histamine, bradykinin, arachidonic acid metabolites) and, more recently, for intercellular signaling chemicals

## Impact of the “Common Cold”

The “common cold” is the most prevalent disease affecting humans and is defined by the expression of symptoms/signs (primarily nasal, nasopharyngeal) consistent with an underlying virus infection (with rhinoviruses predominating and others such as Coxsackie, influenza, parainfluenza, respiratory syncytial virus [RSV], adenovirus contributing). Although they are typically considered to be self-limiting and of short duration, these infections in the young, elderly, and immunocompromised can have significant complications, with high morbidity and excess mortality [12,13]. Moreover, recent cost-analysis studies document a large financial impact of the “common cold” on the microeconomy and macroeconomy secondary to absenteeism and poor work performance, as well as the large monetary outlays for “treatments” with limited efficacy [14•–16•]. A reading of these studies is unnecessary for most adults and children who express their appreciation of the disease’s impact in the oft-raised question: “Why can’t ‘modern’ medicine find a cure for the common cold?” To address that question, one must first define what the “common cold” is and then what a “cure” would represent.

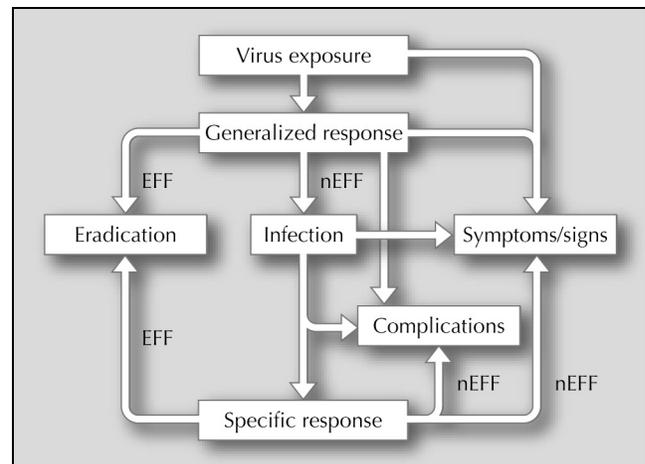


**Figure 1.** A Venn diagram representing our nosologic definition of a vURI. All vURIs are caused by virus infection, but not all vURIs are accompanied by complications or a vSSC. Complications and vSSCs are neither mutually exclusive nor inclusive, and a proportion of attributed vSSCs are misclassified nonviral SSCs (nvSSCs). See text for details. SSC—symptom/sign complex; URI—upper respiratory infection.

## Nosology

There is strong experimental evidence that nasal (nasopharyngeal) infection with upper respiratory virus is incompletely coupled to symptom/sign expression (Fig. 1). Not all infected persons express symptoms/signs, and attenuation of symptoms/signs by terminal signal blockade (*eg*, antihistamines) does not affect viral clearance [17]. Other evidence shows that symptom/sign expression is not prerequisite for the development of complications in infected persons [18,19]. Finally, with few nonexclusive exceptions (for example, fever for influenza), the constellation of symptoms/signs for the “common cold” is not well differentiated from other causes of nasal/nasopharyngeal inflammation (*eg*, allergy/streptococcal infection) [20] and does not discriminate among the different causative viruses [21]. From these observations, we can create a nosologic definition of the “common cold” as a symptom/sign complex (SSC) indicative of generalized nasal inflammation with an exclusionary attribution by default to an underlying viral infection (vSSC). Viral cultures and/or other assays are rarely done for clinical “colds,” and assignment is usually made based on history, seasonality, and/or short duration of SSC presentation. More inclusively, we can define the “common cold” as an upper respiratory virus infection (vURI) with or without the defined vSSC.

The “common cold,” defined by presence of the vSSC, is the entity referenced by the general population in their urgency for a “cure,” because vURI in the absence of vSSC is not self-assessable. However, there are strong theoretical



**Figure 2.** A simple representation of the causal pathways linking viral exposure to infection and viral eradication by activation of the general and specific host defense responses. Note that we assign a multiple set of paths to the development and expression of symptoms/signs and complications (SSCs). Because some of these paths also participate in host defense, caution is advised in seeking a vSSC “cure” in the absence of a vURI “cure.” Not shown are the various feedback paths for downregulation of responses after virus eradication. See text for details. EFF—effective; nEFF—non effective; vURI—viral infection.

concerns that a vSSC “cure” in the absence of a vURI “cure” could be detrimental to the population and to the individual. For example, given the lack of a one-to-one correspondence between vSSC and vURI and/or complications, the introduction of treatments that affect vSSC alone can be expected to drive an increased prevalence of those complications as infectious signaling, and voluntary withdrawal of the contagious individual from social contact would be inhibited. Also, treatments that affect early signaling events upstream to the bifurcation of the event sequences leading to vSSC and to antiviral host defense could potentially be deleterious by downregulating the host response to the virus infection (Fig. 2) and, thereby, allowing dissemination of virus and/or synergistic bacterial pathogens to adjacent anatomical compartments (*eg*, lungs, sinuses, middle ear). These concerns are not applicable to similar strategies used to treat allergic reactions for which the host inflammatory response to an “innocuous” antigen is considered to be the pathology.

## Causality

Although the implications of the introductory quotation from Kant have been criticized for his failure to discriminate between deductive and inductive inference, with the latter founded on probability and refuted by violation, appropriate caution is emphasized with respect to assigning causality to event (*ie*, relatum) relationships. In keeping with Kant’s analysis of causality (as refined by modern scholars), the relata (chemicals, events) included in descriptions of disease pathogenesis and symptom/sign expression need first to be categorized with respect to

immanence (existence in space-time), individuation (grainedness), and adicity (number), and the relationships should be classified with respect to connection (causal vs cotemporal), direction (cause-effect, effect-cause, conjoined effects), and selection (appropriate choice of relata). In analyzing pathways for disease pathogenesis, the relata are most simply viewed as signals and responses, where the response could be a “downstream” second signal. Signal-response relationships have an implicit direction, whereas the direction of signal-signal relationships is defined by empirical data. In both cases, causality is supported if the signals/responses are immanent and proximate in space-time and temporally ordered, with cause preceding effect. A discussion of these issues is rarely found in the medical literature. A good primer for those interested has been written by Schaffer [22].

To illustrate, we use the “simple” signal-response pathway linking histamine nasal challenge to symptom/sign expression [23]. In this case, the primary signal (histamine dose) is immanent, fine-grained (titerable), and unitary, but the classification of responses varies with the level of analysis. There, nasal exposure provokes a dose-related SSC that includes sinus pain, itchy eyes/nose/ears, nasal congestion, rhinorrhea, and sneezing. As an event, the SSC is immanent, has an adicity valence  $\geq 5$ , and is fine-grained (variable magnitude). In comparison, the first two components of the SSC lack documented immanence (not measurable by objective techniques), and all five components have an expected adicity valence of 1 and are fine-grained. By design, the relationship can be defined as causal with a signal-response direction and unbiased selection of stimulus. However, experimental results show that this simple analysis is in error. There, histamine-receptor blockade abolishes sneezing and reduces rhinorrhea but has no effect on the nasal congestion provoked by histamine exposure [24]. A reinterpretation of the description, given these results, assigns a more coarse grainedness to nasal congestion (a summed measure of responses over multiple intermediate pathways) and a higher adicity valence to the primary histamine response (a multiplicity of divergent pathways leading to different responses).

A similar analytic approach can be applied to more complex networks and pathways, but such analyses are complicated by the additional problems of signal redundancy (multiple signals causing identical responses), signal conservation (identical signals used in different, unrelated pathways), response mimicry (different events assigned to the same response), biased selection (choice of a pseudo-signal spatiotemporally co-represented with the true signal or of a multivalent response and pseudo-signal that are both caused by a true [non measured] signal), and null signals (absence of a signal causing a response), among others. Also, there are special problems associated with the application of this analytic method to studies of disease pathogenesis, such as: signal potency; metabolic rate and detectability by assay; the spatial compartmentalization of

the signaling cascade; and accessibility of that compartment to signal-response assay (*eg*, nasal biopsy for intramucosal events), the possibility of signals that act upon themselves (*eg*, feedback inhibition), and the grainedness of the analytic level (*eg*, organ, tissue, cellular, intracellular), among others. In this summary, we cannot present a critical analysis of the misapplication of casual inference in past studies of the common cold, but introduce this material so that investigators, reviewers, and readers correctly interpret co-represented signals/responses. We suggest that, barring empirical support for causality (*eg*, modulated signal leading to altered response), signals that precede and correlate with a response should be referred to as “predictive markers” of that response.

### Signal/Responses During the “Common Cold”

The “common cold” is a viral infection of the nasal/nasopharyngeal mucosa that, upon detection by the host, invokes two interactive and temporally overlapping defense systems, a generalized, innate immune response and a virus-specific, adaptive immune response. Both systems include inflammatory pathways that facilitate and actively recruit antiviral proteins and effector cells to the site of infection. The generalized, innate response occurs early after virus exposure and includes a number of signal-response pathways capable of detecting viral infection, inactivating virus, and destroying infected host cells and other pathways that activate or dampen the adaptive immune response as required [25]. The adaptive immune response is invoked later and includes multiple, redundant, feedback-modulated pathways that limit the extent of infection, bind free virions, kill infected host cells, detect viral clearance, repair mucosal damage, and downregulate inflammation [26]. Because the early, innate response is relatively nonspecific, it is expected that many of the invoked pathways are coincidental and without relevance to viral clearance. Yet, these, as well as all other activated pathways, potentially contribute to the vSSC. Pharmacologic modulation of the signals for these non-relevant pathways and for all innate response pathways after full activation of the adaptive immune response can be expected to reduce the vSSC magnitude without affecting host defense. A similar argument cannot be made for pharmacologic modulation of the signaling pathways of the adaptive immune system, where network complexity precludes simple causal analysis (see earlier). For example, manipulations designed to decrease the vSSC may have adverse consequences with respect to viral clearance because of signal conservation, whereas others designed to enhance antiviral activity may have an opposite effect by feedback inhibition of the requisite pathways for viral clearance and tissue repair.

Early attempts to identify possible signaling molecules synthesized or released during a vURI focused on

established mediators of the nasal allergic reaction and included histamine, bradykinin, and the prostaglandins, leukotrienes, and other arachidonic acid metabolites [1–3,27,28]. The usual format for these studies was daily monitoring for symptoms/signs and periodic assay of recovered nasal lavage fluids for suspected “mediators” in adults experimentally exposed to a “common cold” virus. Largely, the results of those studies were disappointing, with only bradykinin showing a consistently increased concentration during active infection and a temporal correlation with subjective and objective measures of secretion production. In follow-up studies, bradykinin was shown to be present during the symptomatic period of “natural” colds [29] and to provoke nasal congestion, rhinorrhea, sinus pain, and sore throat when applied to the nasal mucosa [23,30]. These data were interpreted as eliminating histamine and the various arachidonic acid metabolites as possible signaling chemicals during a vURI, while establishing a causal relationship between bradykinin and the specified symptoms. More recent studies reported increased histamine metabolites in urine collected during the symptomatic period of experimental influenza infection [31] and increases in leukotriene C4 in nasal lavage fluids during experimental rhinovirus infection [32], but these studies included a small number of subjects, and the data were generally characterized within and between subject variances.

Intervention studies designed to exploit this causal analysis called these interpretations into question. Treatments of experimental colds with specific bradykinin receptor blockers [33] or other therapies that significantly decreased nasal bradykinin levels [34] had no effect on the vSSC or on the individual symptoms (*ie*, demonstrated a causal disassociation between bradykinin level and signs/symptoms). In contrast, whereas studies of antihistamine, steroid, and nonsteroidal interventions documented no significant effects on the vSSC, those results showed significant effects on the specific symptoms/signs that were uniquely provoked by the target chemical in nasal-challenge studies [17,23,35–38]. None of these treatments was reported to significantly affect nasal congestion, decrease the frequency of certain otologic complications, or increase the magnitude or duration of viral shedding (*ie*, showing a disassociation between the pathways leading to host defense, selected symptoms, and selected complications). Treatment of colds with “first generation” antihistamines consistently lessens sneezing, rhinorrhea, and secretion production, but the results of studies using “second generation,” non-sedating antihistamines are less conclusive. Recently, Muether and Gwaltney [39•] presented an interesting hypothesis to explain this difference. They propose that the ability of first-generation antihistamines to suppress sneezing and reduce secretions lies in their ability to cross the blood-brain barrier and block histaminic and muscarinic receptors in the medulla.

These observations emphasize some of the difficulties of causal analysis when applied to complex networks, such as signaling during a vURI. For example, the early stage of a vURI is marked by a large influx of vascular secretions and plasma proteins [27,28], which introduces the substrate for bradykinin synthesis to a nasal mucosa rich in the converting enzyme. This transudation serves to dilute any locally synthesized/released chemicals (*eg*, histamine, prostaglandin, leukotriene), whereas increasing local bradykinin levels by substrate limited synthesis. In terms of causal analysis, the purported signal (bradykinin) and the response (secretion) are directionally inverted (secretions cause bradykinin) or, at a coarser level of analysis, do not have the property of individuation (bradykinin level = secretion quantity). Also, the vSSC and other high acidity response measures are not useful outcomes in intervention studies designed to clarify signal-response pathways and need to be replaced by more individuated responses. This would require much larger sample sizes than most studies have employed, thus entailing higher, and perhaps unreasonable, costs to fund such a research program [40].

More recently, the focus of studies exploring mediation of vURI disease expression has shifted to the proinflammatory cytokines and chemokines, a family of host-derived chemicals that orchestrate and coordinate the inflammatory response to a variety of insults [41]. Although there is a large literature documenting the elaboration of biologically active cytokines and other intercellular-signaling chemicals, including interleukin (IL)-1 $\beta$ , tumor necrosis factor (TNF)- $\alpha$ , IL-6, IL-8, and nitric oxide (NO) by epithelial cells, leukocytes, and other cell populations after virus infection or exposure to cytokine stimuli [42–44], these studies do not easily relate to vSSC expression or the coordinated responses of the host to infection, subjects that are central to the theme of this review, and, consequently, are not discussed. Also not reviewed here is a model system wherein the nasal mucosa is challenged with a specified cytokine [45]. Given that cytokines and chemokines are intercellular-signaling molecules, such studies generate information in a nonrepresentative environment (normal vs inflamed mucosa), making contextual interpretation difficult. Rather, we present a summary of the data for intercellular-signaling chemicals in nasal-lavage fluids recovered from subjects with vURIs caused by different viruses and the supporting evidences that the levels of some of these chemical signals serve as “predictive markers” of the vSSC magnitude. Because of the limited and contradictory results for NO, we defer discussion of this mediator to a later time [46].

In natural vURIs caused by RSV, parainfluenza virus, rhinovirus, and influenza virus, Gern *et al.* [47] focused on the production of the proinflammatory chemokine, IL-8. For control children, they reported a positive correlation between IL-8 nasal-lavage level and age and lesser lavage levels when compared with those for children infected with RSV, parainfluenza virus, and rhinovirus, but not

influenza virus. A correlation between IL-8 level and vSSC was only observed for rhinovirus infection. Other investigators conducted a broader survey of cytokine production during natural colds. For example, Noah *et al.* [48] collected serial lavages from 95 children with a vURI (*ie*, vSSCs) of unidentified etiology and reported increased IL-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$  during the period of acute illness. Roseler *et al.* [49] studied 20 patients with vSSC and five controls and reported increased lavage concentrations of IL-1 $\beta$ , IL-6, and IL-8 but not IL-4 (nonmeasurable in all samples) in vSSC patients only. Kaiser *et al.* [50•] assayed IL-6, TNF- $\alpha$ , interferon (IFN)- $\alpha$ , IFN- $\gamma$ , and IL-10 in lavage fluids and serum from patients with community acquired influenza A infection. They reported significant elevations in all measured cytokines during the period of illness. In correlational analyses, a rapid decrease in viral titer was related to the magnitude of the IFN- $\gamma$  level, and the magnitude of the vSSC (which included temperature as a sign component) was directly related to IL-6 level. Taken together, these observations document local nasal (and perhaps systemic [50•]) production of proinflammatory, anti-inflammatory, and regulatory cytokines during vSSC expression in natural vURIs caused by different viruses. However, this study format does not offer good control over event timing in reference to viral infection nor can it easily provide signal-response data for asymptomatic or subclinical vURIs.

To remedy this, a number of investigative groups studied nasal cytokine production in adults experimentally infected with a known virus. In studies focused on a specific cytokine, RV infection was shown to provoke increased nasal-lavage levels of IL-1 $\alpha$  [42], IL-1 $\beta$  [51], IL-6 [43], IL-8 [44,52,53], granulocyte colony-stimulating factor (G-CSF) [54], and IL-1ra [42]. Of these, IL-8 was constitutively present and was reported to correlate with vSSC in one study [52]. Also, G-CSF was reported to correlate with increased blood neutrophils, and both G-CSF and IL-8 to correlate with nasal neutrophils [54]. A later study of experimental rhinovirus infection that assayed nasal lavages for a panel of cytokines, including IL-1 $\beta$ , IL-6, IL-8, IL-10, and TNF $\alpha$ , reported increased levels of IL-1 $\beta$  and IL-6 in symptomatic, infected subjects but not in asymptomatic subjects. No postexposure changes in IL-8, IL-10, and TNF- $\alpha$  were documented in either the symptomatic or asymptomatic group, but a phase relationship between vSSC magnitude and IL-1 $\beta$  and IL-6 levels was observed [55].

A similar experimental format was used to study the local cytokine response to coronavirus, RSV, and influenza virus infection. In a pilot study using coronavirus challenge, 10 of 20 exposed subjects developed a vSSC. IL-4, IL-6, and G-CSF were not elevated in the nasal-lavage fluids postexposure in either group, whereas IL-1 $\beta$  increased in all subjects, and IFN- $\gamma$  increased in subjects with a vSSC [56]. Also, in a preliminary study of experimental RSV infection, three of 10 subjects inoculated with RSV developed a vSSC and shed virus. In that subset,

nasal-lavage IL-8 levels showed a transient postinoculation increase. During the period of viral shedding, lavage levels of IL-8, released on activation, normal T-cell expressed and secreted (RANTES), macrophage inflammatory protein (MIP)-1 $\alpha$ , and monocyte chemoattractant protein (MCP)-1 all increased [57].

For experimental influenza virus infection, an early study reported a postexposure increase in IL-6 but not IL-4 level in recovered nasal lavages [58]. The patterns of change in nasal IL-6 and nasal, but not systemic, symptoms were appropriately phased for possible causality. In follow-up studies, Cohen *et al.* [59] reported that the greater vSSC during influenza infection for subjects with high baseline psychological stress was also reflected in higher postexposure IL-6 levels in the nasal lavage, and Skoner *et al.* [60] reported that rimantadine treatment (2 days post-influenza exposure) significantly reduced viral shedding, the provoked systemic symptoms, and nasal lavage IL-8 level, but not the provoked nasal symptoms or nasal lavage IL-6 levels. In an experimental study of zanamivir prophylaxis, Fritz *et al.* [61] reported that treatment prevented influenza infection and abolished the postexposure rises in IL-6, IL-10, TNF- $\alpha$ , IFN- $\gamma$ , MCP-1, and MIP-1 lavage levels documented for placebo-treated subjects. More inclusively, Hayden *et al.* studied the nasal lavage and blood levels of IL-1 $\beta$ , IL-2, IL-6, IL-8, IFN- $\alpha$ , TGF- $\beta$ , and TNF- $\alpha$  in 19 volunteers during the course of an experimental influenza infection [9]. They reported that nasal IL-6 and IFN- $\alpha$  peaked early (day 2) and correlated directly with viral titers, temperature, mucus production, and symptom scores. TNF- $\alpha$  peaked later when viral titer was dropping (day 4), and IL-8 peaked late (days 4–6) and correlated only with lower respiratory symptoms. No infection-related changes in nasal lavage IL-1 $\beta$ , IL-2, or TGF- $\beta$  levels were observed.

The more complete data for influenza infection shows a phased series of cytokine elaborations during infection with an early period of nasal symptoms/signs corresponding to IL-6 elaboration, resolution of viral shedding corresponding to TNF- $\alpha$  elaboration, and resolution of upper-respiratory illness (but with possible lower-airway involvement) corresponding to IL-8 elaboration [9]. However, although that study related IL-6 to systemic symptoms (*eg*, temperature), the rimantadine intervention study ascribed IL-8 as a marker of those symptoms (co-modulated) [60]. The results of all reviewed studies support IL-6 as a predictive marker of nasal symptoms/signs for influenza infection, but not for those symptoms/signs during coronavirus (IL-1 $\beta$ , IFN- $\gamma$ ), RSV (IL-8), and rhinovirus (IL-6 or IL-8) infections.

One difficulty in comparing the results of these studies for the same virus or among viruses is the lack of a standard protocol that includes a specific panel of signals and responses to be assayed in all experimental studies. The benefits of adopting such a protocol is exemplified by the previously unpublished data (Gentile *et al.* [58]) presented

**Table 1. Selected responses to nasal challenge of adults with influenza, RSV, and rhinovirus**

Response	Malady	Day								
		0	1	2	3	4	5	6	7	8
Nasal symptoms	Influenza	0.7	1.2	2.9	2.5	2	1.1	0.7	0.2	ND
	RSV	0.2	0.4	0.9	1.3	2	2.2	2.7	3.1	2.6
	Rhinovirus	0	2	3.8	3.8	1.9	1.3	ND	ND	ND
Secretion wt (g)	Influenza	0.8	1.3	4.3	6.8	2.4	1	0.5	0.3	ND
	RSV	0.4	0.6	1.2	0.9	0.8	1.5	2.5	4.3	1.7
	Rhinovirus	0.6	1.9	6.4	8.7	5.9	6.2	ND	ND	ND
IL-1 (log difference)*	Influenza	0	0.02	0.02	0.23	0.15	0.11	0.01	-0.12	ND
	RSV	0	0.14	0.08	0	0.05	0.16	0.6	0.69	0.38
	Rhinovirus	0	-0.08	0.77	0.6	0.48	0.36	0.28	ND	ND
IL-6 (log difference)	Influenza	0	0.13	0.66	0.77	0.79	0.84	0.54	0.22	ND
	RSV	0	0.09	0.16	0.14	0.2	0.55	0.12	0.17	0.45
	Rhinovirus	0	0	0.97	0.97	0.58	0.63	0.46	ND	ND
IL-8 (log difference)	Influenza	0	0.15	0.01	0.22	0.32	0.38	0.3	0.13	ND
	RSV	0	0.14	0.17	0.08	-0.05	0.26	0.62	0.57	0.53
	Rhinovirus	0	-0.12	0.36	0.46	0.45	0.38	0.55	ND	ND
IL-10 (log difference)	Influenza	0	-0.03	0.26	0.33	0.25	0.5	0.56	0.15	ND
	RSV	0	-0.04	0.01	0.02	-0.06	0.05	0.13	0.52	0.41
	Rhinovirus	0	0	0.64	0.57	0.09	0.06	0.04	ND	ND

\* Note that the lavage values of cytokines are adjusted by subtracting the log baseline (day 0) from the log values at each day and consequently have no units, but represent fold increase.

IL—interleukin; ND—not done; RSV—respiratory syncytial virus.

in Table 1 for subjects experimentally infected with rhinovirus type 39 ( $n = 16$ ), influenza A virus ( $n = 26$ ), and RSV ( $n = 13$ ), and followed under identical conditions using standardized assessment methods and assay procedures. The use of standardized measures allows comparisons of response amplitudes and signal-response phasing among viruses. For example, although the nasal symptom/sign complex for rhinovirus and influenza occurs within 3 days after exposure, that for RSV is delayed by as much as 7 days. Importantly, this temporal pattern is also observed for the four assayed cytokines. Also of interest is that for all three viruses, the cytokine that tracks nasal symptoms most closely is IL-1, as opposed to the other previously suggested candidates—IL-6, which remains elevated after nasal symptoms resolve, and IL-8, which peaks after nasal symptoms and remains elevated for an extended time. Such comparisons can also be used to validate or refute hypotheses, as, for example, the suggested causal relationship between IL-6 and fever/elevated temperature reported by Hayden *et al.* [9] for influenza A infection. Clearly, nasal IL-6 is not sole and sufficient as a marker of elevated temperature/fever given the observation that IL-6 is produced in similar magnitude during rhinovirus infection, where elevated temperature/fever is not a component of the vSSC.

Other groups studied upper-airway cytokine expression during “natural” vSSCs and/or vURIs with the specific goal of identifying signals related to lower airway complications. Bonville *et al.* [62] detected increased MIP-1 $\alpha$  and RANTES, chemokines with potent effects on the recruitment, and degranulation of eosinophils and basophils in nasopharyngeal secretions from pediatric patients with

vURI caused by RSV, adenovirus, influenza, and parainfluenza virus infection. Sheeran *et al.* [63] reported that RANTES, MIP-1 $\alpha$ , IL-6, IL-8, and IL-10 nasal-lavage levels were significantly greater in 24 hospitalized children with RSV infection compared with non-ill, control children. These levels correlated with RSV titer, and RANTES level was greater for nonintubated when compared with intubated patients. Hornsleth *et al.* [64] reported that a higher TNF- $\alpha$ :IL-6 ratio for nasal lavages recovered from infants with RSV infection was related to greater illness severity, and in a later, more comprehensive analysis reported that the TNF-R1/RANTES ratio was a more consistent indicator of illness [65]. In a comparison study of RSV and influenza virus infection in children with and without asthma, nasal aspirate levels of IL-11 were significantly greater in asthmatic children with RSV when compared with nonasthmatic children with RSV, and nasal IL-6 levels were greater in RSV-infected children when compared with influenza-infected children. No between-virus or between-asthmatic state differences were observed for nasal IL-8 and IFN- $\gamma$  levels [66]. Also, IFN- $\gamma$  was reported to be present in nasal-lavage fluids from 30/39 (76.9%) infants with RSV infection, but the purported upstream signals for IFN- $\gamma$  synthesis, IL-12, and IL-18 were detectable in only 6/40 (15%) and 11/38 (28.9%), respectively. Nasal IL-18, but not IFN- $\gamma$  nor IL-12, was found in significantly greater concentrations in subjects with nonhypoxic forms of bronchiolitis than in those with vSSC alone [67]. Unfortunately, a synthesis of these observations with respect to linking a cytokine, cytokine ratio, or other signals as a predictive marker to lower-airway involvement during a vURI is not

possible given the seemingly arbitrary choice of assayed chemicals for each study, the inherent differences among studies in design, methods of assessment and illness definition, and the presentation bias for enrolled individuals attributable to the use of a hospital setting.

## Conclusions

A description of the signaling pathways leading to the host antiviral response and accompanying vSSC during a vURI has the laudable goals of reducing illness burden, complications, and viral spread and remains an important focus of continuing research. Although studies of signaling chemicals in natural vURIs can provide guidance in this quest as well as confirmatory evidence for observations made in experimental models (both in vitro and in vivo), their restriction to illnesses with a documented vSSC and the temporal uncertainty of sampling with respect to timing of virus infection make interpretation of these "biased" data difficult, at best. In contrast, assay of secretions and compartmental effector cells (eg, mucosal biopsy, scraping) for chemical signals (and/or their precursors) during the course of an experimental virus infection in combination with signal-specific blockade and/or parallel in vitro characterization of cellular responses offers a unique opportunity to study these pathways and their interactions. An independent but complementary strategy now in its infancy is to include genotyping for cytokine polymorphisms on all subjects enrolled in experimental studies, with the goal of relating these results to cytokine level, illness expression, and host defense. Preliminary studies using this assay showed consistency with respect to genotype effects on natural and experimental RSV infection [68•,69•].

To date, the power of these methodologies has not been exploited because of the small number of subjects included in any one study [40], the presentation of data for individual signals from the same study in different publications [42–44], the non-uniform selection of assessed signals and responses across studies [9,60,70,71], and the misapplication of causal analysis in data interpretation. To overcome these limitations, we recommend that the protocols used by different investigators are standardized to the extent possible (including a standard panel of signals and individuated responses); that archival lavage fluids and DNA samples from all study volunteers are stored indefinitely should later research identify unsuspected signaling chemicals; and that, after independent publication of the study results by an investigative team, contributing investigators submit de-identified data (and appropriate protocols) to a shared data repository for later pathway analyses. Given the expected need for large sample sizes for characterizing the signal-response pathways, implementation of these recommendations would satisfy this requirement as well as allow the generation of specific hypothesis, the rational estimation of sample size for hypothesis testing, and the elimination of bias associated with favored publication of positive results.

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